using AMPLICOR Monitor test v2.0 and HCV bDNA-probe method for a number of cases. HCV core antigen was analyzed by CLEIA method using Lumipulse Ortho HCV antigen kit (Ortho-Clinical Diagnostics Inc., Tokyo, Japan) as described previously.[10] The data were analyzed by Lumipulse *f* (Fujirebio Inc., Tokyo, Japan).

Outcome

Patients who were negative for serum HCV-RNA, according to the AMPLICOR Monitor test, during a 6 month period after completion of the IFN therapy were defined as SVR. Patients positive for HCV-RNA during this period were defined as non-responders.

The basis of non-possible ribavirin use is either hemoglobin <12.0 g/dL, age equal to or more than 70 years old or women equal to or less than 40 years old. A downward modification of the administration method was separated into two groups of major and minor: modifications that canceled ribavirin or changed twice-daily treatment with IFN to once daily were classified as major and other changes were classified as minor.

Viral half-life

Viral half-life was calculated according to the equation:

[Half – life = $T_{1/2}$, viral amount = V (V_0 = before IFN treatment, V_{24} = 24 h after the first IFN administration), time = t (day)]

$$V = V_0 e^{-st} \rightarrow V_{24} = V_0 e^{-s}$$

$$\log V_{24} = \log(V_0 e^{-s}) = \log V_0 + \log e^{-s} = \log V_0 - s$$

$$s = \log V_0 - \log V_{24}$$

$$(1/2)V_0 = V_0 e^{-sT1/2}$$

$$1/2 = e^{-sT1/2}$$

$$\log(1/2) = -\log 2 = \log(e^{-sT1/2}) = -sT_{1/2}$$

$$T_{1/2} = (\log 2)/s$$

$$T_{1/2} = (\log 2)/[\log V_0 - \log V_{24}]$$

Because the competitive RT-PCR method used for groups divided by the quantity of HCV-RNA expresses the amount of HCV using $\log_{10}$, we converted the calculation type from $\log_e$ to $\log_{10}$. The viral half-life used in this study was calculated as follows,

$$T_{1/2} = (\log_{10} 2)/[\log_{10} V_0 - \log_{10} V_{24}]$$

Statistics

Differences between experimental and control groups were analyzed by the Mann–Whitney's *U*-test or the Fisher's exact test. $P < 0.05$ was considered statistically significant.

RESULTS

Clinical effect

AS SHOWN IN Table 1, the SVR rates for patients with a genotype 1b/ high viral load were 28.6% (4/14), 13.6% (3/22) and 25.0% (8/32) in groups of same-day IFN-β/α2b, IFN-α2b monotherapy and IFN-α2b plus ribavirin, respectively. The SVR rates for patients with a genotype 1b/ low viral load were 91.7% (11/12), 27.3% (3/11) and 57.1% (4/7) in the respective groups. By contrast, the SVR rates for patients with a genotype 2a+2b/high viral load were 70.8% (17/24), not tested and 50.0% (3/6) in the respective groups. There was no difference in the clinical effect between groups of therapy.

Patients with problematic ribavirin use and side-effects

Among the 174 patients who received IFN therapy after ribavirin release, ribavirin was not given to 36 (26.1%) of 138 patients in the high virus group (see Table 2a). The reasons were old age (≥70 years of age; 13 patients), invalidity or intolerance at the first IFN therapy (8 patients), women of childbearing age (6 patients), rejection (5 patients) and low levels of hemoglobin (4 patients). As shown in Table 2b, the cases that became withdrawals or downward modification after IFN therapy were 6.8% or 19.2% (major modification 1.4%, minor modification 17.8%) in the group of same-day IFN-β/α2b (73 patients), 18.8% or 2.1% in that of IFN-α2b monotherapy (48 patients), and 17.0% or 24.6% (major modification 3.8%, minor modification 20.8%) in that of IFN-α2b plus ribavirin (53 patients), respectively. Importantly, the combined rate of withdrawal and modification in a group of IFN-α2b plus ribavirin was significantly higher than that in either group of same-day IFN-β/α2b or IFN-α2b monotherapy.

Dynamics of HCV core antigen during the early phase of IFN therapy

We monitored the level of RNA and core antigen during the early phase of the IFN regimen. Based on the results

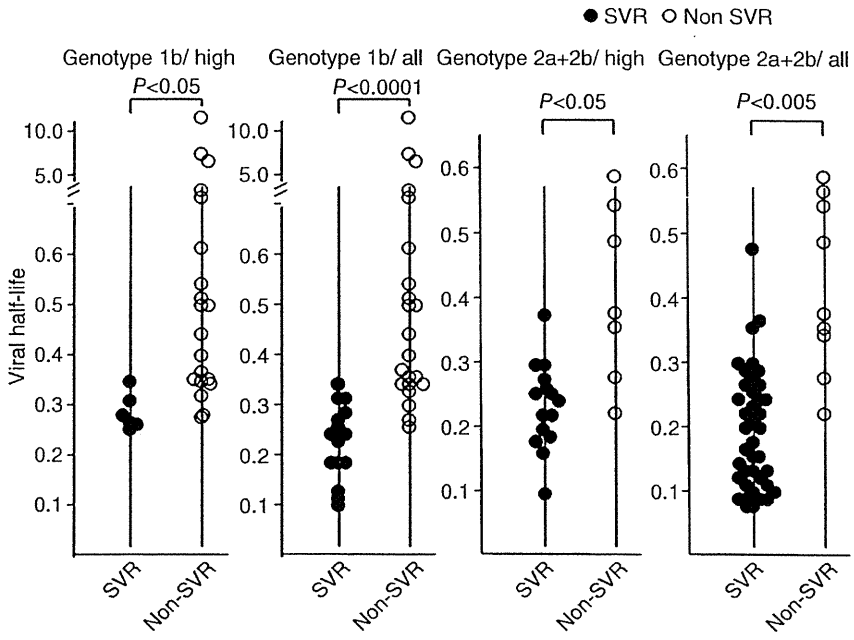

Figure 2 Significant differences can be seen in the early phase of viral half-life between the sustained viral response (SVR) and non-SVR groups. The half-life in patients achieving SVR was significantly lower than that in non-SVR in patients with genotype 1b/high viral load, genotype 1b/all, genotype 2a+2b/high viral load, and genotype 2a+2b/all ($P < 0.05$, $P < 0.0001$, $P < 0.05$ and $P < 0.005$, respectively). The data of three of 20 patients with genotype1b/high were excluded from the analysis because Smirnov's rejection test determined those as abnormal values. The open or closed circles display the half-life in patients achieving non-SVR or SVR, respectively. All patients showing a half-life under 0.2 achieved SVR.

above, HCV core antigen assayed by the CLEIA method appears suitable for the analysis of the dynamics of HCV. As shown in Figure 2, there was a significant difference in the half-life between those patients showing SVR and non SVR in all genotypes of HCV. However, there was no difference in viral half-life among the regimens of same-day IFN-β/α2b, IFN-β alone, IFN-α2b alone and twice-daily treatment with IFN-β (see Fig. 3).

DISCUSSION

THE NUMBER OF patients with hepatocellular carcinoma due to CH-C is still increasing. Currently, IFN treatment is the only effective treatment to eliminate HCV. However, the SVR rate after IFN treatment in CH-C varies according to several factors, such as genotype, quantity of HCV-RNA and the number of muta-

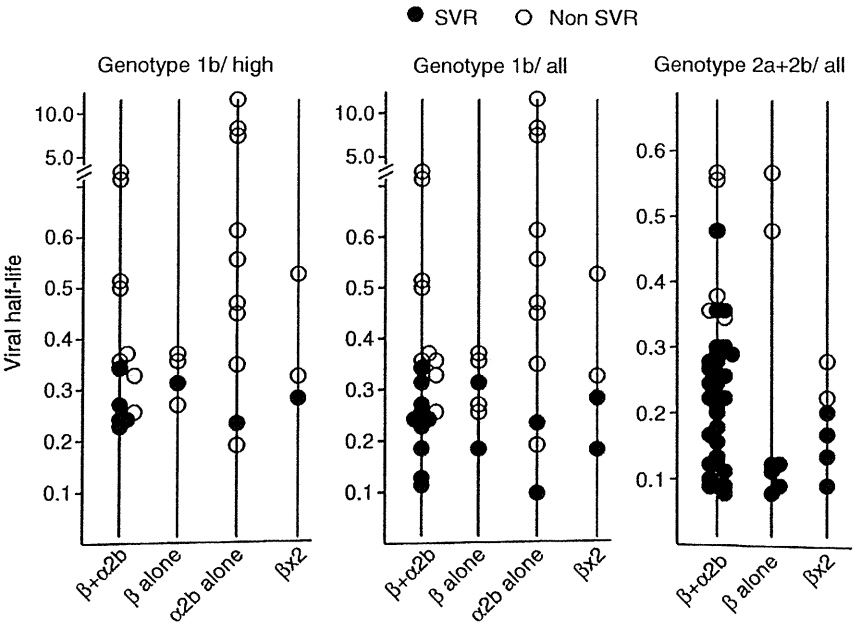

Figure 3 Dynamics of hepatitis C virus core antigen during the early phase of interferon therapy. We calculated the early phase of viral half-life using the viral amount of hepatitis C virus before and 24 h after the first interferon injection. The open or closed circles display the half-life in patients from non-sustained viral response (SVR) or SVR, respectively.

tions in the NS5A region. In particular, the outcome of treatment in genotype1 is poor. Furthermore, the SVR rate with the treatment of IFN alone for the patients of a genotype 1b/high viral load provided particularly poor results (approximately 5%). In addition, the clinical effect for patients of genotype 1 or 2b with a low viral load and of genotype 2a with a high viral load is insufficient.

On this account, various IFN administration methods, such as prolonged interferon treatment[11,12] and combination with ribavirin,[4–8] have been reported to raise the SVR rate. A rise of SVR induced by prolonged interferon treatment is generally acknowledged, although there are problems of patient acceptability and cost.

Since it has been confirmed by various reports that an IFN with ribavirin regimen raises the therapeutic efficacy for CH-C patients with a high viral load, treatment with a combination of IFN and ribavirin is becoming mainstream. However, ribavirin has side-effects, such as anemia and teratogenicity, making it unsuitable for patients with anemia and women of childbearing age. In addition, the side-effects on digestive system are often serious, so it is difficult to use ribavirin for patients aged 70 years or more. As shown in Table 2a, 36 patients (26.1%) were not able to receive ribavirin among 138 patients of the high virus group for whom ribavirin was thought to be desirable. Thus, it is a major problem to determine how to treat patients who are intolerant to ribavirin.

In this study, we attempted to devise an effective IFN regimen without using ribavirin. We anticipated new therapeutic outcomes by treating patients with IFN-β and IFN-α on the same-day. In patients with a genotype 1b/high viral load, the SVR rate of same-day IFN-β/α2b (28.6%) was higher than that in the groups of IFN-α2b monotherapy (13.6%) (see Table 1). In the genotype 1b/ low virus group, regarded as not as effective by IFN monotherapy, the SVR rate for same-day IFN-β/α2b (91.7%) treatment was markedly higher than those in the other two groups.

There was no significant difference among the three therapy groups in terms of withdrawal (Table 2b). However, the combined rate of withdrawal and modification in either group of same-day IFN-β/α2b or IFN-α2b monotherapy was significantly lower than that in a group of IFN-α2b with ribavirin. Although twice-daily treatment with IFN-β established by Okushin *et al.*[13] was similar to same-day IFN-β/α2b regimen in terms of SVR rate for patients of genotype 1b/high and a low viral load, a variety of severe adverse events were caused by the regimen (liver damage, decreased serum albumin, marked thrombocytopenia, and severe proteinuria).[14–16] In our examination, only 4 of 10 patients completed the treatment without downward modification (data not shown), so it is difficult to choose twice-daily treatment with IFN-β as a standard regimen. In our data, the percentage of withdrawal from treatment with IFN-α2b monotherapy seems to be too high. Because we mainly used the IFN-α2b at a dose of 10 MU/day for the group of IFN-α2b monotherapy, some patients became intolerant of such a dose of IFN. On the other hand, since we use 6 MU/day of IFN-α2b at the beginning of treatment for same-day IFN-β/α2b regimen, patients had a period of adjustment. We can draw several conclusions from our data concerning a same-day IFN-β/α2b regimen; (i) almost all cases are able to undergo this treatment, (ii) the SVR rate in whole patients with genotype 1b is higher compared to an IFN-α2b plus ribavirin regimen, (iii) the frequency of stopping and withdrawal of therapy is not high compared to IFN-α2b monotherapy and is significantly lower than IFN-α2b plus ribavirin.

In order to analyze the mechanism by which the same-day IFN-β/α2b regimen is highly effective, we evaluated the dynamics of HCV-RNA and core antigen during the early phase of IFN therapy. As for the AMPLICORE Monitor test, which is generally used for RNA assay, its dynamic range of quantification is 0.6–500 KIU/mL.[17] Therefore, it was thought that the AMPLICORE Monitor test could not be used in an evaluation of dynamics of viral load in this concentration range (data not shown). Under the conditions used in this study the HCV core Ag determined by the CLEIA method shows high assay accuracy ranging from 20 to 20 000 fmol/L. Thus, we concluded that this methodology was a very reliable laboratory procedure to monitor HCV dynamics (data not shown). Based upon these data, we used the HCV core Ag by CLEIA method for analyses of the differential dynamics of HCV amount during the early phase of each IFN therapy regimen. Because there was a significant difference of half-life between patients showing SVR and non-SVR in all genotypes of HCV, we focused on the differential half-life of HCV-RNA by the treatments between same-day IFN-β/α2b and the others. Interestingly, there was no significant difference in the HCV half-life during the early phase of IFN therapy among the regimens of same-day IFN-β/α2b, β alone, α2b alone and twice-daily treatment with IFN-β (see Fig. 3). These data show that the efficacy of same-day

IFN-β/α2b was not through shortening of the viral half-life.

Because both IFN-α or β monotherapy is less effective than our same-day IFN-β/α2b regimen, we next have to consider the mechanism of the differential effects. IFN-α and β together bind the IFN-α receptor (R) 1 and 2, and make a complex to activate Jak1 and Tyk2.[18] These first stages of IFN action appear to be the same. However, IFN-β bind much stronger to IFN-α R1 (α chain) and 2 (β chain), thereby changing the structure of the complex.[19] However, IFN-α and IFN-β binds the same α and β_L subunits of the IFNR, yet differences in signaling and biological effects exist between them. Domanski *et al.* demonstrated that IFN-α and IFN-β utilize different regions of the intracellular domain of the β_L subunit to generate an antiviral state.[20–24] Furthermore, recent studies have revealed intracellular signal transductions by IFN-α and β occurring in a different manner. Although signal transductions by IFN-α were stopped in Tyk2-deficient cells, IFN-β induced the expression of an upstream region of the IFN-regulated human gene without Tyk2.[25] This finding suggests that additional signaling mechanisms should be triggered by IFN-β. Above all, despite using the same receptor, IFN-β has at least another function to activate and generate an antiviral state, compared to IFN-α. The same-day IFN-β/α2b regimen stimulates two or more signaling pathways, which may be why our IFN-β/α2b regimen shows a higher SVR rate compared to other treatment regimens. Next, we focused on immunological modification for the effect of IFN treatments, because immunological response is required in the fight against HCV. Hiroishi *et al.* reported that high- titer infection with HCV may suppress the cytotoxic T lymphocyte responses.[26] We hypothesized that immunological responses against HCV (genotype 1b/high viral load) were more impaired in the patients with stably high viral load. In the same-day β/α2b but not IFN-α2b monotherapy, the SVR rate in patients with genotype 1b/stably high viral load was markedly lower than that with 1b/moving type (moving type patients were defined as individuals with a high viral load who showed a low viral load more than once during a one-year period before the start of IFN treatment; data not shown). These data may indicate the reason for the higher effect of same-day IFN-β/α2b was due to the higher SVR rate, compared to that of IFN-α2b monotherapy.

In conclusion, same-day β/α2b treatment had few cases where therapy was discontinued and showed a high SVR rate. This regimen is an effective treatment, especially for cases where ribavirin is unsuitable.

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Insulin resistance raises the risk for recurrence of stage I hepatocellular carcinoma after curative radiofrequency ablation in hepatitis C virus-positive patients: A prospective, case series study

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Aim: Several studies have reported that insulin resistance raises the risk of primary hepatocellular carcinoma (HCC). We conducted a prospective, case series study to test the impact of insulin resistance on the recurrence after curative radiofrequency ablation (RFA) of stage I HCC in HCV-positive patients.

Methods: From January 2006 to December 2007, 226 consecutive patients underwent treatment for primary HCC at our institutions, including 37 stage I cases. Among them, 33 were HCV-positive, and three, six and 24 received curative surgery, transarterial chemoembolization or RFA, respectively. In the 24 patients treated with RFA, recurrence-free survival was analyzed using the Kaplan–Meier method. The factors contributing to recurrence of HCC were subjected to univariate and multivariate analyses using the Cox proportional hazards model. Insulin resistance was estimated by the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR).

Results: Kaplan–Meier analysis showed that the recurrence-free survival was lower in patients with higher HOMA-IR (>2.3, $P = 0.0252$) or with lower serum albumin level (<3.3 g/dL, $P = 0.0004$). In the univariate analysis, HOMA-IR ($P = 0.0420$) and albumin ($P = 0.0036$) were significantly associated with recurrence of HCC. Multivariate analysis revealed albumin (odds ratio = 0.01, 95% confidence interval = 0.0002–0.015, $P = 0.0001$) and HOMA-IR (odds ratio = 3.85, 95% confidence interval = 1.57–14.2, $P = 0.0015$) to be independent predictors for recurrence of HCC.

Conclusion: Serum albumin level and HOMA-IR were independent risk factors for recurrence of stage I HCC after curative RFA in HCV-positive patients. Patients with these factors require closer surveillance.

Key words: hepatitis C virus, hepatocellular carcinoma, Homeostatic Model Assessment of Insulin Resistance, insulin resistance, radiofrequency ablation, recurrence

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is prevalent worldwide, especially in Africa and the Western Pacific Region. HCC is the third most common cause of cancer death in men and the fifth most common in women; every year, more than 600 000 people die from this disease (www.who.int/whosis/).

Risk factors for the development of primary HCC include viral infection such as hepatitis B virus (HBV) and hepatitis C virus (HCV), alcohol consumption, aflatoxin and immune-related hepatitis.[1] Regarding risk factors for recurrence, several studies have suggested male sex, presence of cirrhosis, high α-fetoprotein (AFP), large tumor foci, multiplicity of tumors, pathologically high-grade atypia of tumor cells and presence of portal venous invasion of tumor.[2–6] Recently, several epidemiological studies have revealed a close association between diabetes mellitus (DM) and HCC. Wideroff *et al.*[7] described the standardized incidence ratios in Denmark for primary liver cancer in subjects with DM compared with the general population as 4.0 (95% confidence interval [CI] = 3.5–4.6) and 2.1 (95% CI = 1.6–2.7) for men and women, respectively. El-Serag *et al.*[8] reported that DM increased the risk of chronic non-alcoholic liver disease and HCC in male patients without concomitant

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liver disease in the USA. Furthermore, patients with chronic hepatitis and cirrhosis tend to experience complications with DM or to show insulin resistance.[9] This is particularly the case for patients with HCV infection and non-alcoholic fatty liver disease (NAFLD), including its most severe form, non-alcoholic steatohepatitis (NASH), which can lead directly to HCC.[10–12] The HCV core protein induced insulin resistance by increasing tumor necrosis factor-α which disrupts tyrosine phosphorylation of insulin receptor substrate-1.[13] Thus, DM including insulin resistance seems to be closely associated with various liver diseases that can lead to HCC, although the impact of insulin resistance on the recurrence of HCC has not been evaluated.

In this study, to identify the impact of insulin resistance on recurrence after initial curative treatment for HCC, we designed a prospective, case series analysis to examine recurrence-free survival in consecutive patients with stage I HCC, stratified by Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) level, which is commonly used for measuring insulin resistance.[14,15] In particular, we focused on HCV-positive patients who were treated with radiofrequency ablation (RFA).

METHODS

Patients

FROM JANUARY 2006 to December 2007, 226 primary HCC patients underwent initial treatment at our institutions, and 199 of them were followed to the end of this study (April 2008). Among them, we had 37 consecutive patients with stage I HCC that met all the criteria: a single tumor of 2 cm or less diameter, with no vascular invasion, no lymph-node invasion and no distant metastasis.[16]

Hepatocellular carcinoma nodules were detected by imaging modalities including abdominal ultrasonography, dynamic computed tomography (CT), dynamic magnetic resonance imaging (MRI) and abdominal arteriography. Diagnosis of HCC was made from a typical hypervascular tumor stain on angiography and typical dynamic-study findings of enhanced staining in the early phase and attenuation in the delayed phase. Etiologies for HCC were HCV in 33 patients, HBV in two and others in two.

Treatment, follow up and determination of recurrence

Three patients were treated with surgical resection, six with transarterial chemoembolization (TACE) and 28 with RFA. Among them, we only recruited those who were positive for HCV and treated with RFA ($n = 24$). Therapeutic effect was judged to be curative using dynamic CT or MRI with total disappearance of imaging characteristics of HCC as described above.

Patients were thereafter followed on an out-patient basis using serum tumor markers such as AFP and protein induced by vitamin K absence or antagonists II (PIVKA-II) every month, and by abdominal ultrasound, dynamic CT scan or dynamic MRI every 3 months. Recurrent HCC was diagnosed using the imaging modalities described earlier as the appearance of another lesion different from the primary one. The follow-up period was defined as the interval from the date of initial treatment until the date of diagnosis of recurrence, or until April 2008 if HCC did not recur. We defined the local tumor progression at the initial HCC site as censored.

Statistical analysis

Baseline characteristics were compared using the Student's *t*-test for continuous variables or χ^2-test for categorical variables. Recurrence-free survival was estimated using the Kaplan–Meier method, and differences between curves were examined by log–rank test. There were 13 possible predictors for recurrence of HCC after the initial curative treatment: sex, age, body mass index (BMI), Child–Pugh classification, serum albumin level, total bilirubin level, alanine aminotransferase (ALT) activity, platelet count, prothrombin time, HOMA-IR (defined as fasting plasma glucose [mg/dL] × fasting immunoreactive insulin [μU/mL] / 405), hemoglobin A_{1c} (HbA_{1c}), and serum tumor markers (AFP and PIVKA-II). The parameters, that proved to be significant by log–rank test, were then subjected to the univariate and multivariate analyses using the Cox proportional hazards model. Statistical significance was declared if the *P*-value was 0.05 or less. In addition, we employed the quantitative insulin sensitivity check index (QUICKI), which directly correlates with the glucose clamp method,[17] to supplement the evaluation of insulin resistance by HOMA-IR: QUICKI = 1 / (log [immunoreactive insulin] + log [fasting plasma glucose]).

RESULTS

Patients' baseline characteristics and laboratory data

THE BASELINE CHARACTERISTICS and laboratory data of 24 patients (15 men and nine women, median age 73 years) are shown in Table 1. Twenty

Table 1 Baseline demographic and clinical characteristics

		Normal range
Sex (male/female)	15/9	
Age (years)	73 (61–82)	
BMI	22.3 (19.5–33.5)	
Child–Pugh classification (A/B/C)	20/4/0	
Follow-up period (days)	365 (60–770)	
ALB (g/dL)	3.75 (2.4–4.4)	3.9–4.9
ALT (IU/L)	48.5 (21–98)	7–40
T-Bil (mg/dL)	0.96 (0.6–2.1)	0.2–1.2
PLT ($\times 10^4/\mu L$)	8.85 (4.1–21)	14.1–32.7
PT (%)	74 (56–118)	70–120
FPG (mg/dL)	107 (75–155)	70–110
FIRI (μg/dL)	10.8 (2.78–32.2)	2–10
HOMA-IR	2.96 (0.76–7.39)	<1.6
HbA1c (%)	5.2 (3.7–7.2)	<5.6
AFP (ng/dL)	26.7 (2.2–203)	<20
PIVKA-II (mAU/mL)	24 (9–127)	<40

Values are median (range).
AFP, α-fetoprotein; ALB, albumin; ALT, alanine aminotransferase; BMI, body mass index; FIRI, fasting immunoreactive insulin; FPG, fasting plasma glucose; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; PIVKA-II, protein induced by vitamin K absence or antagonists II; PLT, platelets; PT, prothrombin time; T-Bil, total bilirubin.

patients were classified into Child–Pugh class A, four patients into class B and none into class C. The median follow-up period was 365 days (range 60–770 days), and no patient died during the study.

Possible risk factors for recurrence of HCC

No local tumor progression was diagnosed in this study period. Seven patients experienced the defined recurrence in the liver, but no one showed distant metastasis. One-year recurrence-free survival in total patients was 64%; Figure 1(a,b) shows Kaplan–Meier curves for recurrence-free survival according to HOMA-IR level (≤2.3 and >2.3), which produced significant difference ($P = 0.0252$). Serum albumin level (≥3.3 and <3.3 g/dL; $P = 0.0004$) was also a significant variable (Fig. 1c).

The Cox proportional hazards model was used to analyze risk factors for recurrence of stage I HCC after the curative RFA, using the 13 variables described earlier (Table 2). HOMA-IR level (odds ratio [OR] = 1.66, 95% CI = 1.01–2.72, $P = 0.0420$), and serum albumin level (OR = 0.08, 95% CI = 0.01–0.45, $P = 0.0036$) were identified as significant risk factors by univariate analysis. Multivariate analysis identified albumin (OR = 0.01,

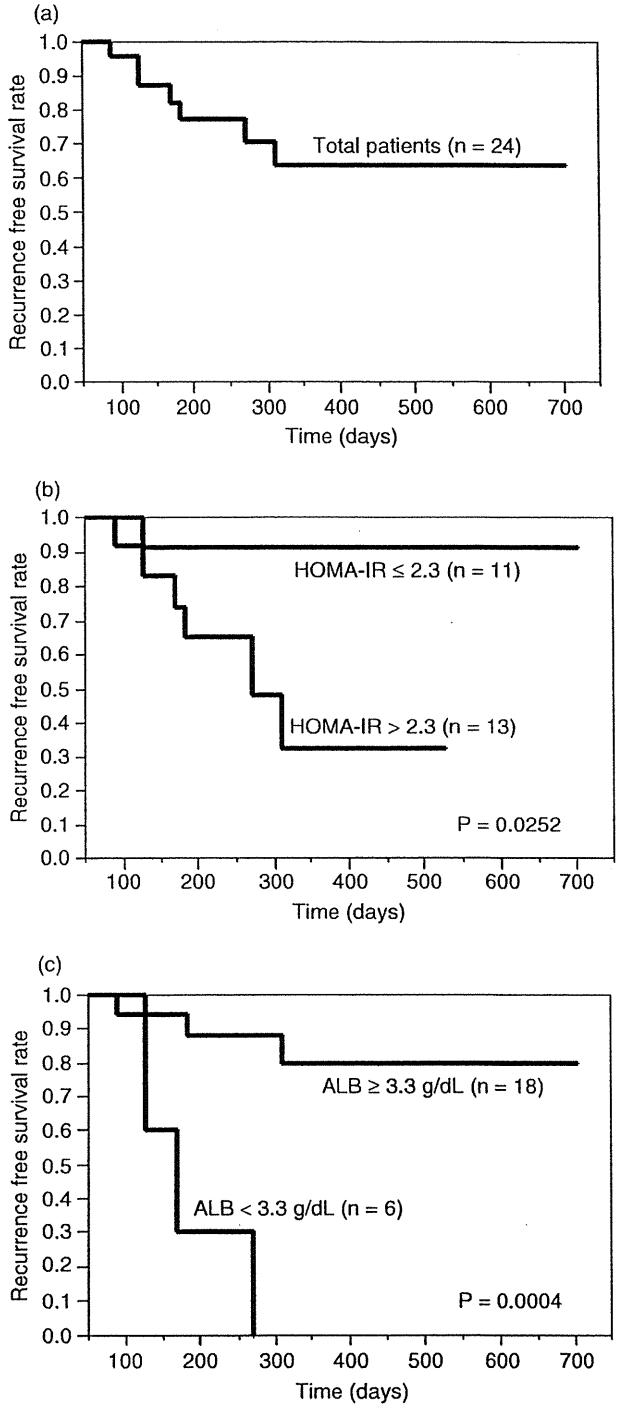

Figure 1 Kaplan–Meier curves for recurrence-free survival in (a) total patients and in subgroups divided according to (b) Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) level or (c) serum albumin level. ALB, albumin.

Table 2 Univariate analyses of possible risk factors for recurrence of hepatocellular carcinoma by Cox proportional hazards model

	95% CI			
	OR	Lower	Upper	*P*-value
Men (vs women)	1.20	0.25	8.41	0.8242
Age (years)	1.06	0.93	1.23	0.3451
BMI	0.90	0.59	1.22	0.6036
Child B (vs A)	4.81	0.60	31.3	0.1253
ALB (g/dL)	0.08	0.01	0.45	0.0036
T-Bil (mg/dL)	2.75	0.27	19.7	0.3603
ALT (IU/L)	0.99	0.95	1.02	0.6923
PLT ($\times 10^4/\mu L$)	0.86	0.65	1.05	0.1770
PT (%)	0.95	0.87	1.01	0.1617
HOMA-IR	1.66	1.01	2.72	0.0420
HbA1c (%)	0.69	0.27	1.53	0.3850
AFP (ng/dL)	1.00	0.99	1.02	0.1242
PIVKA-II (mAU/mL)	1.00	0.96	1.03	0.6172

OR is shown with a unit increase in continuous variables.
AFP, α-fetoprotein; ALB, albumin; ALT, alanine aminotransferase; BMI, body mass index; CI, confidence interval; FIRI, fasting immunoreactive insulin; FPG, fasting plasma glucose; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; OR, odds ratio; PIVKA-II, protein induced by vitamin K absence or antagonists II; PLT, platelets; PT, prothrombin time; T-Bil, total bilirubin.

95% CI = 0.0002–0.15, $P = 0.0001$) and HOMA-IR level (OR = 3.85, 95% CI = 1.57–14.2, $P = 0.0015$) as significant independent risk factors for recurrence.

Table 3 shows the patients' baseline characteristics and laboratory data divided according to HOMA-IR level (≤2.3 and >2.3). No significant differences were noted between the two subgroups except fasting plasma glucose and fasting immunoreactive insulin. Two patients in the HOMA-IR 2.3 or less subgroup took oral hypoglycemic drugs, sulfonylurea derivatives and voglibose. Three patients in the HOMA-IR more than 2.3 subgroup took oral hypoglycemic drugs; two took sulfonylurea derivatives and one took pioglitazone. No patient received insulin treatment.

We supplementally analyzed the data by excluding the patients under treatment with these oral hypoglycemics and also the patients with fasting plasma glucose above 140 mg/dL, in order to avoid possible unreliability in HOMA-IR evaluation. In nine patients, each remaining in HOMA-IR of 2.3 or less and HOMA-IR of more than 2.3, serum albumin (OR = 0.02, 95% CI = 0.0002–0.40, $P = 0.0060$) and HOMA-IR (OR = 3.49, 95% CI = 1.45–13.8, $P = 0.0033$) were still significant.

In a similar manner, evaluation of insulin sensitivity by QUICKI gave the results that lower QUICKI (≤0.33,

Table 3 Baseline demographic and clinical characteristics of patients classified according to HOMA-IR level

	HOMA-IR ≤2.3 ($n = 11$)	HOMA-IR >2.3 ($n = 13$)	*P*-value
Sex (male/female)	7/4	8/5	0.9157
Age (years)	70 (61–82)	74 (63–80)	0.1846
BMI	23.55 (19.5–33.5)	22.1 (19.5–25.1)	0.1219
Follow-up period (days)	393 (155–701)	337 (60–770)	0.2785
Child–Pugh classification (A/B)	10/1	10/3	0.3483
ALB (g/dL)	3.9 (2.4–4.4)	3.4 (2.7–4.4)	0.3304
ALT (IU/L)	40 (21–98)	53 (25–80)	0.6103
T-Bil (mg/dL)	0.8 (0.7–2.1)	1.0 (0.6–1.7)	0.6655
PLT ($\times 10^4/\mu L$)	8.5 (4.1–21)	8.9 (4.9–13.9)	0.5766
PT (%)	72 (56–95.5)	74 (58–118)	0.9953
FPG (mg/dL)	90 (75–119)	109 (86–155)	0.0151
FIRI (μg/dL)	7.98 (2.78–10.8)	14.1 (7.86–32.2)	0.0005
HOMA-IR	1.79 (0.76–2.27)	3.76 (2.91–7.39)	<0.0001
HbA1c (%)	5.05 (3.7–7.2)	5.3 (4.1–6.8)	0.6848
AFP (ng/dL)	23.2 (2.2–153.2)	28 (8–203)	0.7339
PIVKA-II (mAU/mL)	21 (9–127)	28 (9–67)	0.8071
Presence of oral hypoglycemic drugs (yes/no)	2/9	3/10	0.7678
Presence of insulin treatment (yes/no)	0/11	0/13	1.0000

Values are median (range).
AFP, α-fetoprotein; ALB, albumin; ALT, alanine aminotransferase; BMI, body mass index; FIRI, fasting immunoreactive insulin; FPG, fasting plasma glucose; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; PIVKA-II, protein induced by vitamin K absence or antagonists II; PLT, platelets; PT, prothrombin time; T-Bil, total bilirubin.

i.e. impaired insulin sensitivity) was associated significantly with the increased risk of HCC recurrence (OR = 7.97, 95% CI = 1.32–152, $P = 0.0213$).

DISCUSSION

SEVERAL EPIDEMIOLOGICAL STUDIES have revealed the association of DM with cancer incidence and cancer mortality for various organs such as the liver, biliary tract, pancreas, endometrium, kidney, colon, bladder and breast.[7,18–21] The mechanism by which insulin acts as a carcinogenic factor is currently a focus of interests. First, insulin functions as a growth factor by phosphorylating insulin receptor substrate 1 and activating the downstream mitogen-activated protein kinase cascade, which affects cellular proliferation.[22,23] Second, hyperinsulinemia increases peripheral lipolysis and hepatic accumulation of free fatty acids, and the excess β-oxidation in mitochondria and microsome leads to the production of reactive oxygen species[24,25] that play a significant role in carcinogenesis.[26,27] Adipocyte-secreted cytokines (adipokines) such as tumor necrosis factor-α and interleukin-6 also play a significant role in both insulin residence and carcinogenesis.[28,29] Thus, these factors could cooperatively induce the insulin resistance and carcinogenesis.

We demonstrated in the present study that a higher HOMA-IR level increases the risk of early recurrence after initial curative RFA of stage I HCC in HCV-positive patients. This finding basically agrees with previous studies[7,8,18] that suggested an association between insulin resistance and carcinogenesis as described above, but HbA_{1c} level did not predict recurrence (Table 2). This might be explained by the clinical relevance that HbA_{1c} level in patients with liver cirrhosis is often underestimated because of anemia. Therefore, the results of the present study suggest that the role of hyperinsulinemia is more important than that of hyperglycemia as reflected by HbA_{1c} in the recurrence of HCC. We therefore should pay attention to levels not only of glucose and HbA_{1c} but also of insulin when we follow patients who are at risk for HCC.

Interventional modalities to improve insulin-resistance could be a key to prevent the primary or recurrent HCC in patients complicated with such metabolic disorders. For instance, metformin and thiazolidine derivatives could be potential candidates for this purpose.[30,31] Oral branched-chain amino acid (BCAA) granules might be a candidate for preventing HCC recurrence in DM cases because, in addition to improving hypoalbuminemia,[32,33] this agent improves insulin resistance without stimulating insulin secretion.[34] Improvements of insulin resistance and glucose tolerance by BCAA have been reported in clinical trials.[35,36] Furthermore, Muto *et al.*[37] described that oral supplementation with BCAA granules inhibited liver carcinogenesis in HCV-positive liver cirrhosis with DM and obesity. Such effect of BCAA is also supported in experimental models.[38,39] These reports,[32–39] together with our present findings (Table 2 and Fig. 1b), suggest that insulin resistance is a significant risk factor for early recurrence of HCC and thus might be a critical target to prevent the recurrence and development of second primary HCC.

A limitation of this study is that the therapeutic effect of the primary HCC was judged as curative by imaging diagnosis but not by surgical pathology. Although the recurrent HCC developed apart from the primary tumor, we could not totally differentiate the recurrent lesion and a second primary HCC. A higher recurrence rate in this study (Fig. 1a) might be explained by this fact. Advanced medical imagings such as positron emission tomography would help solving such limitations in future study of this kind. Furthermore, the basic question, if de novo, namely, the first or second primary liver carcinogenesis is regulated by insulin resistance, should be addressed by recruiting HCC-free cirrhotics. However, such study requires a larger sample size and a longer observation period. Instead, we focused on the recurrent HCC, including possible second primary tumors, which develop at a 2–3-fold higher incidence than the first primary one.

Other study limitations are the short observation period, the small number of recruited patients and also the small number of detected events. Such a sample size essentially raises the possibility of β-error, including the absence of the statistical power of AFP and PIVKA-II for predicting the recurrence (Table 2). Previous reports agree that these tumor markers are risk factors of HCC recurrence,[2,6] although some criticisms remain.[4,5] In addition, we should state that the small number of events particularly restricts the reliability of multivariate analysis, while the calculation itself was possible in our study.

In conclusion, we presented for the first time that insulin resistance is significantly associated with the early recurrence of stage I HCC after curative RFA in HCV-positive patients. Increased HOMA-IR, which sensitively reflects insulin resistance, might be a useful biomarker for prediction of high-risk patients who cause early recurrence of HCC.

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