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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Post-challenge hyperglycemia is a significant risk factor for the development of hepatocellular carcinoma in patients with chronic hepatitis C

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Abstract
Background Several epidemiological studies have reported that diabetes mellitus is a risk factor for hepatocellular carcinoma (HCC) in hepatitis C virus (HCV)-positive patients. However, it is unclear whether or not post-challenge hyperglycemia is a risk factor. The purpose of this study was to determine the association between post-challenge hyperglycemia and hepatocarcinogenesis in HCV-positive patients.
Methods A total of 203 HCV-RNA-positive subjects (108 males, mean age 54.3 ± 10.8 years; 95 females, mean age 56.6 ± 10.3 years; genotype 1b/2a/2b/3a: 152/38/12/1) who underwent liver biopsy and a 75-g oral glucose tolerance test, and who were treated with interferon (IFN) were enrolled in this study. None of the subjects had been treated with antidiabetic drugs. The subjects underwent ultrasonography and/or computed tomography every 6 months after the end of the IFN therapy.
Results Thirteen patients, including one patient who achieved a sustained viral response (SVR) with IFN, developed HCC. On multivariate analysis, male sex, age >65 years, excessive alcohol consumption, non-SVR, liver steatosis area >5% in liver specimens, and 120-min post-challenge hyperglycemia were risk factors for the development of HCC. After matching subjects for sex, age, alcohol intake, and response to the IFN therapy, advanced fibrosis stages [hazard ratio (HR) 2.8], liver steatosis (HR 5.4), and 120-min post-challenge hyperglycemia (HR 4.9) were significant risk factors for the development of HCC. Furthermore, after matching for the fibrosis stage, liver steatosis (HR 5.7) and 120-min post-challenge hyperglycemia (HR 6.9) remained as significant factors for HCC development.
Conclusion Post-challenge hyperglycemia is an independent risk factor for HCC in HCV-positive patients.

Keywords Hyperglycemia · Oral glucose tolerance test · Hepatocellular carcinoma · Hepatitis C

Abbreviations

HCV	Hepatitis C virus
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
DM	Diabetes mellitus
HR	Hazard ratio
BMI	Body mass index
ALT	Alanine aminotransferase
HbA1c	Hemoglobin A1c
IFN	Interferon
OGTT	Oral glucose tolerance test
PCR	Polymerase chain reaction
HOMA-IR	Homeostasis model assessment for insulin resistance
SVR	Sustained viral response

Introduction

Chronic hepatitis C virus (HCV) infection is a disease that can progress to cirrhosis and hepatocellular carcinoma (HCC) [1, 2]. Several factors associated with HCC

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development in chronic HCV have been reported, including male sex, older age at infection, excessive alcohol consumption, coinfection with hepatitis B virus (HBV), and variations in HCV itself [3–6]. Recent epidemiological studies have shown that diabetes mellitus (DM) is also a risk factor for HCC in patients with chronic hepatitis C [7, 8], although studies in Taiwan revealed no association between DM and HCC [9, 10]. Therefore, it is still unclear whether or not DM is a significant risk factor for HCC.

Furthermore, despite the findings of these epidemiological studies, several issues remain unresolved. First, not all of the subjects in these studies underwent glucose tolerance tests, and DM was defined based on inconsistent criteria, with some studies defining diabetes based on the use of antidiabetic drugs such as insulin, the presence of fasting hyperglycemia, and/or abnormal levels of hemoglobin A1c (HbA1c). Accordingly, it is not clear whether specific components of DM, particularly post-prandial hyperglycemia, are risk factors for HCC. Second, no study has evaluated whether insulin resistance or hyperinsulinemia, which might develop in advance of hyperglycemia, is associated with the development of HCC. Third, it is unclear whether DM remains a risk factor for HCC after accounting for pathological liver findings such as fibrosis, inflammation, and steatosis, which are acknowledged risk factors for HCC [11–14]. Fourth, because many HCV-positive patients receive interferon (IFN) therapy, it is essential to consider the response to IFN therapies in such studies.

Therefore, considering these limitations of earlier studies, and the unanswered questions, we conducted a prospective cohort study of subjects with chronic hepatitis C, who underwent a 75-g oral glucose tolerance test (OGTT), liver biopsy, and IFN therapy.

Patients and methods

Patients

Overall, 203 HCV-positive subjects who underwent liver biopsy and a 75-g OGTT between 2002 and 2007 and who were treated with IFN were enrolled in this study (Table 1). All of the subjects were positive for serum HCV-RNA detected by polymerase chain reaction (PCR). Criteria for inclusion in the study were: hemoglobin $\geq$12 g/dl, leukocyte count $\geq$3,000/mm^3, platelet count $\geq$90,000/μl, and serum creatinine levels within the normal range. Patients were excluded if they had decompensated liver disease; were hepatitis B surface antigen-positive; or had a history of liver transplantation, neoplastic disease (including HCC), severe cardiac or chronic pulmonary disease, autoimmune disease, a psychiatric disorder, or severe retinopathy; or were planning on becoming pregnant. In this study, subjects who met the criteria of both fasting glucose level $\geq$126 mg/dl and HbA1c $\geq$6.5%, were diagnosed as having overt DM and excluded because they should be treated for DM prior to IFN therapy. Subjects who were treated with antidiabetic drugs or subcutaneous insulin infusion were excluded because it was difficult to perform the 75-g OGTT and analyze its results, and because it is unclear whether antidiabetic drugs affect HCC occurrence.

Because the duration of IFN therapy differed among the subjects, the end of the IFN regimen was defined as the start of the study observation period. The endpoint of this study was HCC occurrence. The protocol was approved by the Local Review Board in accordance with the ethical guidelines of the Declaration of Helsinki (1975, as revised in 1983). Written informed consent was obtained from all patients.

Physical examination, serum biochemistry, and OGTT

Body mass index (BMI) was calculated as the body weight in kilograms divided by the square of the height in meters (kg/m^2). Venous blood samples were taken from all patients at around 0800 hours after a 12-h overnight fast, to determine blood cell count and blood chemistry. Serum HCV-RNA levels were analyzed by reverse-transcriptase PCR (nested PCR or Amplicor; Roche Diagnostic Systems, CA, USA) and HCV genotypes were determined by reverse-transcriptase PCR (Roche Diagnostic Systems, CA, USA).

Insulin resistance was evaluated by the homeostasis model assessment for insulin resistance (HOMA-IR), using the following equation [15]: HOMA-IR = fasting insulin (μU/ml) × fasting glucose (mg/dl)/405. The reference values for fasting glucose level and fasting insulin level in our clinical laboratory are 70–110 mg/dl and 4–24 μU/ml, respectively. However, we considered subjects with HOMA-IR of >2.5 as showing insulin resistance, according to a previous report [16].

All subjects underwent a 75-g OGTT. Samples were collected at baseline and every 30 min after glucose ingestion for 120 min to measure glucose and insulin levels. All examinations were performed up to 3 months before starting IFN therapy.

Liver histology

Liver needle biopsies were performed percutaneously with a 16-G needle (Super-Core™ semi-automatic biopsy instrument; InterV Clinical Products, Dartmouth, MA, USA) up to 3 months before starting IFN therapy. All subjects enrolled this study underwent liver biopsy. The

Table 1 Clinical characteristics of the patients

	Total number of patients (n = 203)	Patients with HCC (n = 13)
Age (years)[a]	55.4 ± 10.6	63.1 ± 6.5
Female % (M/F, n)	46.8 (108/95)	7.7 (11/1)
Alcohol consumption		
Excessive/daily/social or none, n	21/30/152	4/0/9
BMI[a]	23.5 ± 3.0	23.7 ± 2.7
IFN therapy history (naïve/>2), n	132/71	6/7
ALT (IU/l)[a]	71.3 ± 55.0	76.3 ± 44.8
Platelets ($\times 10^4$/μl)[a]	16.3 ± 6.2	12.1 ± 3.2
AFP (ng/ml)[a]	15.0 ± 37.8	14.3 ± 9.6
Viral load ($\times 10^6$ IU/ml)[a]	1.8 ± 1.5	1.5 ± 1.1
Genotype (1b/2a/2b/3a)[a]	152/38/12/1	12/0/1/0
Fasting glucose (mg/dl)[a]	86.7 ± 9.3	89.9 ± 14.4
Fasting insulin (μU/ml)[a]	9.4 ± 5.5	10.9 ± 7.8
HOMA-IR[a]	2.0 ± 1.3	2.7 ± 1.9
Liver histology		
A0/A1/A2/A3, n	1/71/106/25	0/3/8/2
F0/F1/F2/F3/F4, n	2/91/63/37/10	0/1/6/5/1
Steatosis <5/5–9/>10%, n	175/15/13	7/3/3
Response to IFN therapy; SVR, n (%)	89 (44.3)	1 (7.7)

HCC hepatocellular carcinoma, *BMI* body mass index, *IFN* interferon, *ALT* alanine aminotransferase, *AFP* alpha-fetoprotein, *HOMA-IR* homeostasis model assessment for insulin resistance, *SVR* sustained viral response

[a] Data are expressed as means ± SD

liver biopsy specimen was fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin–eosin and Azan for histological evaluation. Pathological liver fibrosis and inflammation activity were evaluated according to the METAVIR scoring system (stages 0–4 for fibrosis and grades 0–4 for inflammatory activity) [17]. The area of steatosis in the liver specimen was calculated using Image J 1.42 (http://rsb.info.nih.gov/ij/). All liver biopsy specimens were evaluated by three experienced pathologists who were unaware of the clinical conditions of the patients.

Therapy and follow-up protocol

Between 2002 and 2003, all of the treatment-naïve (hereafter, 'naïve') patients with genotype 1b/high viral load (>100 KIU/ml) and patients refractory to prior IFN therapy were treated with either IFNα2a or IFNβ plus oral ribavirin at body weight-dependent doses (total dose: 600 mg for patients <60 kg; 800 mg for patients weighing 60–80 kg; 1,000 mg for patients weighing ≥80 kg). Between 2004 and 2007, all of the naïve patients with genotype 1b/high viral load and patients refractory to prior IFN therapy were treated with either pegylated (Peg)-IFNα2a (180 μg/week subcutaneously) or Peg-IFNα2b (1.5 μg/kg/week subcutaneously) plus oral ribavirin in body weight-dependent doses. Patients with genotype 1b were treated for 48 weeks, while all other patients were treated for 24 weeks. Between 2002 and 2003, non-genotype 1b and low viral load (<100 KIU/ml) naïve patients were treated with IFNα2a or IFNβ for 24 weeks, and between 2004 and 2007, such patients were treated with Peg-IFNα2a (180 μg/week subcutaneously) monotherapy for 24 weeks. Patients were not randomized to therapy and the selection of the therapeutic protocol was at the study physicians' discretion.

Ultrasonography and/or computed tomography were performed every 6 months in all patients. At 6 months after the end of treatment, patients with a negative qualitative HCV-RNA test were considered to have a sustained viral response (SVR). Patients with a negative qualitative HCV-RNA test at the end of therapy and a positive HCV RNA text after therapy were considered to show relapse. Patients who never achieved viral clearance during therapy were considered non-responders.

Statistical analysis

Comparisons between groups were made using the Mann–Whitney U test for continuous variables and the χ^2 test for categorical data. Changes in biological parameters in each group were assessed using paired t tests. Continuous variables are summarized as means ± SD. Differences were considered significant at $P < 0.05$. The Cox proportional hazard regression model was used for univariate and multivariate analyses to determine the risk of HCC occurrence. Significant variables on univariate analyses were included in the multivariate analyses. In the multivariate analyses, up to three subjects without HCC

occurrence were randomly selected for each patient with HCC, and were matched by sex, age, alcohol intake, response to IFN therapy, and fibrosis stage. Statistical analyses were performed with SPSS II (SPSS Japan, Tokyo, Japan).

Results

Subject characteristics

The clinical characteristics of the 203 patients (108 males, mean age 54.3 ± 10.8 years; 95 females, mean age 56.6 ± 10.3 years) enrolled in this study are summarized in Table 1. The average observation time was 52.0 ± 19.5 months. Twenty-one (10.3%) patients were classified as having excessive alcohol intake (>50 g ethanol per day). Using the METAVIR scoring system, fibrosis was staged as F0 in two patients (1%), F1 in 91 (44.8%), F2 in 63 (31%), F3 in 37 (18.2%), and F4 in 10 (4.9%). All liver biopsy specimens taken before therapy showed typical features of chronic HCV infection, including infiltration of lymphocytes in Glisson's capsule, piecemeal necrosis, and periportal fibrosis. The average area of steatosis in the liver specimens was 2.6 ± 3.1%. During the observation period, 13 patients, including one patient who achieved SVR with IFN therapy, developed HCC (12 males and 1 female, mean age 62.8 ± 6.7 years).

OGTT results

The serum glucose and insulin levels during the 75-g OGTT are shown in Fig. 1a, b. In patients who developed HCC (HCC group), the glucose levels at 30 ($P = 0.002$), 90 ($P = 0.033$), and 120 ($P = 0.001$) min, and the insulin levels at 30 min ($P = 0.017$) were significantly higher than those in patients without HCC (non-HCC group). There were no significant differences in fasting glucose or insulin levels between the HCC group and the non-HCC group.

Univariate and multivariate analyses of risk factors for HCC

On univariate analyses, male sex [hazard ratio (HR) 10.5], age >65 years (HR 5.3), excessive alcohol consumption (HR 4.6), non-SVR after IFN therapy (HR 9.5), advanced liver fibrosis (HR 2.9), α-fetoprotein >10 ng/ml (HR 4.6), liver steatosis area >5% (HR 5.7), and 120-min post-challenge hyperglycemia (>200 mg/dl; HR 6.3) were significant risk factors for the development of HCC (Table 2). BMI, fasting glucose, fasting insulin, insulin levels during the 75-g OGTT, HOMA-IR, cholesterol, and triglyceride were not associated with the development of HCC. Furthermore, viral load and genotype, and IFN therapy protocols were not associated with the development of HCC.

On multivariate analyses, male sex, age >65 years, excessive alcohol consumption, non-SVR, liver steatosis area >5%, and 120-min post-challenge hyperglycemia were risk factors for the development of HCC (Table 3). When we limited the analyses to the HCC group ($n = 13$) and non-HCC patients ($n = 30$) matched for sex (male; $n = 27$), age (>65 years; $n = 8$), alcohol intake (excessive alcohol intake; $n = 8$), and response to IFN therapy (SVR; $n = 3$), advanced fibrosis stage (HR 2.8), liver steatosis area >5% (HR 5.4), and 120-min post-challenge hyperglycemia (HR 4.9) were significant risk factors for the development of HCC. When we matched patients for fibrosis stage (advanced fibrosis stage; $n = 10$) as well as the above factors (male; $n = 27$, age >65 years; $n = 9$, excessive alcohol intake; $n = 8$, SVR; $n = 3$), liver steatosis area >5% (HR 5.7), and 120-min post-challenge hyperglycemia (HR 6.9) remained as significant factors associated with the development of HCC.

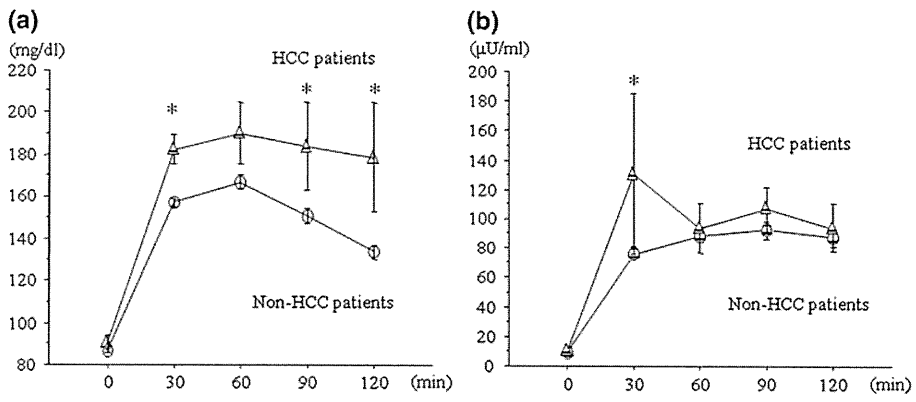

Fig. 1 **a** Serum glucose levels and **b** insulin levels on 75-g oral glucose tolerance test (OGTT). *Open triangles* patients who developed hepatocellular carcinoma (*HCC*). *Open circles* patients without HCC. *$P < 0.05$ by Mann–Whitney *U* test. *Error bar* ± standard deviation

Table 2 Univariate analyses: comparison of the risk factors for HCC between 13 patients with HCC and non-HCC patients

Variable	HCC ($n = 13$)	Non-HCC ($n = 190$)	HR (95% CI)
Male	12 (92.3)	96 (50.5)	10.5 (1.4–81.0)*
Age >65 years	6 (46.2)	29 (15.3)	5.3 (1.8–16.0)*
Excessive alcohol consumption[a]	4 (30.8)	17 (8.9)	4.6 (1.4–15.0)*
Response to IFN therapy; non-SVR	12 (92.3)	101 (53.2)	9.5 (1.2–73.2)*
Fibrosis stage; F3 and F4	6 (46.2)	41 (21.6)	2.9 (1.1–8.7)*
BMI >25	5 (38.5)	53 (27.9)	1.5 (0.5–4.7)
AFP >10 ng/ml	8 (61.5)	50 (26.3)	4.6 (1.4–15.2)*
Steatosis >5%	6 (46.2)	22 (11.6)	5.7 (1.9–17.1)*
Fasting glucose ≥126 mg/dl	1 (7.7)	2 (1.1)	6.4 (0.8–50.0)
Fasting insulin ≥15 μU/ml	2 (15.4)	29 (15.3)	0.9 (0.2–4.0)
HOMA-IR ≥3	2 (15.4)	33 (17.4)	0.7 (0.2–3.4)
120-min post-challenge hyperglycemia[b]	5 (38.5)	15 (7.9)	6.3 (2.0–19.1)*

Data are expressed as numbers (%)

HR hazard ratio, *CI* confidence interval, *BMI* body mass index, *IFN* interferon, *SVR* sustained viral response, *AFP* alpha-fetoprotein, *HOMA-IR* homeostasis model assessment for insulin resistance

* $P < 0.05$

[a] More than 50 g ethanol/day

[b] Serum glucose level was more than 200 mg/dl at 120 min on 75-g oral glucose tolerance test (OGTT)

Table 3 Multivariate analyses: comparison of the risk factors for HCC between 13 patients with HCC and non-HCC patients

Variable	HCC	Non-matched		Sex, age, alcohol intake, response to IFN matched		Sex, age, alcohol intake, response to IFN, fibrosis stage matched	
	$n = 13$	$n = 190$	HR (95% CI)	$n = 30$	HR (95% CI)	$n = 30$	HR (95% CI)
Male	12 (92.3)	96 (50.5)	18.8 (2.2–161.4)*	Matched	–	matched	–
Age >65 years	6 (46.2)	29 (15.3)	9.9 (2.5–39.9)*	Matched	–	Matched	–
Excessive alcohol consumption[a]	4 (30.8)	17 (8.9)	7.2 (1.4–37.5)*	Matched	–	Matched	–
Response to IFN therapy; non-SVR	12 (92.3)	101 (53.2)	20.4 (2.1–200.9)*	Matched	–	Matched	–
Fibrosis stage; F3 and F4	6 (46.2)	41 (21.6)	6.3 (1.2–33.3)*	8 (26.7)	2.8 (1.0–11.3)*	Matched	–
AFP >10 ng/ml	8 (61.5)	50 (26.3)	1.3 (0.4–1.8)	15 (50)	0.5 (0.7–3.1)	15 (50)	0.4 (0.1–2.2)
Steatosis >5%	6 (46.2)	22 (11.6)	5.6 (1.4–22.6)*	3 (10)	5.4 (1.1–27.3)*	2 (3.3)	5.7 (1.2–27.1)*
120-min post-challenge hyperglycemia[b]	5 (38.5)	15 (7.9)	19.5 (3.7–104.1)**	4 (13.3)	4.9 (1.3–18.9)*	2 (6.7)	6.9 (1.7–28.4)*

Data are expressed as numbers (%)

HR hazard ratio, *CI* confidence interval, *IFN* interferon, *SVR* sustained viral response, *AFP* alpha-fetoprotein

* $P < 0.05$, ** $P < 0.001$

[a] More than 50 g ethanol/day

[b] Serum glucose level was more than 200 mg/dl at 120 min on 75-g OGTT

Clinical characteristics of patients with post-challenge hyperglycemia

The clinical characteristics of 20 patients with 120-min post-challenge hyperglycemia on the 75-g OGTT and the remaining 183 patients are summarized and compared in Table 4. Fasting glucose levels and the HCC occurrence rate were significantly higher in patients with post-challenge hyperglycemia. On the other hand, the SVR rates were not significantly different, being 40% in patients with post-challenge hyperglycemia and 44.8% in patients without post-challenge hyperglycemia. The rate of patients with advanced liver fibrosis was higher in patients with post-challenge hyperglycemia than in the other patients, although the difference was not statistically significant.

Table 4 Clinical characteristics of the patients with/without 120-min post-challenge hyperglycemia

	Post-challenge hyperglycemia		*P* value
	With ($n = 20$)	Without ($n = 183$)	
Age (years)[a]	58.2 ± 8.3	55.1 ± 10.8	0.223
Female % (M/F, *n*)	35 (13/7)	48 (95/88)	0.265
Alcohol consumption			
Excessive or habitual/social or none, *n*	2/18	28/155	0.523
BMI[a]	23.9 ± 2.5	23.4 ± 3.0	0.477
ALT (IU/l)[a]	68.1 ± 35.1	71.7 ± 56.6	0.781
Platelets ($\times 10^4/\mu l$)[a]	16.0 ± 6.0	16.3 ± 6.2	0.830
AFP (ng/ml)[a]	21.0 ± 44.4	14.4 ± 37.0	0.458
Viral load ($\times 10^6$ IU/ml)[a]	1.4 ± 1.3	1.8 ± 1.6	0.205
Genotype (1b/non-1b), *n*	15/5	137/46	0.989
Fasting glucose (mg/dl)[a]	96.5 ± 15.9	85.8 ± 8.5	<0.001
Fasting insulin (μU/ml)[a]	9.4 ± 4.5	9.3 ± 5.6	0.978
HOMA-IR[a]	2.4 ± 1.4	2.0 ± 1.2	0.228
Liver histology			
A0–1/A2–3, *n*	4/16	68/115	0.128
F0–2/F3–4, *n*	12/8	144/39	0.060
Steatosis <5/5–9/>10%, *n*	17/0/3	158/15/10	0.122
Response to IFN therapy; SVR, *n* (%)	8 (40)	82 (44.8)	0.681
HCC occurrence, *n* (%)	5 (25)	8 (4.4)	<0.001

BMI body mass index, *IFN* interferon, *ALT* alanine aminotransferase, *AFP* alpha-fetoprotein, *HOMA-IR* homeostasis model assessment for insulin resistance, *SVR* sustained viral response

[a] Data are expressed as means ± SD

Cumulative HCC occurrence rate

The cumulative HCC occurrence rates in the patients with 120-min post-challenge glucose levels of ≥200 and those with levels of <200 mg/dl are shown in Fig. 2a. While the HCC occurrence rates at 3 and 5 years were 3.3 and 4.3% in patients with 120-min glucose <200 mg/dl, the corresponding rates were 15.0 and 28.1% in patients with 120-min glucose ≥200 mg/dl. There was a significant difference in the HCC occurrence rate between patients with 120-min glucose <200 versus those with ≥200 mg/dl ($P < 0.001$).

Figure 2b shows the cumulative HCC occurrence rates in patients with a liver steatosis area of >5% and those with a liver steatosis area of ≤5%. The rates at 3 and 5 years were 14.3 and 20.4% in patients with a liver steatosis area of >5% versus 2.9% and 4.7%, respectively, in patients with a liver steatosis area of ≤5%. There was a significant difference in the HCC occurrence rate between patients with a liver steatosis area of ≤5% versus those with a liver steatosis area of >5% ($P < 0.001$).

Comparison of 75-g OGTT results between patients with a liver steatosis area of >5% and those with a liver steatosis area of ≤5%

The serum glucose and insulin levels during the 75-g OGTT in patients with a liver steatosis area of >5% and those with a liver steatosis area of ≤5% are shown in Fig. 3a, b. There were no differences in glucose levels between the two groups. In contrast, fasting and 30-min insulin levels were significantly higher in patients with a liver steatosis area of >5% versus those with a liver steatosis area of ≤5% (fasting insulin: 11.4 ± 6.0 vs. 9.0 ± 5.3 μU/ml, $P = 0.035$; 30-min insulin: 118.9 ± 147.6 vs. 73.4 ± 51.8 μU/ml, $P = 0.003$).

Discussion

This study has revealed that post-glucose challenge hyperglycemia is an independent risk factor for the development of HCC in chronic hepatitis C patients without overt DM or those who are not being treated with antidiabetic drugs.

Although it is unclear why post-challenge hyperglycemia influences hepatic carcinogenesis, we assumed that the mechanism might involve oxidative stress associated with an acute increase in glucose levels. Four of the five patients with HCC and glucose levels of >200 mg/dl at 120-min after the glucose load had normal fasting glucose levels. A previous study showed that acute glucose fluctuations caused greater oxidative stress than sustained chronic hyperglycemia in patients with type 2 DM [18]. Moreover, the activation of oxidative stress as a result of hyperglycemia plays an important role in the pathogenesis of

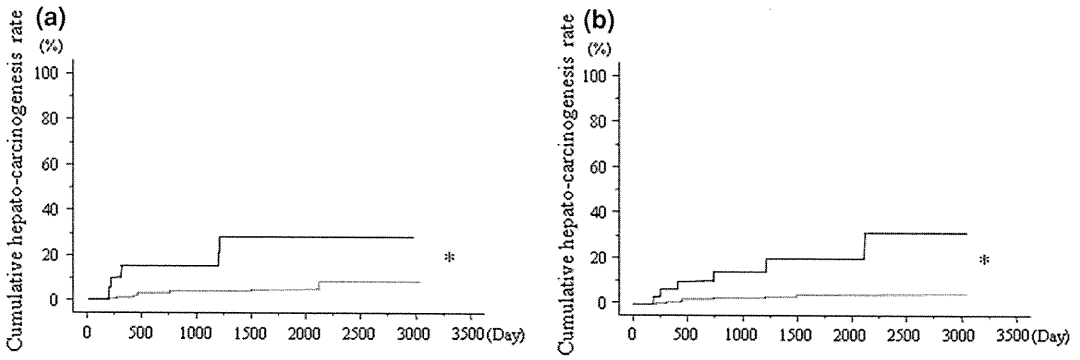

Fig. 2 **a** The cumulative HCC occurrence rates in patients with 120 min post-challenge hyperglycemia (serum glucose level more than 200 mg/dl at 120 min on 75-g GTT; *thick line*) and patients without hyperglycemia (serum glucose level less than 200 mg/dl at 120 min on 75-g GTT; *thin line*). **b** The cumulative HCC occurrence rates in patients with liver steatosis of more than 5% (*thick line*) and patients with liver steatosis of 5% or less (*thin line*). $*P < 0.001$ by log-rank test

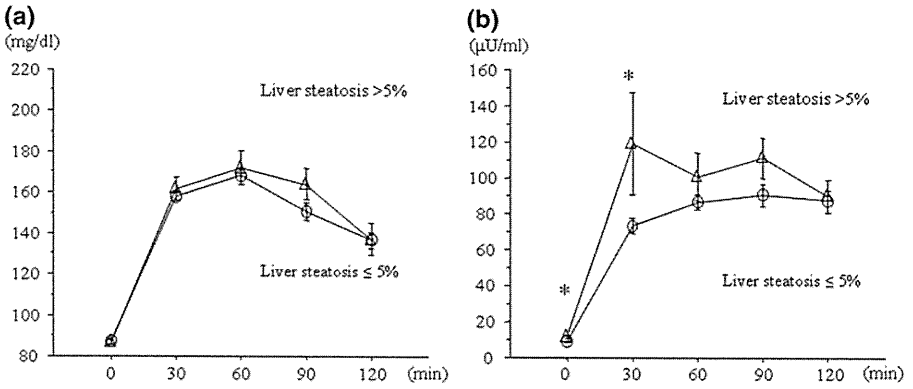

Fig. 3 **a** Serum glucose levels and **b** insulin levels on 75-g OGTT. *Open triangles* patients with liver steatosis of more than 5%. *Open circles* patients with liver steatosis of 5% or less. $*P < 0.05$ by Mann–Whitney *U* test. *Error bar* ± standard deviation

diabetic complications and carcinogenesis [19]. It was also reported that DNA damage caused by oxidative stress could be associated with hepatocarcinogenesis [20, 21]. Because it was previously demonstrated that the post-challenge glucose level was correlated with post-prandial glucose and HbA1c levels [22, 23], the patients with post-challenge hyperglycemia were assumed to have been exposed to daily fluctuations in glucose levels and oxidative stress. These findings might explain why the post-challenge glucose level, but not fasting glucose, was associated with HCC occurrence in the present study. However, further studies that include the assessment of oxidative stress are needed to elucidate the association between acute glucose fluctuations and hepatocarcinogenesis.

Hyperinsulinemia caused by insulin resistance is a well-known carcinogenic factor in several organs, including the liver [24, 25]. It has been shown that HCV itself, including its core protein, induces insulin resistance by impairing the insulin signaling pathway [26, 27]. It was also reported that insulin resistance was more severe in chronic HCV-infected patients than in patients with chronic hepatitis caused by another etiology [28, 29]. However, our study failed to show any associations between HOMA-IR or fasting insulin and the development of HCC. Instead, we found that hepatic steatosis was an independent risk factor for HCC. Moreover, we found significant differences in the fasting and 30-min insulin levels after a glucose load, but not in glucose levels at any time, between patients with and without steatosis, so that HOMA-IR and the area under the curve of insulin concentrations during the 75-g OGTT were higher in patients with liver steatosis (data was not shown). These findings suggest that hyperinsulinemia or insulin resistance might influence hepatic carcinogenesis via hepatic steatosis.

Konishi et al. [30] reported that post-challenge hyperglycemia, but not insulin resistance, was a risk factor for HCC occurrence in chronic hepatitis C patients. Although their results are similar to our own, there is a difference in terms of whether or not hepatic steatosis is a risk factor for HCC occurrence. The discrepancy between these two studies might be due to the methods used to measure liver steatosis. In our study, an image analyzer was used to precisely measure the fat-occupied area, thus allowing us to include the actual area of steatosis in the analyses.

Liver fibrosis can not only cause hepatic cancer, but it can also cause insulin resistance and glucose intolerance.

To overcome any potential bias due to liver fibrosis, we performed case-matched multivariate analyses. These analyses showed that post-challenge hyperglycemia and hepatic steatosis were associated with the development of HCC, independent of hepatic fibrosis.

DM is generally diagnosed based on pre- or post-prandial blood glucose, HbA1c, or glycoalbumin levels. However, it was previously reported that fasting glucose, HbA1c, and glycoalbumin were inadequate tests for the diagnosis of impaired glucose tolerance in patients with advanced liver fibrosis [31, 32]. Although the measurement of post-prandial blood glucose might be an easy method to determine post-challenge hyperglycemia, these values are likely to fluctuate according to the meal content or the length of time after the meal. Therefore, we believe that OGTTs are an indispensable and useful method to detect post-challenge hyperglycemia and to predict the risk of HCC in chronic HCV-infected patients.

It is still unclear what stage in the progression of glucose intolerance carries the greatest risk for HCC. This is because the earlier cohort studies that investigated possible associations between DM and HCC occurrence did not use consistent diagnostic criteria for "overt DM" [7–10]. Because the glucose levels at 120 min during an OGTT are more precise and sensitive parameters for the diagnosis of glucose intolerance than the evaluation of pre- and/or post-prandial hyperglycemia [33, 34], our data suggest that the stages of DM/glucose intolerance preceding "overt DM" may also be associated with HCC occurrence.

Our study revealed significant differences in glucose levels not only at 120 min, but also at 30 and 90 min during OGTTs, between the HCC and non-HCC patients. Furthermore, the 30- and 90-min glucose levels were significant risk factors for HCC on univariate analyses (30 min > 175 mg/dl: HR 4.3, 95% CI 1.4–13.1; 90 min > 175 mg/dl: HR 3.6, 95% CI 1.2–11.1). However, these HRs were smaller than the HR for 120-min and they were not significant on multivariate analysis. Interestingly, according to previous studies, such as DECODE and DECODA, 120-min post-challenge glucose levels were associated with increased risks for macrovascular events and heart disease-related death [35, 36]. Although the mechanisms underlying these associations are not yet fully understood, it seems that 120-min post-challenge hyperglycemia is an important factor involved in several events.

There is no doubt that the eradication of HCV with IFN is an effective approach to reduce the risk of HCC in chronic HCV-infected patients [37]. Our data indicate that the SVR achieved by IFN treatment is a significant factor that inhibits the development of HCC. Recently, it was reported that HCV infection per se downregulated the cell surface expression of the glucose transporter [38]. We have previously reported that the eradication of HCV contributes to improvements in insulin resistance and post-challenge hyperglycemia [39]. These findings suggest that the eradication of HCV by IFN therapy contributes to improvements in glucose intolerance. According to our present results, however, post-challenge hyperglycemia was independent of the IFN response, which means that patients with both glucose intolerance and sustained HCV infection are at increased risk for HCC. These results indicate that improvement of glucose intolerance should be considered as one of the strategies to prevent HCC in patients with chronic hepatitis C, particularly those in whom HCV cannot be eradicated.

A limitation of our study is that the severity of glucose intolerance might change following IFN therapy, because of HCV eradication or because of adverse effects of IFN such as anorexia and body weight loss. To confirm whether or not glucose intolerance is a true risk factor for HCC, future studies should include continued assessment of glucose tolerance following IFN therapy.

In conclusion, the assessment of post-challenge hyperglycemia using a 75-g OGTT is useful for estimating the risk of HCC in HCV-positive patients. Future studies are needed to elucidate the underlying mechanism and identify possible treatments to further reduce the risk of HCC.

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Editorial

Can exercise be a new approach for chronic hepatitis C?

Hepatitis C virus (HCV) is a major cause of chronic liver disease with an estimated 170 million carriers worldwide, and leads to cirrhosis and hepatocellular carcinoma (HCC).[1,2] Although therapies for chronic hepatitis C (CHC) have improved substantially during the last two decades, the sustained virological response rate is still around 50% in patients infected with HCV genotype 1 and around 80% in patients infected with HCV genotype 2 or 3. Therefore, we need to take measures to restrain the disease progression, together with the development of stronger antiviral treatments.

Recently, several host and virus-related factors have become well-established predictors of response to antiviral therapy and clinical outcome in CHC patients.[3–5] Among these, obesity and its associated metabolic complications are increasingly recognized as independent risk factors for diminished response to therapy and more severe liver disease.[6–8]

Concerning the response to antiviral therapy, it has been reported that obesity or metabolic syndrome is a risk factor for non-response to pegylated interferon (PEG-IFN) monotherapy or PEG-IFN plus ribavirin (RBV) independent of genotype and presence of cirrhosis in CHC patients.[9,10] Regarding the insulin resistance strongly associated with metabolic syndrome, Romero-Gómez *et al.*[11] showed that increased homeostasis model assessment as an index of insulin resistance is an independent predictor of decreased viral response to PEG-IFN plus RBV therapy for CHC. The author and colleagues also previously reported that the whole-body insulin sensitivity index, which is calculated by a 75-g oral glucose tolerance test and correlated with whole-body (primarily muscle) insulin sensitivity, is a highly specific marker for predicting the antiviral effect of PEG-IFN plus RBV therapy.[12] These findings suggest that excessive fat accumulation and accompanying insulin resistance in the liver and muscle definitely interfere with the antiviral effect of IFN. Two recent studies evaluated the effects of adding insulin sensitizers, pioglitazone[13] or metformin,[14] to the standard antiviral therapy. However, neither drug provided sufficient additive effects. There are no studies to date assessing whether lifestyle interventions impact on the response to antiviral treatment.

Many previous reports indicated that metabolic abnormalities, including liver steatosis, obesity and diabetes, can worsen the clinical course of CHC.[15–20] These findings suggest that obesity, especially visceral adiposity, and accompanying insulin resistance and glucose intolerance can vigorously cause progression of fibrosis and hepatocarcinogenesis in CHC patients, although it is well known that HCV itself can induce insulin resistance and oxidative stress in infected hepatocytes.[21]

Taking these observations together, lifestyle modifications should take priority and are likely to be very important for management for CHC patients. There is a report that weight reduction is associated with decreases in serum liver enzymes, hepatic steatosis and fibrosis scores in obese patients with CHC.[22] However, there is little information to date concerning the effects of physical exercise for CHC patients.

In this issue of *Hepatolology Research*, Konishi *et al.*[23] show that aerobic exercise can improve insulin sensitivity and decrease serum leptin secreted from adipose tissue. Since a previous study indicated that high serum leptin is a negative predictive factor for response to IFN,[24] this new study suggests the possibility that aerobic exercise may improve the response to antiviral treatment in CHC patients. However, they could not show significant changes in serum adiponectin, interleukin-6 and tumor necrosis factor-α, which are well-known to be more important cytokines than leptin for insulin resistance in metabolic disorders, non-alcoholic fatty liver disease (NAFLD) and CHC. The authors should more carefully address these non-comprehensive results.

Despite many lines of evidence indicating the effectiveness of physical exercise for improvement of NAFLD, the precise mechanisms by which it reduces hepatic steatosis remain unknown. Generally, it is thought that physical exercise can reduce visceral adiposity, decrease fatty acid delivery to the liver and improve insulin sensitivity at the skeletal muscle level, resulting in decreased hepatic steatosis. Recent data from animal studies using Otsuka Long-Evans Tokushima Fatty rats indicate that daily physical activity can directly stimulate lipid oxidation and inhibit lipid synthesis in the liver through activation of the

adenosine monophosphate (AMP)-activated protein kinase pathway and upregulation of hepatic mitochondrial function.[25,26]

In HCV infection, HCV itself is known to modulate lipid homeostasis by increasing lipogenesis via sterol regulatory element-binding protein activation and reducing oxidation and lipid export, leading to steatosis.[27] If physical exercise can also modulate HCV-induced lipid metabolism abnormalities in the liver, it will provide new strategies for managing or treating CHC patients. Future studies will need to address the exact role of physical activity with or without weight loss and its beneficial effects on the histological features of CHC, or focus on how the intensity or duration of exercise is appropriate for CHC patients.

Finally, clinical physicians have experienced difficulties in clinical practice in making patients adhere to structured programs of physical activity for metabolic syndrome or NAFLD.[28] Therefore, if lifestyle modifications are applied to clinical management of CHC, a multidisciplinary team approach, including not only physicians but also dieticians and physical activity specialists, is needed to maximize adherence to physical exercise intervention.

The time has come that we, as hepatologists, must seriously commit to lifestyle-related disorders, similar to diabetologists and endocrinologists.

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Postoperative Infectious and Non-Infectious Complications after Hepatectomy for Hepatocellular Carcinoma

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ABSTRACT

Background/Aims: Hepatic resection for hepatocellular carcinoma (HCC) is associated with a relatively high morbidity rate. This study investigated risk factors for morbidity after resection of HCC that were related to perioperative management and operative techniques.

Methodology: Five hundred and thirty HCC patients who underwent hepatectomy between 1992 and 2008 were divided into three groups: 51 patients with infectious complications during their hospital stay (infectious group), 67 patients with non-infectious complications (non-infectious group) and 412 patients without complications who were discharged within 21 days after hepatectomy (uncomplicated group).

Results: Non-infectious complications decreased significantly over time. Although infectious complications also decreased, the change was not significant. The overall survival rate of the groups with complications was significantly worse than that of the uncomplicated group ($p<0.0005$). Univariate and multivariate analyses showed that an operating time >300min and bile leakage were independent risk factors for infectious complications, while a platelet count $\leq 13 \times 10^4$/mL, cirrhosis and operative blood loss >1,000mL were risk factors for non-infectious complications.

Conclusions: To achieve zero morbidity, it is important to avoid bile leakage and minimize blood loss during resection of HCC in patients with cirrhosis.

KEY WORDS: Liver cancer, Hepatectomy, Postoperative complications.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide (1). Although the majority of cases are still found in Asia and Africa, recent studies have shown that the incidence and mortality rate of HCC are rising in North America and Europe (2,3). There has been an increase of reports on non-surgical therapeutic options for small HCC, such as percutaneous ethanol injection therapy (PEIT) (4), microwave coagulation therapy (MCT) (5), and percutaneous radiofrequency ablation (RFA) (6), but the best treatment for patients with small tumors remains controversial. Liver transplantation is theoretically the optimal treatment for HCC because it is the only method of treating both the tumor and the underlying liver disease. Replacement of the diseased liver is not only the best anticancer treatment, but it is also the best method of preventing new tumors from arising and avoiding the life-threatening complications of cirrhosis. For HCC patients with cirrhosis, transplantation based on the Milan criteria has a better outcome than hepatectomy with respect to both survival and recurrence (7-10). However, the limited availability of donor organs makes liver transplantation problematic (11,12). In Japan, liver transplantation is still not a practical option for many HCC patients, because the national health insurance scheme only covers transplantation for patients with decompensated cirrhosis whose tumors fit the Milan criteria. Therefore, resection is considered to be a reasonable first-line treatment for Japanese patients with small tumors and underlying chronic liver disease.

In Japan, most HCCs are associated with chronic hepatitis and liver cirrhosis induced by infection with the hepatitis B or C viruses. Due to advances in perioperative management, anesthesia and operative techniques, performance of hepatectomy for HCC has become more common. However, the postoperative mortality rate remains high compared with that for other types of surgery in patients who have cirrhosis or chronic hepatitis. The morbidity rate of patients with cirrhosis undergoing liver resection has recently been reported to be as high as 20-70%, with a mortality rate of 5-21% (13-18). In fact, the recent mortality rate is typically <2% at high-volume centers in Japan (19-21). However, relatively high morbidity rates remain problematic after liver resection for HCC. The postoperative course does not always proceed as expected due to various intraoperative stresses, including blood loss and ischemia. Therefore, perioperative predictors of morbidity after hepatectomy are needed.

The present study was performed to investigate risk factors for morbidity after resection of HCC

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that were related to perioperative management and operative techniques.

METHODOLOGY

Patients

Between February 1992 and December 2008, a total of 530 patients with HCC underwent R0 resection at our institution, which was defined as macroscopic removal of all tumors. Nineteen patients died in hospital, while the remaining 513 were followed up as outpatients and were retrospectively reviewed. None of the patients received postoperative adjuvant therapy before recurrence was detected.

During the 8-year period from 1992 to 1999 (first period), 181 patients underwent hepatic resection, compared with 118 patients during the 3-year period from 2000 to 2002 (second period), 126 patients from 2003 to 2005 (third period) and 105 patients from 2006 to 2008 (fourth period). These four periods were set on the basis of changes in perioperative management and surgical techniques. In the first period, patients with a poor hepatic functional reserve underwent limited resection. The liver was coagulated with a microwave tissue coagulator (22) along the resection line for both hemostasis and tissue destruction. After this, the liver parenchyma was transected by the crushing method. After hemostasis was confirmed, air was injected into the bile duct to detect sites of biliary leakage. Intermittent clamping of the portal vein and hepatic artery (Pringle's maneuver) was not done to avoid aggravation of portal hypertension. During the second period, bipolar cautery with saline irrigation (23) was employed for hepatic resection. During the third period, patients with a poor hepatic functional reserve, such as patients with an indocyanine green retention test result ≥30% were excluded from surgery due to the introduction of less invasive treatment, such as lipidolization, PEIT or MCT for patients with small tumors and poor liver function. Also, resection of the hepatic parenchyma was done by using the Cavitron Ultrasonic Surgical Aspirator (CUSA) and bipolar cautery with saline irrigation. In the fourth period, Pringle's maneuver and Belghiti's hanging maneuver were used during most major resections (24). Also, an intraoperative bile leakage test with indocyanine green solution was performed routinely since 2006.

Perioperative/postoperative complications and deaths were recorded to investigate the morbidity and mortality of hepatectomy. Postoperative complications were defined and classified by the modified Clavien system (25). Briefly, grade I was any deviation from the normal postoperative course that did not require special treatment, while Grade II required pharmacological treatment. Grade III required surgical or radiological intervention with (IIIb) or without (IIIa) general anesthesia. Grade IV was life-threatening complications involving dysfunction of one (IVa) or multiple (IVb) major organs, and Grade V was death. Among complications ranked as grade IV or V, liver failure/insufficiency was defined by the occurrence of any of the following: postoperative encephalopathy associated with hyperbilirubinemia (total bilirubin level >5mg/dL for more than 5 days); intractable pleural effusion or ascites (requiring diuretics, thoracocentesis or abdominal paracentesis on 2 or more occasions, or continuous drainage); or variceal bleeding (26,27). Intra-abdominal haemorrhage was diagnosed by examining the drainage from the abdominal cavity. Although mechanical ileus requiring nasointestinal tube drainage did not occur, paralytic ileus was observed and was defined by oral intake of less than 500mL/day for at least 3 days. Renal insufficiency was defined by oliguria (urine output of less than 500mL/day) with sustained elevation of serum creatine to above 1.5mg/dL. Portal vein thrombosis was diagnosed by enhanced computed tomography (CT). Angina pectoris was defined on the basis of typical chest pain and by electrocardiographic findings. Intra-abdominal abscess was diagnosed from inflammatory symptoms combined with the findings on ultrasonography or computed tomography. Wound infection/dehiscence was defined as a deep wound opening irrespective of whether bacterial infection was confirmed. Bacterial enteritis was diagnosed from abdominal symptoms with or without detection of a pathogen. Pneumonia was diagnosed from respiratory symptoms and X-ray findings with or without proof of bacterial infection.

Patients were divided into three groups: 51 patients who had infectious complications during their hospital stay (infectious group), 67 patients who had non-infectious complications (non-infectious group) and 412 patients without complications who were discharged within 21 days after hepatectomy (Uncomplicated group). Assignment to the groups with infectious and non-infectious complications was based on the initial complication after the operation, since many patients had multiple complications including liver failure.

Clinicopathological variables and surgery

Before surgery, each patient underwent conventional liver function tests, measurement of the indocyanine green retention rate at 15min (ICGR15) and ^{99m}-Technetium-diethylenetriamine penta-acetic acid-galactosyl human serum albumin (^{99m}Tc-GSA) liver scintigraphy (28). Hepatitis screening was done by measurement of hepatitis B surface antigen (HBsAg) and hepatitis C antibody (HCVAb). The levels of α-fetoprotein (AFP) and protein induced by vitamin K absence or antagonism-II (PIVKA-II) were also measured in all patients. Surgical procedures were classified according to the Brisbane terminology proposed by Strasberg *et al.* (29). Anatomic resection was defined as resection of the tumor together with the related portal vein branches and the corresponding hepatic territory. Anatomic resection was classified as hemihepatectomy (resection of half of the liver), extended hemihepatectomy (hemihepatectomy plus removal of additional contiguous segments), sec-

tionectomy (resection of two Couinaud subsegments (30)), or segmentectomy (resection of one Couinaud subsegment). All of the other non-anatomic procedures were classified as limited resection. The tumors treated by limited resection consisted of both peripheral tumors and central tumors. Peripheral tumors and those with extrahepatic growth were managed by partial hepatectomy because this method was able to achieve an adequate surgical margin. Conversely, central tumors located near the hepatic hilum or major vessels were only treated by nucleation because it was too difficult or dangerous to remove enough of the liver to obtain an adequate margin. One senior pathologist reviewed each specimen for histologic confirmation of the diagnosis. The width of the surgical margin was measured as the distance from the tumor edge to the line of resection.

Follow-up

All of the patients who survived were followed-up after discharge with a physical examination, liver function tests, US, CT or magnetic resonance imaging (MRI) being performed at least every 3 months to check for intrahepatic recurrence. Chest radiographs were also obtained to detect pulmonary metastasis and chest CT was done if the plain radiograph showed abnormalities. Bone metastases were diagnosed by bone scintigraphy.

When recurrence of HCC was detected from changes of tumor markers or imaging findings, recurrence limited to the remnant liver was treated by transcatheter arterial chemoembolization, lipidolization, re-resection or percutaneous local ablation therapy such as RFA. After detection of extrahepatic metastases, active treatment was performed in patients with a good hepatic functional reserve (Child-Pugh class A or B) and good performance status (0 or 1), while other patients were only given radiation therapy for bone metastases to relieve symptoms. Surgical resection was done in patients with a solitary extrahepatic metastasis and no intrahepatic recurrence.

Prognostic factors

We performed univariate and multivariate analysis of 33 clinicopathological factors to identify independent variables related to the postoperative occurrence of infectious or non-infectious complications in the 530 HCC patients. The patient factors studied were age, gender, HBsAg, HCVAb, diabetes mellitus, liver function (including albumin, total bilirubin, cholinesterase, prothrombin time, platelet count, alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyltransferase (γ-GTP), and ICGR15, maximum removal rate of technetium-99m-diethylenetriamine penta-acetic acid-galactosyl human serum albumin (GSA (Rmax) and Child-Pugh class), and esophageal and/or gastric varices. The tumor factors studied were AFP, PIVKA-II, histological features (including tumor diameter), number of tumors, differentiation, microscopic capsule formation, surgical margin, microvascular invasion, grade of fibrosis, and tumor stage according to the TNM classification (31). The operative factors that we studied were the operating time, blood loss, perioperative blood transfusion, operative procedure, sutures and bile leakage. All of the variables shown to be significant by univariate analysis were subsequently examined in a multivariate logistic regression model to identify independent predictors of postoperative infectious or non-infectious complications.

Statistical analysis

Results were expressed as the mean±SD. Continuous variables were evaluated with the unpaired Student's *t*-test, the Mann-Whitney U test, or the Kruskal-Wallis test as appropriate. Categorical data were compared with the chi-square test and Fisher's exact test, where appropriate. The Kaplan-Meier life table method was used to calculate the disease-free survival rate and overall survival rate as of June 2010, and differences of survival were estimated with the generalized logrank test. In all analyses, $p<0.05$ was considered to indicate statistical significance.

RESULTS

Patient demographics are summarized in **Table 1**. There were 181 patients during the first period, 118 during the second period, 126 during the third period, and 105 during the fourth period. The mean age of the patients gradually increased across the four periods. The number of patients positive for HBsAg during the first and second periods was substantially higher than during the third and fourth periods. The preoperative serum albumin level was higher in the first period than in the second and third periods. Results for operative blood loss, blood transfusion, postoperative hospital stay and morbidity were all worse in the first period than in the fourth period. The incidence of infectious complications gradually decreased, although not significantly, across the four periods. However, the incidence of non-infectious complications in the third and fourth periods was significantly lower than in the first and second periods.

Among our 530 consecutive patients receiving hepatectomy for HCC, infectious and non-infectious complications were observed in 51 and 67 patients, respectively (**Table 2**). Grade V infectious complications consisted of three cases of intra-abdominal abscess with septic shock, while grade V non-infectious complications included ten cases of liver failure, three of intra-abdominal hemorrhage and one of portal vein thrombosis. Four and two patients from the infectious and non-infectious groups, respectively, recovered after intensive care and they were classified as having grade IVb complications.

Risk factors for postoperative infectious complications

Clinicopathological variables were compared between the 51 patients with infectious complications and the 479 patients with non-infectious

TABLE 1 Comparison of Surgical Valuables between Each Period

	First period (Feb 1992-Dec 1999)	Second period (Jan 2000-Dec 2002)	Third period (Jan 2003-Dec 2005)	Fourth period (Jan 2006-Dec 2008)	*p*-value
Number	181	118	126	105	
Age (years)	61.4±9.3	65.9±8.2	66.3±9.0	68.9±8.4	<0.0001
Gender (male/female)	145/36	93/25	100/26	83/22	0.9932
HBsAg (+/-)	44/137	24/94	11/115	11/94	0.0007
HCVAb (+/-)	125/56	89/29	85/41	63/42	0.1024
ICGR15 (%)	18.2±9.6	20.3±10.4	19.0±10.4	18.6±11.8	0.1581
Albumin (g/dL)	3.81±0.42	3.67±0.42	3.65±0.41	3.69±0.53	0.0179
Total bilirubin (mg/dL)	0.92±0.34	0.85±0.40	0.88±0.58	0.88±0.32	0.2132
Prothrombin time (%)	90±13	89±12	85±14	89±12	0.0507
ALT (U/L)	51±33	57±29	52±32	50±28	0.1083
AFP (ng/mL)	6225±36150	1903±10688	1648±3981	4405±20480	0.1787
Surgical procedure (limited/anatomic)	125/56	94/24	112/14	72/33	0.0001
Operating time (min)	299±116	268±99	305±111	306±121	0.03
Operative blood loss (mL)	1833±2379	1224±1256	1165±938	980±899	0.0076
Blood transfusion (+/-)	107/74	48/70	51/75	23/82	<0.0001
Tumor size (cm)	4.27±3.26	3.56±2.96	4.10±2.93	4.40±3.41	0.1221
No. of tumors	1.84±1.67	1.44±0.96	1.26±0.64	1.51±1.46	0.073
Associated liver disease (normal or hepatitis/cirrhosis)	100/81	66/52	84/42	64/41	0.1923
Postoperative hospital stay (days)	38±41	26±19	23±28	17±9	<0.0001
Morbidity	41 (23%)	32 (27%)	20 (16%)	8 (8%)	0.001
Mortality	10 (6%)	2 (2%)	3 (2%)	2 (2%)	0.1830
Infectious complications	17 (9%)	15 (13%)	13 (10%)	6 (6%)	0.3589
Non-infectious complications	34 (19%)	19 (16%)	10 (8%)	4 (4%)	0.0006

HBsAg, hepatitis B surface antigen; HCVAb, hepatitis C virus antibody; ICGR15, indocyanine green retention rate at 15 minutes; ALT, alanine aminotransferase; AFP, α-fetoprotein.

TABLE 2 Postoperative Complications in 118 Patients Undergoing Hepatectomy

Complication	No. of patients	Grade of surgical complications						
		I	II	IIIa	IIIb	IVa	IVb	V
Infectious events								
Intra-abdominal abscess	40 (33.9)		6	24		4	3	3
Wound infection/dehiscence	7 (5.9)			4	3			
Bacterial enteritis	2 (1.7)		1			1		
Pneumonia	2 (1.7)		1				1	
Non-infectious events								
Liver failure/ insufficiency	54 (45.8)		10	30		2	2	10
Intra-abdominal haemorrhage	6 (5.1)			1	1	1		3
Ileus	3 (2.5)		2	1				
Renal failure/insufficiency	2 (1.7)		1			1		
Portal vein thrombosis	1 (1.0)							1
Angina pectoris	1 (1.0)			1				

Values in parentheses are percentages.

complications or without complications. A total of 33 independent clinicopathological variables, including 19 preoperative, 6 operative and 8 pathologic variables, were analyzed as possible risk factors for postoperative infectious complications, and five variables were found to be significant: GSA-Rmax, operating time, operative blood loss, blood transfusion and bile leakage (**Table 3**).

Multivariate analysis using a logistic regression model that incorporated these five factors identified two significant independent variables, which were an operating time >300min and bile leakage (**Table 4**). In particular, bile leakage increased the risk of postoperative infectious complications by 14.71 times.

Risk factors for postoperative non-infectious complications

Clinicopathological variables were compared between the 67 patients with non-infectious complications and the 463 patients with infectious complications or without complications. As a result, 16 significant factors were identified: age, HBsAg, HCVAb, ICGR15, total bilirubin, cholinesterase, prothrombin time, platelet count, ALT, GSA-Rmax, AFP, operating time, operative blood loss, blood transfusion, number of tumors, and associated liver disease (**Table 5**).

Multivariate analysis using a logistic regression model that incorporated these 16 factors identified three significant independent variables, which were a platelet count $\leq 13 \times 10^4/\mu L$, operative blood loss >1,000mL, and cirrhosis (**Table 6**). When a patient had operative blood loss >1,000mL, the risk of postoperative non-infectious complications was increased by 2.38 times.

Outcome

The disease-free survival rate and overall survival rate of the three groups are compared in

Figure 1. The disease-free survival rates of the patients without complications, with infectious complications and with non-infectious complications were respectively 36.2%, 30.7%, and 33.3% at 3 years; 22.6%, 27.9% and 12.2% at 5 years; and 16.7%, 24.8% and 9.1% at 7 years. The overall survival rates of the patients without complications, with infectious complications and with non-infectious complications were respectively 75.1%, 57.6% and 49.1% at 3 years; 59.6%, 39.2% and 40.7% at 5 years; and 45.8%, 29.8% and 38.2% at 7 years. Although disease-free survival was not significantly different among the three groups (p=0.4515), overall survival showed a significant difference (p<0.0005).

DISCUSSION

Patients with HCC represent a high-risk group for major hepatectomy. Unlike patients undergoing liver resection for metastatic cancer or benign liver conditions, HCC patients usually have underlying liver disease. The morbidity rate of patients with cirrhosis undergoing liver resection was recently reported to be as high as 20-70%, with a mortality rate of 5-21% (13-18). However, the recent mortality rate is usually <2% at high-volume centers in Japan (19-21). The surgical mortality rate in the current study was 2.0%, but the morbidity rate was still relatively high at 8% (**Table 1**). In this study, postoperative complications were recorded according to the modified Clavien classification (**Table 2**) of surgical complications, which allows any deviation from the normal perioperative course to be documented (25).

Among our 530 consecutive patients undergoing resection of HCC, infectious and non-infectious complications were observed in 51 and 67 patients, respectively (**Table 2**). Designation of infectious and non-infectious complications was based on the nature of the initial postoperative complication.

TABLE 3 Risk Factors for Infectious Complications after Resection of HCC

	Infectious group (n=51)	Uncomplicated and non-infectious groups (n=479)	*p*-value
Age (years)	65.9±8.3	65.0±9.3	0.4853
Gender (male/female)	42/9	379/100	0.5875
HBsAg (+/-)	4/47	86/393	0.0675
HCVAb (+/-)	39/12	323/156	0.1872
Child-Pugh class (A/B)	47/4	431/48	0.6192
Diabetes mellitus (+/-)	10/41	103/376	0.7534
ICGR15 (%)	20.6±11.1	18.8±10.4	0.2401
Albumin (g/dL)	3.65±0.47	3.72±0.44	0.2524
Total bilirubin (mg/dL)	0.92±0.35	0.85±0.40	0.2132
Cholinesterase (U/L)	117±50	127±64	0.2922
Prothrombin time (%)	87±12	88±13	0.4796
Platelet count (x10^4/μL)	13.7±6.0	15.0±7.5	0.2601
ALT (U/L)	56±34	52±38	0.5514
ALP (U/L)	291±142	316±171	0.3110
γ-GTP (U/L)	94±102	93±93	0.9387
GSA-Rmax (mg/min)	0.409±0.154	0.480±0.208	0.0394
AFP (ng/mL)	434±1519	3905±24888	0.3202
PIVKA-II (mAU/mL)	1471±5031	2603±9975	0.4248
Esophageal and/or gastric varices (+/-)	15/36	117/362	0.4338
Surgical procedure (limited/anatomic)	43/8	360/119	0.1453
Sutures (silk/non-silk)	48/3	409/70	0.0854
Operating time (min)	358±111	288±111	<0.0001
Operative blood loss (mL)	1942±1608	1239±1731	0.009
Blood transfusion (+/-)	32/19	197/282	0.003
Tumor size (cm)	3.84±1.89	4.13±3.26	0.5399
No. of tumors	1.65±1.32	1.54±1.31	0.5727
Histology (well/mod/poor)	7/38/3	67/340/34	0.8978
Microscopic capsule formation (+/-)	43/7	408/66	0.9882
Microvascular invasion (+/-)	29/22	204/275	0.0509
Microscopic surgical margin (+/-)	6/45	39/440	0.3776
Associated liver disease (normal or hepatitis/cirrhosis)	24/27	290/189	0.0625
Tumor stage (I+II/III+IV)	37/14	323/156	0.4567
Bile leakage (+/-)	23/28	23/456	<0.0001

HBsAg, hepatitis B surface antigen; HCVAb, hepatitis C virus antibody; ICGR15, indocyanine green retention rate at 15 minutes; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ-GTP, γ-glutamyltransferase; GSA-Rmax, regional maximum removal rate of ^{99m}-Technetium-galactosyl human serum albumin; AFP, α-fetoprotein; PIVKA-II, protein induced by vitamin K absence/antagonism-II.

TABLE 4 Risk Factors for Infectious Complications after Resection of HCC According to Multivariate Analysis

Variable	Odds ratio	95% CI	*p*-value
Operating time >300min	3.38	1.13-10.1	0.0291
Bile leakage (+)	14.71	6.45-33.33	<0.0001

CI, confidence interval.

TABLE 5 Risk Factors for Non–Infectious Complications after Resection of HCC

	Non-infectious group (n=67)	Uncomplicated and infectious groups (n=463)	*p*-value
Age (years)	61.6±9.1	65.5±9.2	0.0012
Gender (male/female)	50/17	371/92	0.2976
HBsAg (+/-)	4/63	86/378	0.0102
HCVAb (+/-)	55/12	307/156	0.0095
Child-Pugh class (A/B)	57/10	421/42	0.1322
Diabetes mellitus (+/-)	9/58	104/359	0.0917
ICGR15 (%)	21.6±10.2	18.6±10.4	0.0309
Albumin (g/dL)	3.62±0.40	3.73±0.45	0.0626
Total bilirubin (mg/dL)	0.95±0.35	0.84±0.40	0.0401
Cholinesterase (U/L)	110±46	128±64	0.0252
Prothrombin time (%)	84±13	89±13	0.0016
Platelet count (x10^4/μL)	12.0±5.8	15.3±7.5	0.0007
ALT (U/L)	74±59	49±42	0.0302
ALP (U/L)	311±133	314±173	0.8767
γ-GTP (U/L)	94±66	93±98	0.9515
GSA-Rmax (mg/min)	0.382±0.163	0.486±0.207	0.0006
AFP (ng/mL)	9055±43393	2763±19105	0.042
PIVKA-II (mAU/mL)	3732±12937	2317±9041	0.284
Esophageal and/or gastric varices (+/-)	21/46	111/352	0.1924
Surgical procedure (limited/anatomic)	51/16	352/111	0.9866
Sutures (silk/non-silk)	61/6	396/67	0.2208
Operating time (min)	334±135	289±108	0.0026
Operative blood loss (mL)	2098±2290	1192±1607	0.0001
Blood transfusion (+/-)	41/26	188/275	0.0015
Tumor size (cm)	4.38±3.36	4.06±3.13	0.4395
No. of tumors	2.05±2.02	1.48±1.16	0.0009
Histology (well/mod/poor)	7/51/2	67/327/35	0.4316
Microscopic capsule formation (+/-)	61/6	390/67	0.2078
Microvascular invasion (+/-)	35/32	198/265	0.1442
Microscopic surgical margin (+/-)	6/61	39/424	0.8839
Associated liver disease (normal or hepatitis/cirrhosis)	25/42	289/174	<0.0001
Tumor stage (I+II/III+IV)	42/25	318/145	0.3257
Bile leakage (+/-)	2/65	44/419	0.0765

HBsAg, hepatitis B surface antigen; HCVAb, hepatitis C virus antibody; ICGR15, indocyanine green retention rate at 15 minutes; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ-GTP, γ-glutamyltransferase; GSA-Rmax, regional maximum removal rate of ^{99m}-Technetium-galactosyl human serum albumin; AFP, α-fetoprotein; PIVKA-II, protein induced by vitamin K absence/antagonism-II.

TABLE 6 Risk Factors for Non–Infectious Complications after Resection of HCC According to Multivariate Analysis

Variable	Odds ratio	95% CI	*p*-value
Platelet count ≤13×10^4/μL	2.41	1.15-5.04	0.02
Operative blood loss >1,000mL	2.38	1.25-4.52	0.0083
Cirrhosis	1.79	0.92-3.51	0.0471

CI, confidence interval.