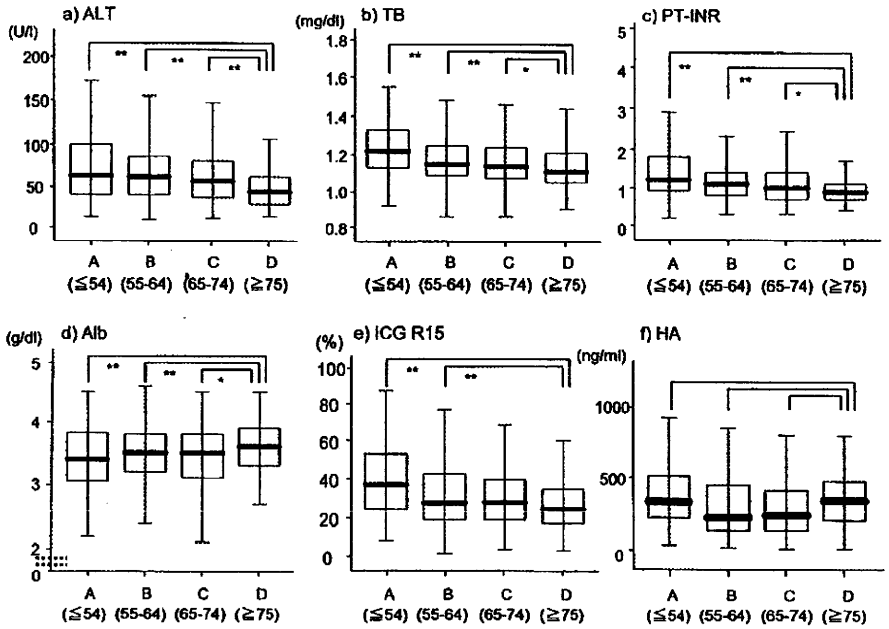

Figure 4. A comparison of age at initial diagnosis of HCC and liver functions. The average values of (a) ALT, (b) TB and (c) PT-INR in the D group were significantly lower than the values in the other groups (ALT: D vs. A, B and C, **P<0.01; TB and PT-INR: D vs. A and B, **P<0.01, D vs. C, *P<0.05). The average value of (d) Alb in the D group was significantly higher than that in the other groups (D vs. A and B, **P<0.01; D vs. C, *P<0.05). The average value of (e) ICG R15 in the D group was significantly lower than the value in the A and B groups (D vs. A and B, **P<0.01). The average value of (f) HA did not show a significant difference. *P<0.05, **P<0.01 between the indicated groups.

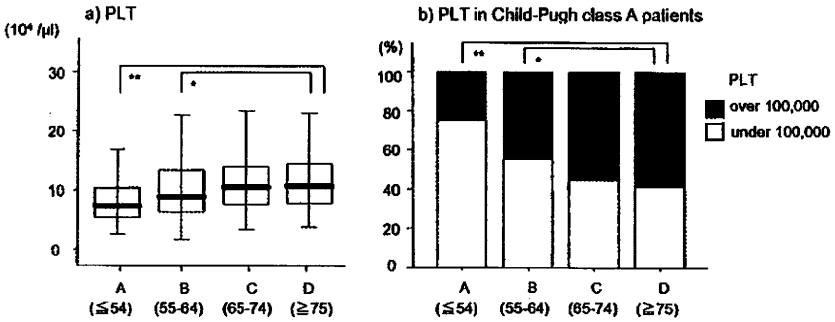

Figure 5. Comparison of age at initial diagnosis of HCC and PLT, and PLT in Child-Pugh class A patients. (a) The average value of PLT in the D group was significantly higher than the value in the A (**P<0.01) and B (*P<0.05) groups. (b) The rate of 100,000 or more of PLT in Child-Pugh class A patients of the D group was significantly higher than that in the A (**P<0.01) and B (*P<0.05) groups. *P<0.05, **P<0.01 between the indicated groups.

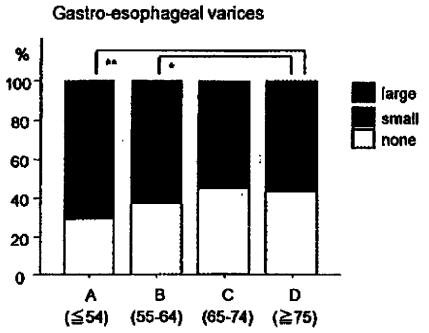

Figure 6. The age at initial diagnosis of HCC and the endoscopic form factor grade of varices. The number of patients with large varices was the lowest in the D group and the highest in the A group. The number of patients with large varices in the D group was significantly lower than that in the A (**P<0.01) and B (*P<0.05) groups. *P<0.05, **P<0.01 between the indicated groups.

noted most frequently in the D group, and it was significantly higher than that in the A group (P<0.05) (Fig. 2d).

Comparison of liver function. The percentages of Child-Pugh class A patients were 59.8, 64.5, 70.1 and 79.5%, in the A, B, C and D groups, respectively. Therefore, the percentage of Child-Pugh class A patients increased with age, and the percentage of Child-Pugh class A patients in the D group was higher than the other groups (D vs. A, B, P<0.01; D vs. C, P<0.05) (Fig. 3). The average values of ALT, TB and PT-INR were lower with age, and these average values in the D group were significantly lower than those in the other groups (ALT: D vs. A, B and C, P<0.01; TB, PT-INR: D vs. A and B, P<0.01; D vs. C, P<0.05) (Fig. 4a-c). The average value of Alb in the D group was significantly higher than that in the other groups (D vs. A and B, P<0.01; D vs. C, P<0.05)

Table II. Characteristics of the HCC tumors of the 1096 patients at initial diagnosis of HCV-related HCC.

	A group (87) 42-54 years of age	B group (363) 55-64 years	C group (514) 65-74 years	D group (132) 75-87 years	P-value
Number of tumors					<0.05, D vs. C
Solitary	37	156	217	59	
Multiple	50	207	297	73	
Tumor size					<0.05, D vs. A and B
≤3 cm	61	238	312	73	
>3 cm	26	125	202	59	
PVTT					NS
Present	7	45	66	11	
Absent	80	318	448	121	
TNM staging					NS
I	14	88	106	20	
II	37	120	171	51	
III	30	107	174	49	
IV	6	48	63	12	
AFP (ng/ml)					NS
≤20	28	120	185	53	
21-200	41	158	183	42	
>200	18	85	146	37	
Extrahepatic metastasis					NS
Yes	2	20	18	3	
No	85	343	496	129	

PVTT, portal vein tumor thrombosis; AFP, α-fetoprotein.

(Fig. 4d). The average value of ICG R15 was the lowest in the D group, and the average value of ICG R15 in the D group was significantly lower than that in the A and B groups (D vs. A and B, P<0.01) (Fig. 4e). The average value of HA was the lowest in the C group, thus indicating a strong variation and no significant difference (Fig. 4f). The average value of PLT was the highest in the D group, and the average value of PLT in the D group was significantly higher than that in the A and B groups (D vs. A, P<0.01; D vs. B, P<0.05) (Fig. 5a). When the Child-Pugh class A patients were divided per 100,000/μl of PLT and compared, the rate of 100,000 or more in the D group increased with age, and it was higher than the A and B groups (D vs. A, P<0.01; D vs. B, P<0.05) (Fig. 5b). The number of patients with large varices decreased with age, and the number of patients with large varices in the D group was lower than that in the A and B groups (D vs. A, P<0.01; D vs. B, P<0.05) (Fig. 6).

Comparison of characteristics of the HCC tumors. The number of patients with small HCCs ≤3 cm in the D group was lower than that in the A and B groups (D vs. A and B, P<0.05), and the number of patients with solitary HCC tumor in the D group was higher than that in the C group (D vs. C, P<0.05) (Table II). There were no statistical differences in the prevalence of PVTT, tumor stage, AFP or extrahepatic metastasis.

Pathological findings of liver biopsies from noncancerous areas. The pathological findings of patients for whom liver biopsies from noncancerous areas were performed are documented in Table III. Four of the 18 patients showed liver cirrhosis with activity ≥2 and fibrosis 4 in the Desmet classification. Fourteen of the 18 patients showed chronic hepatitis that was milder than activity 2 and fibrosis 3 in the Desmet classification.

Discussion

An elder is generally defined as anyone 65 years of age or older, but this definition is not standardized worldwide. In Japan, the average age at initial diagnosis of HCC is 65 years or older (13,14,22). Therefore, it is not appropriate to draw the dividing line at 65 years of age in order to define the characteristics of HCC that presents at old age (23). On the other hand, it has been reported that HCV-related HCC which develops in individuals in their 40s exhibits different characteristics from HCV-related HCC which develops in individuals 50 years of age or older, since HCC in individuals in their 40s is independently related to heavy drinking and the presence of HBV coinfection (24). It is therefore not appropriate that younger patients in their 40s and older patients who are 65 years of age or older are treated as a similar group, even when HCC patients are divided by a line drawn at 70 years

Table III. Pathological findings of liver biopsies from non-cancerous areas and the liver function of 18 patients with HCC 75 years of age or older.

No.	Age	Gender	Histology Desmet classification	PLT (x10⁴/μl)	ALT (U/l)	TB (mg/dl)	PT-INR	Alb (g/dl)	ICG R15 (%)	HA (ng/ml)
1	78	F	A1F1	9.4	87	1.10	1.09	3.6	30.6	287
2	80	M	A2F4	7.9	79	0.70	1.03	3.3	14.3	237
3	82	F	A2F2	37.5	37	0.50	0.97	4.1	9.7	123
4	85	F	A2F2	8.3	28	0.50	1.06	4.1	16.8	470
5	75	M	A2F2	13.8	44	0.90	1.03	3.5	28.9	265
6	77	M	A2F2	17.5	64	1.30	1.13	4.0	30.3	225
7	75	M	A2F1	12.5	22	0.90	0.97	4.1	14.2	62
8	80	F	A2F2	15.3	54	1.00	1.12	3.4	45.7	1340
9	76	M	A2F4	8.3	62	0.80	1.18	3.5	24.5	557
10	87	M	A2F2	8.1	43	0.90	1.05	3.8	22.1	503
11	78	M	A1F1	16.3	33	0.95	1.05	4.5	16.8	126
12	76	M	A2F4	13.9	77	1.20	1.16	3.8	16.2	405
13	75	M	A3F4	8.9	33	1.40	1.16	3.7	48.2	619
14	76	F	A1F2	8.0	52	0.90	1.08	3.7	18.0	260
15	82	F	A2F3	11.0	37	2.20	1.20	3.1	23.8	1360
16	76	F	A2F2	11.9	45	0.70	1.02	4.2	9.6	353
17	76	M	A1F2	13.3	40	0.40	1.04	3.4	25.9	732
18	77	M	A2F3	10.1	54	1.02	1.01	4.1	45.2	163
Average	78.3			12.9	49.5	0.97	1.08	3.77	24.5	449

PLT, platelet count; ALT, serum alanine aminotransferase; TB, serum total bilirubin; PT-INR, prothrombin time-international normalized ratio; Alb, serum albumin; ICG R15, indocyanine green retention rate at 15 min; HA, serum hyaluronic acid.

of age as reported in previous studies (14,15,25,26). We therefore divided the elderly into the C group (>65 years of age and <75 years) and the D group (≥75 years of age), and the non-elderly into the A group (<54 years of age) and the B group (>55 and <65 years of age) to create 4 groups. The clinical characteristics of HCC in each group were thus examined in further detail by comparing each group, while particularly focusing on the D group.

Consequently, the most significant characteristic in patients with HCV-related HCC in the D group was that hepatic reserve was maintained and HCC occurred against a background of liver disorder with mild inflammation and fibrosis. In our study, levels of ALT and TB, which are well-known markers of inflammatory necrosis in the liver, were significantly lower in the D group than in all of the other groups. Notably, some studies found that alcohol promotes the progression of background chronic liver disease and consequently enhances carcinogenesis of the liver or that alcohol directly promotes carcinogenesis of the liver (27-29). In our study, the A group of younger HCC patients included many heavy drinkers of alcohol with severe inflammation in addition to high values of ALT and TB, as was previously found by Shimauchi *et al* (24). This finding corroborates reports that HCC frequently occurs in association with severe inflammation (30). Conversely, it was assumed to be one of the main reason that the D group had many patients with low values of ALT and TB, since the D group included many patients who did not habitually consume alcohol.

It has been reported that in chronic liver disease, changes in the platelet count correlate with the degree of fibrosis in liver histology (31-33). As a result, the platelet count is regarded as a marker for fibrosis. In our study, the platelet count in the D group was significantly higher than that in the A and B groups (younger than 65 years of age), and the D group in particular showed the highest platelet count of 100,000 or more in the Child-Pugh class A patients. The study of 18 patients in the D group was assessed by means of a liver biopsy, and the background liver showed chronic hepatitis of less than F3 except for 4 cases of F4, thus indicating that many patients with mild fibrosis had not advanced to liver cirrhosis.

Gastro-esophageal varices are the most common clinical manifestation of portal hypertension in patients with liver cirrhosis. Nakayama *et al* (34) reported that as the endoscopic form factor grade of varix increased, the incidence of the occurrence of HCC also increased, and in particular, a form 3 (large size) factor of varices was an independent predictor for HCC. In our study, many of the patients in the D group either did not have varices or only had small varices, and also the number of patients with large varices in the D group was significantly smaller than the non-elderly groups (younger than 65 years of age). Therefore, it was assumed that many of the patients in the D group had not advanced to liver cirrhosis, and many cases of HCC had thus occurred against a background of chronic liver disease that was not conducive to carcinogenesis.

Alb, PT-INR and ICG R15 values are well-known markers of hepatic reserve in the liver. In our study, the average values of Alb, PT-INR and ICG R15 in the D group were higher than in all of the other groups, showing the highest rate in the Child-Pugh class A patients. Generally, it has been reported that a decreased number of hepatocytes and decreased liver weight due to aging generally results in a decreased regeneration capacity of hepatocytes and a decreased hepatic blood flow (35-38). In addition, it has been suggested that the elderly often have a latent nutrient disturbance or metabolic anomalies, thus causing a declining trend in Alb and ICG R15 (39,40). However, in our study, many cases of HCC in the D group were present along with low values of ALT and TB, unlike the non-elderly group. Therefore, it appears that the progression of chronic liver disease was not promoted, and the hepatic reserve was consequently well-maintained in the elderly HCC group, despite the normal decline in the physiological liver function with age. As noted above, many cases of HCC in the elderly occur against a background of chronic liver disease with mild inflammation and fibrosis, and it is assumed that there are some characteristic factors which contribute to hepatocarcinogenesis in the elderly.

In recent years, age has become a point of focus in the progression of HCV-persistent infection and fibrosis or carcinogenesis. Mahmood *et al* (41) reported that age was the most important factor contributing to a high value of reactive oxygen metabolites in the blood associated with HCV-related chronic liver disease. Moreover, the involvement of oxidative stress has been previously reported in aging itself, which is believed to cause a decrease in various organ functions and immunity (42). The elderly are susceptible to oxidative stress due to a decrease in the SOD value related to the antioxidation mechanism and the decrease in NK cell activity (42,43). Therefore, hepatocarcinogenesis in the elderly may occur in association with only mild inflammation or fibrosis.

In addition, an increase in the rate of women is cited as one of the characteristics of HCC of the elderly. It has been reported that sex hormones such as estrogen and immune response play an important role in hepatocarcinogenesis (44,45). Since the levels of sex hormones decrease in ageing women, the risk of the incidence of HCC in elderly females increases.

In conclusion, we found that HCV-related HCC in the elderly occurred against a background of chronic liver disease with mild inflammation and fibrosis.

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Original Article

Adipocytokine involvement in hepatocellular carcinoma after sustained response to interferon for chronic hepatitis C

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Aim: Interferon (IFN) dramatically reduces the risk of hepatocellular carcinoma (HCC) after a sustained virological response (SVR) to chronic hepatitis C (CH-C). However, HCC still develops in some patients after SVR. To evaluate metabolic factors in patients with HCC occurring after SVR and to determine whether insulin resistance and adipocytokines were involved in this etiology.

Methods: We examined clinical and biochemical features, histological findings and serum levels of adipocytokine prior to IFN therapy and at the detection of HCC in nine patients who were diagnosed with HCC. As controls, 27 patients were included who showed SVR but had not been diagnosed with HCC for at least 5 years after SVR.

Results: Three of four patients who developed HCC within 5 years after SVR showed liver cirrhosis when HCC was diagnosed. Prior to IFN therapy, four of nine HCC patients were diagnosed as having type 2 diabetes mellitus. Serum levels of leptin and insulin, Homeostatic Model of Assessment of Insulin Resistance and body mass index (BMI) were significantly higher and serum adiponectin was significantly lower in HCC patients at the time of HCC detection than in control patients more than 5 years after SVR. Six HCC patients had increased BMI and one HCC patient had a decreased BMI during the observation period.

Conclusion: Hepatic fibrosis may be tightly related to the emergence of HCC after SVR. Insulin resistance and adipocytokine disorders may be implicated in hepatocarcinogenesis after SVR, in part by promoting hepatic fibrosis.

Key words: adipocytokine, adiponectin, hepatocellular carcinoma, insulin resistance, leptin, sustained virological response.

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common cancers worldwide, especially in South-East Asia.[1] Hepatitis C virus (HCV) infection is a major risk factor for HCC. Seventy to eighty percent of Japanese patients with HCC are infected with HCV.[2] Interferon (IFN) is an antiviral agent against HCV that can eradicate the virus and which improves hepatic inflammation and fibrosis,[3] and is also believed to prevent clinical complications, including the development of HCC. IFN therapy for HCV was proven to lower the incidence of HCC, especially in patients who are treated successfully with IFN and show a sustained virological response (SVR) to the therapy.[4] However, previous studies have revealed that HCC still develops in 1.5–4.0% of patients with SVR.[5–11] Although advanced age,[6–11] male sex,[6,8] an advanced histological stage of hepatic damage,[6–10] higher aspartate aminotransferase levels,[11] lower platelet counts,[11] alcohol intake[7,9] and hepatic steatosis[10] before IFN therapy are thought to be risk factors for HCC, the mechanism of carcinogenesis after SVR is not yet fully understood.

The liver is one of the major organs regulating glucose metabolism. Patients with chronic liver disease

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frequently present with glucose intolerance which is referred to as hepatogenous diabetes with insulin resistance and hyperinsulinemia. The prevalence of glucose intolerance in HCV-related chronic liver disease is higher than in other liver diseases[12] and it has been suggested that HCV directly causes hepatic insulin resistance and hyperinsulinemia.[13,14] Previous studies have shown that the incidence of HCC has been increased 2–4-fold in patients with diabetes mellitus (DM)[15,16] and some reports have indicated that insulin resistance may be involved in cell growth and the carcinogenesis of HCC.[17,18] Meanwhile, several recent studies have reported that obesity increases the risk of malignancy,[19] including HCC.[20] In human obesity, intra-abdominal visceral fat content is strongly correlated with glucose intolerance.[21] In the obese state, adipose tissue promotes inflammation and macrophages migrating into adipose tissue secrete adipocytokines, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1). Enlarged adipose cells also secrete these adipocytokines and free fatty acids (FFA). These adipocytokines and FFA can induce insulin resistance in the whole body.[22–24]

Adiponectin is an adipose tissue-specific plasma protein and is specifically secreted from fat tissue. Hypoadiponectinemia may be implicated in the pathogenesis of the various disorders which comprise metabolic disease,[25] and may be related to the pathogenesis of liver disease through cells expressing the adiponectin receptor in the liver.[26] Leptin is another circulating hormone secreted by adipocytes which acts as an important signaling molecule in energy regulation and food intake,[27] and modulates multiple physiological and pathophysiological states.[27,28]

Meanwhile, adiponectin is thought to be involved in the carcinogenesis of several cancers.[29,30] Adachi *et al.* showed that an adiponectin receptor, T-cadherin, was selectively expressed in intratumoral capillary endothelial cells in HCC, suggesting a positive role for T-cadherin in mediating angiogenesis in HCC.[31] In addition, leptin may also play an important role in the processes of initiation and progression of various human cancers.[32,33] Chen *et al.* reported that leptin induced proliferation and inhibited apoptosis in human HCC cells.[34] Zhou *et al.* showed that leptin stimulated Hep G2 cell proliferation through increased DNA synthesis and the enhancement of mitotic activity.[35] Ribatti *et al.* have reported an involvement of leptin and the leptin receptor in angiogenesis in human HCC.[36]

In this study, we investigated whether obesity, insulin resistance, leptin and adiponectin were involved in the development of HCC in patients after SVR undergoing IFN treatment for chronic hepatitis C (CH-C).

METHODS

Patients

THE SUBJECTS IN this study were nine patients who were diagnosed with HCC at Kurume University Hospital after being treated with IFN therapy for CH-C between 1993 and 2003 and exhibiting SVR. Seven patients were treated with IFN at Kurume University Hospital or affiliated hospitals, and two were treated with IFN at other hospitals. The diagnosis of HCC was based on histological examination for eight patients and on computed tomography (CT), magnetic resonance imaging (MRI) and elevation of tumor markers in one patient. One thousand one hundred and forty-three patients received IFN therapy for CH-C and 308 patients achieved SVR between 1993 and 2003 at Kurume University Hospital. Out of these patients, we selected 27 patients who met the following requirements as control: (i) periodically observed at our hospital after SVR and had no evidence of HCC for over 5 years from the time of SVR until the last outpatient visit; (ii) blood samples before IFN treatment and at following outpatient visit were stored; and (iii) hepatitis B virus surface antigen (HBsAg) was negative in the serum. All control patients and six HCC patients were followed up after SVR. These patients received ultrasonography (US), computed tomography (CT) and examination of tumor markers at least once per year. HCC were detected 1 year or later after SVR regarding six patients developing HCC with periodic observation. Another three patients developing HCC did not undergo periodic observation from the time of SVR to the detection of HCC. Intervals from SVR until detection of HCC regarding these three patients were 106, 156 and 108 months, respectively. SVR was defined as being serum HCV RNA negative for more than 6 months after the termination of IFN therapy. Serum HCV RNA was measured using the COBAS AmpliPrep/COBAS TaqMan HCV Test (Roche Molecular Systems, Branchburg, NJ, US). This newly established technique has excellent sensitivity for the detection of HCV RNA and is more sensitive than conventional methods of detection.[37]

We had less information prior to their IFN therapy about the two HCC patients treated with IFN at other hospitals. Upon detection of their HCC, both patients

were seropositive for anti-HCV antibodies, seronegative for HBsAg and negative for serum HCV RNA as determined by the COBAS AmpliPrep/COBAS TaqMan HCV Test. The other seven HCC patients and 27 control patients were all seropositive for anti-HCV antibodies, positive for serum HCV RNA and seronegative for hepatitis B antigen prior to IFN therapy. The HCV RNA load was quantitated by competitive reverse-transcription polymerase chain reaction, a branched-DNA probe assay, or by the Amplicor-HCV monitor assay.[38–40] HCV genotype was determined according to previously described methods.[41] We excluded patients who had a coexisting liver disease, such as autoimmune hepatitis or primary biliary cirrhosis. Past occurrence of HCC and treatment history for HCC were also part of the exclusion criteria. Informed consent was obtained from each patient and the study was approved by the Ethical Committee of Kurume University. The study was carried out according to the ethical guidelines of the 1975 Declaration of Helsinki.

Metabolic parameters

We compared the clinical characteristics of the nine HCC patients and 27 control patients just before the start of IFN therapy. Body mass index (BMI) was calculated as weight in kilograms/height in meters squared. We obtained BMI values prior to IFN therapy from seven HCC patients treated with IFN in Kurume University Hospital or affiliated hospitals, and from 23 out of 27 control patients. We obtained BMI values for nine HCC patients at the time of HCC diagnosis, and for 22 of the control patients more than 5 years after SVR. The high molecular weight (HMW) form of adiponectin, leptin, insulin and glucose were measured from blood samples stored at −80°C. Blood samples were obtained from five HCC patients and all control patients within 9 months before the start of IFN therapy. Additional samples were obtained within 9 months after the time of SVR in three patients who developed HCC and from all control patients. Blood was also collected later as the last time point sample at the time of HCC diagnosis for nine HCC patients and more than 5 years after SVR for all the control patients. All samples were obtained at fasting time.

Serum insulin levels were measured using an enzyme-linked immunoassay kit (Lumipulse Presto Insulin; Fujirebio, Tokyo, Japan). Plasma glucose levels were measured using the hexokinase-glucose-6-phosphate dehydrogenase method. Insulin resistance was calculated from fasting levels of glucose and insulin using the Homeostatic Model of Assessment (HOMA) method. The formula for the HOMA model is: insulin resistance (HOMA-IR) = fasting glucose (mg/dL) × fasting insulin (μU/mL) / 405. Adiponectin is present in serum in a number of forms, and often exists as a trimer or hexamer of HMW.[42] Adiponectin in the HMW form has been reported to play a primary role in hepatic and whole-body insulin sensitivity and to have significant anti-inflammatory effects.[43] For this reason, we measured serum HMW adiponectin in this study. The levels of HMW adiponectin were determined using a HMW adiponectin assay kit (Fujirebio). Leptin levels were determined using a human leptin RIA kit (Linco Research, St Charles, MO, USA). Values of each metabolic parameter at each time point were compared between the HCC patients and controls.

Histopathology

We compared the histological features of liver tissue before IFN therapy in HCC patients and controls and also compared these with liver tissue at the time of detection of HCC. Liver biopsies were performed on 32 patients treated with IFN at Kurume University Hospital or at affiliated hospitals less than 1 year before the start of IFN therapy. One HCC patient underwent a biopsy 8 years before IFN therapy and showed cirrhosis at that point, and no biopsy was performed on one of the control patients. Assessment of the histological diagnosis was made according to the classifications of Desmet *et al.*[44] Hepatic steatosis was also graded by a modification of the Brunt scoring system as described[45,46] using the following categories: steatosis less than 5%, 5–33%, 33–66% and patients with over 66% steatosis were categorized as grades 0, 1, 2 and 3, respectively. All tissue samples were evaluated by two independent hepatologists. We also examined non-cancerous hepatic lesions resected with HCC from seven patients. These seven cases were assessed for histological tumor differentiation[47] in the resected specimen and one other case was examined using a specimen obtained by aspiration tumor biopsy.

Statistical analysis

Statistical analysis was performed using SPSS ver. 12.0J. Fisher's exact probability test was used to compare categorical data. Differences between two groups were measured using the Mann–Whitney *U*-test. A relationship between different continuous variables was measured by linear regression analysis. $P < 0.05$ was considered statistically significant.

Table 1 Characteristics of nine patients with HCC after sustained virological response

Age at detection of HCC (years)†	61 (54–75)
Maximal tumor diameter (mm)†	26.8 (16–43)
No. of tumors (1/2/3)	7/1/1
Tumor stage (I/II/III)‡	1/5/3
Differentiation (well/moderately)	3/5§
Child–Pugh score (at detection of HCC)¶	A5 for all
Therapy (resection/RFA/radiation)	7/1/1
Periodic observation	
Before detection of HCC (yes/no)	6/3

†Mean (range).
‡Based on the tumor–node–metastasis (TNM) classification.[48]
§Tumor histology was not available for one case.
¶Classified according to Child–Pugh classification.[49]
HCC, hepatocellular carcinoma; RFA, radiofrequency ablation.

RESULTS

Characteristics of nine patients with HCC after SVR

THE CHARACTERISTICS OF nine SVR patients who developed HCC are summarized in Table 1. Eight patients underwent curative therapy, including resection for seven patients and percutaneous radiofrequency ablation for one patient. Another patient was evaluated for resection, but ultimately received radiation therapy due to dementia.

Table 2 shows the clinical and histological profiles of HCC patients. Histological fibrosis worsened in three patients and did not change in one patient out of the six in whom we could compare liver histology at both time points. Four out of five alcohol consumers with HCC continued drinking alcohol after IFN therapy, and only patient 6 stopped drinking after IFN therapy. Four patients developed HCC within 5 years after SVR and five patients developed HCC more than 5 years after SVR. Three of four patients in the former group showed liver cirrhosis when HCC was diagnosed, and no patient showed liver cirrhosis in the latter group although histological data of two patients were not available.

Comparison of baseline characteristics of SVR patients with and without HCC after SVR

A comparison of the baseline characteristics of SVR patients with and without HCC is summarized in Table 3. Patients with regular smoking were significantly more in the HCC group than in the control group ($P = 0.046$). Before the start of IFN therapy, four out of nine HCC patients and none of the controls were diagnosed with type 2 DM, a significant difference between the two groups ($P = 0.002$). The average observation period from the time of SVR to the detection of HCC for the HCC group was 81.7 months (range

Table 2 Clinical and histological profiles of SVR patients with HCC

	Before IFN therapy			At the detection of HCC			DM	Alcohol (23 g/day)	Interval (months)§
	G/S†	Steatosis‡	BMI	G/S†	Steatosis‡	BMI			
Patient 1	2/2	1	23.4	1/4	0	23.7	–	–	22
Patient 2	2/4	2	26.5	1/4	1	28.9	+	–	38
Patient 3	2/1	0	19.3	1/4	1	23.1	–	–	42
Patient 4¶	2/2	1	27.8	1/1	0	29.6	–	+	48
Patient 5	2/2	0	20.2	N/A	NA	24.1	–	+	80
Patient 6	2/2	1	29.8	2/1	0	27.6	+	+	106
Patient 7¶	NA	NA	NA	NA	NA	24.5	+	–	108
Patient 8¶	1/1	2	23.3	1/2	1	24.9	–	+	135
Patient 9¶	NA	NA	NA	1/1	1	26.9	+	+	156

†G and S refer to hepatic inflammatory grading (A0–3) and fibrotic staging (F0–4), respectively.
‡Graded 0–3.
§Interval from SVR until detection of HCC.
¶Adiponectin, leptin, insulin and HOMA-IR prior to IFN therapy could not be acquired for patients 4 and 8. Detailed information for IFN therapy, laboratory and histological data, and metabolic parameters prior to IFN therapy could not be acquired for patients 7 and 9.
BMI, body mass index; DM, type 2 diabetes mellitus; HOMA-IR, Homeostatic Model of Assessment of Insulin Resistance; HCC, hepatocellular carcinoma; IFN, interferon; NA, not available; SVR, sustained virological response.

Table 3 Comparison of baseline characteristics between SVR patients with and without HCC after SVR

	HCC group	Control group	*P*-value†
Age at IFN induction (years)‡	53.0 (44–65)	51.3 (23–74)	NS
Sex (male/female)	5/4	11/16	NS
Alcohol (≥23 g/day) (+/–)	5/4	6/21	NS
Smoking (+/–)	6/3	7/20	0.046
Type 2 diabetes mellitus (+/–)	4/5	0/27	0.002
HBcAb (+/–)	5/4	11/16	NS
HCV viral load before IFN (high/low)§	2/5	8/18	NS
Virus genotype (1/2)	3/3	15/12	NS
IFN (a/b/a + b/consensus)	5/2/0/0	19/4/2/2	NS
(with RBV¶: PEG IFN††)	(3:0)	(4:1)	
Total IFN amount ($\times 10^4$) (mean)	59 800	56 392	NS
Observation period after SVR (months)‡	81.7 (22–156)	102 (61–149)	NS
AST (IU/L)‡‡	75.9 ± 49.9	62.2 ± 44.7	NS
ALT (IU/L)‡‡	69.3 ± 40.7	78.3 ± 70.4	NS
Total bilirubin (mg/dL)‡‡	1.0 ± 0.6	0.8 ± 0.2	NS
Albumin (g/dL)‡‡	3.7 ± 0.3	4.0 ± 0.2	NS
Prothrombin time (%)‡‡	93.2 ± 12.4	97.8 ± 16.4	NS
Platelet ($\times 10^4$/mL)‡‡	12.3 ± 3.4	15.4 ± 5.3	NS
Hyaluronic acid (ng/mL)‡‡	187.5 ± 153.4	122.9 ± 212.6	0.045
α-Fetoprotein (ng/mL)‡‡	42.4 ± 81.4	11.0 ± 29.2	NS
Child–Pugh score (5/6/7)	5/0/1	24/1/0	NS

All data were based on the patients from whom it was available for each characteristics.
†Comparison between patients with HCC (HCC group) and without HCC (control group) after SVR. *P*-values were calculated with Fisher's exact probability test and the Mann–Whitney *U*-test.
‡Mean (range).
§When the serum HCV RNA level was more than 1 Meq/mL by branched DNA assay, more than 10^6 copies/mL by competitive reverse transcription polymerase chain reaction, or more than 10^5 copies/mL by the Amplicor-HCV monitor assay, it was determined to be a high viral load.
¶Number of the patients in whom Ribavirin was used in combination.
††Number of patients in whom pegylated interferon was used.
‡‡Means ± standard deviation.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBcAb, hepatitis B core antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; NS, not significant.

22–156), and the average time from SVR to the outpatient visit when we obtained the last follow-up results for the control group was 102 months (range 61–149). There was no significant difference in the length of the observation period between the two groups. Serum levels of hyaluronic acid prior to IFN therapy were significantly higher in the HCC group than in the control group ($P = 0.045$).

Changes in adipocytokines during the observation period

Our findings on metabolic parameters are summarized in Table 4. Prior to IFN therapy and at the time of SVR, serum levels of adiponectin and leptin were not significantly different between the HCC group and the control group. However, adiponectin was significantly lower in the HCC group upon HCC detection than in the controls at their follow-up visit more than 5 years after SVR ($P = 0.030$), and leptin was significantly higher in HCC patients than in controls at that time ($P = 0.036$). When we only analyzed patients for whom we had data both prior to IFN therapy and at the last time point, percentage of change per year in adiponectin level was significantly lower ($P = 0.046$) and that in leptin level was significantly higher ($P = 0.003$) in the HCC patients than in controls. Figure 1(A) shows changes in adiponectin and Figure 1(B) shows changes in leptin in both groups during the observation period.

In all cases in both the HCC group and control group, serum leptin levels were positively correlated with serum insulin levels and HOMA-IR but not with adiponectin levels or BMI at the last time point (data not

Table 4 Metabolic parameters of the study population

	Adiponectin (μg/mL)		
	Before IFN Tx	at SVR	Last Time point†
HCC group	10.8 ± 8.6 (5)	8.8 ± 4.0 (3)	5.0 ± 2.7 (9)
Control group (27)‡	8.5 ± 4.1	8.0 ± 4.2	8.1 ± 4.0
P-value§	NS	NS	0.03
	Leptin (ng/mL)		
	Before IFN Tx	At SVR	Last Time point†
HCC group	8.9 ± 2.9 (5)	8.4 ± 2.1 (3)	11.1 ± 6.9 (9)
Control group (27)‡	6.4 ± 4.7	6.5 ± 5.7	7.0 ± 6.8
P-value§	NS	NS	0.036
	BMI		
	Before IFN Tx		Last Time point†
HCC group	24.3 ± 3.9 (7)		25.9 ± 2.4 (9)
Control group	21.7 ± 2.6 (23)		21.9 ± 2.5 (22)
P-value§	NS		0.001
	Insulin (μU/mL)		
	Before IFN Tx	At SVR	Last Time point†
HCC group	16.4 ± 10.6 (5)	9.3 ± 1.3 (3)	11.5 ± 6.9 (9)
Control group (27)‡	8.9 ± 6.6	8.7 ± 9.8	6.3 ± 6.8
P-value§	0.022	NS	0.03
	HOMA-IR		
	Before IFN Tx	At SVR	Last Time point†
HCC group	5.0 ± 4.9 (5)	1.6 ± 0.6 (3)	2.9 ± 2.1 (9)
Control group (27)‡	1.8 ± 1.7	1.7 ± 1.7	1.3 ± 0.9
P-value§	0.014	NS	0.004

Data are shown as the means ± standard deviation.
P-values were calculated with the Mann–Whitney *U*-test.
†Data at the time of HCC diagnosis in the HCC group and at an outpatient visit more than 5 years after SVR for control group.
Number in parenchyma means the number of cases involved in the analysis.
‡The number of control group cases was 27 except BMI analysis.
§Comparison between patients with HCC (HCC group) and without HCC (Control group) after SVR.
HCC, hepatocellular carcinoma; HOMA-IR, Homeostatic Model of Assessment of Insulin Resistance; IFN, interferon; NS, not significant; SVR, sustained virological response; Tx, therapy.

shown). In addition, adiponectin levels were inversely correlated with serum insulin and HOMA-IR but not with BMI at the last time point (data not shown). Prior to IFN therapy, leptin and adiponectin levels were not correlated with other metabolic parameters (data not shown). At the time of SVR, serum leptin levels were positively correlated with serum insulin levels and HOMA-IR, however, serum adiponectin levels were not correlated with these parameters and leptin levels (data not shown).

Variation in BMI and insulin resistance during the period of observation

Prior to IFN therapy, the BMI of HCC patients and control patients were not significantly different (Table 4). However, at the last time point, the BMI of HCC patients was significantly higher than that of control patients ($P = 0.001$). Figure 1(C) shows the changes in BMI during the period of observation. For six out of seven HCC patients, BMI was higher at the time of HCC detection compared to before IFN therapy. When we only analyzed patients for whom we had data both prior to IFN therapy and at the last time point, percentage of change per year in BMI values was significantly higher in the HCC patients than in controls ($P = 0.048$). Prior to IFN therapy, serum insulin and HOMA-IR were significantly higher in HCC patients than in controls ($P = 0.022$ and $P = 0.014$, respectively) (Table 4). In addition, serum insulin and HOMA-IR were significantly higher in HCC patients than in controls at the last time point ($P = 0.003$ and $P = 0.004$, respectively). Percentage of change per year in the insulin level or HOMA-IR in the HCC patients were not significantly different from those in the controls (data not shown). At the time of SVR, there was no significant difference in serum insulin and HOMA-IR between these two groups.

Histological features of the study population

Table 5 shows the histological features of the study population. When patients were divided into the hepatic inflammation groups A0–1 and A2–3, the histological activity grade at HCC detection was significantly improved compared to that before IFN therapy ($P = 0.029$). The histological activity grade prior to IFN therapy was not significantly different between the HCC group and the control group. When patients were divided into the fibrosis groups F0–1 and F2–4, the histological fibrotic stage at HCC detection was not significantly improved compared to that before IFN therapy, and fibrotic stage prior to IFN therapy was not significantly different between the HCC group and the control group. When patients were divided into the hepatic steatosis groups grade 0–1 and grade 2–3, there was no significant difference in the two groups before IFN therapy. Hepatic steatosis at the time of HCC detection was not significantly different from before IFN therapy in the HCC group.

DISCUSSION

FOR OUR STUDY, we selected patients who had been followed for more than 5 years after SVR with no detectable HCC as a control group because we wanted to be sure to exclude patients who developed HCC from the control group as much as possible. When we divide the patients in the HCC group into two groups – one group in which patients developed HCC within 5 years after SVR and another group in which patients developed HCC more than 5 years after SVR – three of four patients in the former group showed liver cirrhosis when HCC was diagnosed. This observation suggests that liver cirrhosis is tightly related to the development of HCC with a relatively short interval after SVR.

Insulin is known to be an important factor not only for a variety of metabolic pathways, but also for cell proliferation.[17] One of the major functions induced by elevated serum insulin is the activation of the mitogen-activated protein kinase cascade which has effects on cell proliferation.[50] Saito *et al.* reported that hyperinsulinemia activated the growth of human HCC cells from patients with liver cirrhosis.[18] Insulin resistance has also been shown to induce fibrotic progression in the liver with CH-C.[51] Advanced hepatic fibrosis is known to be a major risk factor for the occurrence of HCC in patients with CH-C,[52] even after SVR.[6–10] In our study, liver fibrosis was not significantly improved at the time of detection of HCC compared with before IFN therapy, and had progressed in three patients and was unchanged in another one out of the seven patients for whom we were able to compare the histological findings at both time points, although previous studies had shown an improvement of hepatic fibrosis after SVR to IFN therapy.[53] Kawaguchi *et al.* have reported that clearance of HCV improves insulin resistance.[14] In this study, both the HCC group and control group also showed a decline in serum insulin levels and HOMA-IR after SVR, and significant differences in these parameters between the two groups were not found once at the time of SVR; however, the values in the HCC group were still relatively high at the time of detection of HCC (normal

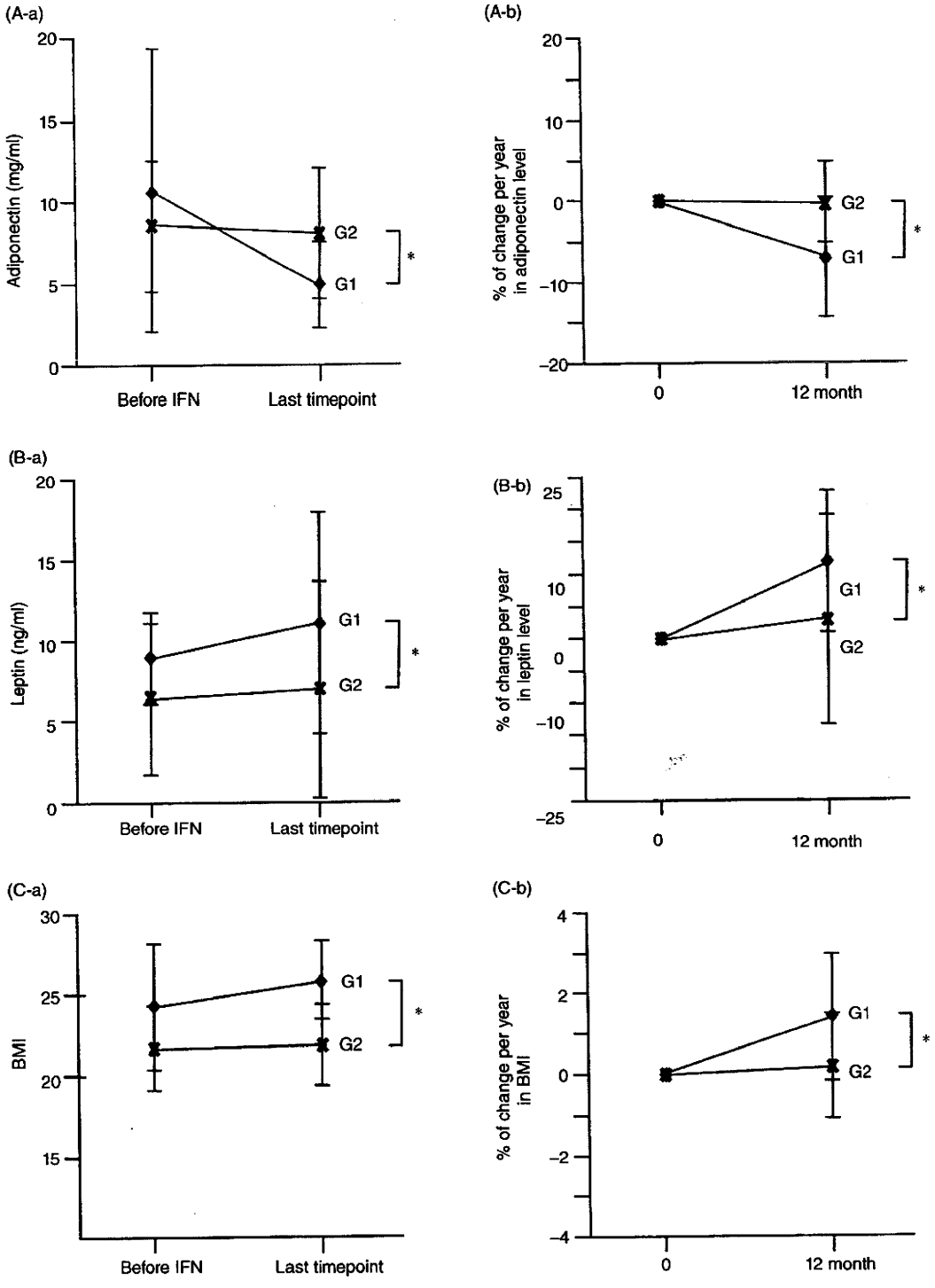

Figure 1 Changes in metabolic parameters during the period of observation in sustained virological response (SVR) patients. Hepatocellular carcinoma (HCC) group (G1) comprised SVR patients who developed HCC, and control group (G2) comprised those who did not develop HCC. (A-a) Changes in the high molecular weight form of adiponectin in the serum (B-a) changes in serum leptin and (C-a) changes in body mass index (BMI). Serum samples were collected and BMI values were obtained before the start of interferon (IFN) therapy. Serum samples and BMI values were also obtained as the last time point sample when HCC was diagnosed in the HCC group and at an outpatient visit more than 5 years after SVR for the control group. Percentages of change per year in the level of the high molecular weight form of adiponectin (A-b), leptin level (B-b) and BMI value in each group were shown. We only included patients for whom both time points were available in this analysis. The data represent means ± standard deviation. *Statistically significant differences between the indicated groups ($P < 0.05$).

range: insulin 1.84–12.2 μU/mL, HOMA-IR ≤1.6) and were significantly different from those of the control group at the last time point. Hyperinsulinemia might be one of the reasons why hepatic fibrosis was not shown to improve in the SVR patients developing HCC.

Adiponectin has been reported to increase insulin sensitivity[54] and to inhibit hepatic fibrosis,[55] however, adiponectin paradoxically decreases with the accumulation of visceral fat.[56] Therefore, hypoadiponectinemia resulting from obesity can cause insulin resistance and accelerate hepatic fibrosis. Two out of three HCC patients in which histological fibrosis worsened showed very low ratio of adiponectin at the last time point to that before IFN therapy (0.2 and 0.51), and another one case that lacked the data before IFN therapy showed very low value of adiponectin at HCC detection (2.7 μg/mL).

Serum leptin levels are positively correlated with BMI as fat tissue increases.[57] Leptin has inhibitory effects on insulin resistance,[58] however, the risk of insulin resistance rises with obesity and in these patients, increased leptin may not be sufficient to improve insulin resistance. It is conceivable that the serum level of leptin is simply a reflection of the degree of insulin resistance.[59] Leptin facilitates hepatic fibrosis through the induction of TNF-α, the proliferation of hepatic stellate cells and stimulation of the sympathetic nervous system.[55,60] In addition, leptin have been reported to be associated with proliferation of HCC.[31,34–36] Taken together, bodyweight gain leads to insulin resistance, increased leptin and decreased adiponectin, and these metabolic alternations may induce the initiation and progression of HCC, in part by promoting hepatic fibrosis in the HCC group.

Serum leptin levels have been reported to decrease after the end of IFN therapy for hepatitis C and then to recover to pretreatment levels after a long follow up.[61] One study reported that serum adiponectin was increased at 6 months after IFN therapy,[62] but another reported decreased adiponectin at 12 weeks after IFN therapy.[63] Thus, the influence of IFN on the levels of serum adiponectin for an extended period is unclear.

Fibrotic stage prior to IFN therapy was not significantly different between the HCC group and the control group ($P = 0.106$), although the number of patients in both groups was very low. Serum levels of hyaluronic acid prior to IFN therapy were significantly higher in the HCC group than in the control group ($P = 0.045$), therefore, we could not disclaim an association between hepatic fibrosis prior to IFN therapy and the occurrence of HCC after SVR.

Table 5 Histological features of the study population

	Inflammatory grading A 0/1/2/3		Fibrotic staging F 0/1/2/3/4		Steatosis Grade 0/1/2/3	
	HCC group	Control group	HCC group	Control group	HCC group	Control group
Before IFN	0/1/6/0	2/11/13/0	0/2/4/0/1	3/14/7/2/0	2/3/2/0	11/13/1/1
At HCC detection	*0/6/1/0	–	0/3/1/0/3	–	3/4/0/0	–

*$P = 0.029$ when A0–1 and A2–3 were compared between the two time points of the HCC group using the Mann–Whitney *U*-test. Prior to IFN therapy, the activity grade was not significantly different between the HCC group and control group. When divided into F0–1 and F2–4 groups for hepatic fibrosis, and when divided into grade 0–1 and grade 2–3 for hepatic steatosis, there was no significant difference between the two groups before IFN therapy and between the two time points of HCC group.
HCC, hepatocellular carcinoma; IFN, interferon.

An association between smoking and hepatic fibrosis, and also an association between smoking and HCC were reported previously.[64,65] Ratio of patients with smoking was significantly higher in the HCC group than in the control group. Therefore, smoking might facilitate hepatic fibrosis and increase the risk of HCC synergistically with the other risk factors in our study.

It is possible that undetectable HCC may have already developed in patients in this study before IFN therapy, based on information about the estimated doubling time of HCC.[52] Even so, abnormalities in metabolic factors prior to IFN therapy and alterations of these factors after IFN therapy might affect the progression of HCC.

This study was conducted at a single medical center and the number of enrolled patients was limited. As a result, the number of patients developing HCC after SVR and of controls was fairly low, and thus the accuracy of our statistical analysis was limited. A large-scale study and careful analysis are needed to confirm our results, which indicate the importance of metabolic factors in the hepatocarcinogenesis process after SVR.

In conclusion, hepatic fibrosis may be tightly related to the emergence of HCC after SVR, and insulin resistance and adipocytokine disorders may be implicated in hepatocarcinogenesis after SVR, in part by promoting hepatic fibrosis. This study is the first to report the correlation between the development of HCC after SVR and metabolic factors including insulin resistance, obesity and adipocytokine levels.

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Original Article

Recent progress in the management of hepatocellular carcinoma detected during a surveillance program in Japan

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Aim: This study explored recent improvements in the management of hepatocellular carcinoma (HCC) diagnosed during surveillance.

Methods: The subjects were 1074 patients with HCC, subdivided into three groups. Group A comprised 211 patients for whom HCC was detected during periodic follow-up examinations at Kurume University School of Medicine, Group B comprised 544 patients diagnosed with HCC during periodic follow-up examinations at other institutions, and, Group C comprised 319 patients with HCC detected incidentally or because of symptoms.

Results: In 1995–2000 and 2001–2006, 91% and 91% of group A, 68% and 70% of group B, and 27% and 26% of group C patients with HCC, respectively, met the Milan criteria. For groups A and B, the proportions of patients with Child–Pugh class A and use of promising treatment increased in the later periods compared to those diagnosed during the earlier periods (group A, Child–Pugh class A, 72% vs 58% [$P = 0.040$], receiving treatment, 90% vs 70% [$P < 0.0001$]; group B, Child–Pugh class A, 71% vs 62% [$P = 0.031$]; receiving treatment, 72% vs 52% [$P < 0.0001$], respectively). The cumulative survival rates of the 405 patients with HCC detected in the latter 6 years tended to be better than those for patients diagnosed in the former 6 years (350 patients) (4 years, 58% vs 50% [$P = 0.0349$]).

Conclusion: The use of promising treatment and prognosis have improved in the last 6 years for patients with HCC diagnosed through surveillance relative to those identified in 1995–2000.

Key words: carcinoma, cirrhosis, hepatocellular surveillance, prognosis.

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common malignancies worldwide[1] and is the leading cause of death in patients with cirrhosis.[2] HCC commonly occurs in patients with chronic liver diseases related to hepatitis C virus (HCV) or hepatitis B virus (HBV) infection, with a reported incidence of HCC in patients with HCV of 1–8% per annum.[3–7] Several cohort studies have shown that surveillance by abdominal ultrasonography (US) and α-fetoprotein (AFP) assay for patients with cirrhosis can detect early-stage HCC and thus have the potential to reduce mortality.[4–13] However, the results of surveillance are controversial including cost effectiveness[14,15] due to the high annual incidence of HCC, the target population and frequency of surveillance, available treatment for HCC, management of cirrhosis, and possibly the US equipment and skill of the US examiner.

The advent of new imaging techniques for tumor staging and improved criteria for selection of patients for liver transplantation (LT), hepatic resection (HR) and locoregional ablative therapies (LAT) has improved survival rates in patients with HCC.[2,16–23] Based on recent technological improvements in LAT, radiofrequency ablation therapy (RFA) has become more effective than percutaneous ethanol injection therapy (PEI) for patients with early-stage HCC.[22,23] Moreover, recent progress in managing complications related to cirrhosis has prolonged the life of many patients with cirrhosis.[2,24,25] These factors have contributed to the reported increase in survival of cirrhotic patients with HCC detected during surveillance over the three quinquennia (1987–2001).[7]

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We reported previously that surveillance for HCC at our Department of Liver Disease, Kurume University School of Medicine, successfully detected early-stage HCC, allowing a better chance of receiving promising treatment in 574 patients diagnosed from 1995–2001.[13] In addition, Kurume University School of Medicine recently introduced RFA for the treatment of early-stage HCC,[22,23] interferon (IFN) therapy for patients with cirrhosis, follow up after curative treatment of HCC[26,27] and management of cirrhosis-related complications by nutritional therapists.[24,25]

The present study explored the effects of recent improvements in managing HCC diagnosed through surveillance in Japanese hospitals.

METHODS

Patients

THE STUDY COMPRISED 1074 Japanese patients with HCC diagnosed at Kurume University School of Medicine from January 1995 to December 2006. The diagnosis of HCC was established by histopathology and/or imaging studies (US, computed tomography [CT], angiography, CT angiography, and magnetic resonance imaging [MRI]), and/or on high plasma levels of tumor markers such as AFP, lens culinaris agglutinin reactive AFP (AFP-L3), and des-γ-carboxy prothrombin (DCP). Patients were subdivided into three groups according to the manner of HCC detection: group A, 211 patients found to have HCC during periodic follow-up examination at Kurume University School of Medicine; group B, 544 patients found to have HCC during periodic follow-up examination in other institutions; and, group C, 319 patients found to have HCC incidentally or because of symptoms.

Surveillance program

Surveillance of 211 subjects in group A included patients of all ages, those with chronic hepatitis and cirrhosis, patients with a background of infection by HCV or HBV, and those suffering from alcoholism or other chronic liver diseases. The surveillance program was based on US examination and AFP determination every 3 months. The need for concomitant examination by CT, MRI and DCP was decided by the referring physician (hepatologist or gastroenterologist). During the subsequent surveillance period, imaging and tumor marker studies, together with physical examinations and routine biochemical testing, were repeated every 3 months. The 544 patients of group B showed nodular liver lesions or elevated AFP or DCP during periodic follow up in other institutions performed at approximately 6-month intervals. The classification of 319 patients into group C was based on a nodular liver lesion detected incidentally or at examination for symptoms and on patient interview, but not at periodic follow-up examination.

Treatment strategy

When a diagnosis of HCC was established at Kurume University School of Medicine, the following treatment options were assessed:

1 LT was only considered after 2003 and was based on HCC meeting the Milan criteria[20] with Child–Pugh class C cirrhosis, as set by the health insurance system in Japan.
2 HR was particularly assessed in patients with localized HCC and preserved hepatic reserve capacity.
3 Non-surgical treatments, such as PEI, microwave coagulation therapy (MCT), RFA, transarterial chemoembolization (TACE), hepatic arterial infusion chemotherapy (HAIC), systemic chemotherapy and radiotherapy were assessed when LT and HR were contraindicated or when the patient refused surgical treatment. The most appropriate therapeutic procedure was selected according to the tumor status and underlying cirrhosis. LAT such as PEI, MCT and RFA was considered in patients with one to three tumor nodules of 30 mm or less in diameter that were devoid of vascular invasion and not associated with extrahepatic metastasis. TACE, HAIC and systemic chemotherapy or radiotherapy were considered in patients with a maximum tumor size of 30 mm, more than three tumors, presence of vascular invasion and/or presence of extrahepatic metastasis.
4 Best supportive care was assessed when the patient had little hepatic reserve capacity or when the patient refused any treatment for the HCC.

Outcome measures

Outcome measures were compared between the two 6-year periods. In January 1995 to December 2000 and January 2001 to December 2006, 512 patients (79 of group A, 271 of group B, 162 of group C) and 562 patients (132 of group A, 273 of group B, 157 of group C), respectively, were diagnosed with HCC. In each group, we compared the following parameters between periods: (i) hepatic function tests and Child–Pugh class; (ii) tumor characteristics including size and number of HCC nodules, presence of vascular invasion and presence of extrahepatic metastasis; (iii) Milan criteria for

HCC (single nodule ≤50 mm in diameter or two to three tumor nodules, each measuring ≤30 mm in diameter), that were devoid of vascular invasion and not associated with extrahepatic metastasis);[20] (iv) treatment of HCC; and (v) cumulative survival of patients with HCC.

Statistical analysis

We used the χ^2-test,[2] Fisher's exact and Mann–Whitney *U*-tests, where appropriate, to evaluate differences in clinical features of patients and in tumor characteristics. Survival was analyzed by the Kaplan–Meier method and survival curves were compared by the log-rank test. Survival was confirmed up to 30 September 2007. Data were analyzed using the statistical software package SPSS for Windows ver. 10.0. $P < 0.05$ was considered significant.

RESULTS

Clinical features of patients

TABLE 1 SUMMARIZES the clinical profile of the 1074 patients with HCC. Child–Pugh class A was reported in 141 group A patients (67%), 363 of group B (67%) and 228 of group C (71%). Patients with cirrhosis numbered 174 in group A (82%), 427 in group B (78%) and 213 in group C (67%). The median tumor sizes in groups A–C were 18.0, 24.0 and 50.0 mm, respectively.

Of the 1074 patients, 650 (61%) with HCC met the Milan criteria, including 192 of group A (91%), 374 of group B (69%) and 84 of group C (28%). With regard to treatment, none of the patients received LT, while 27 (13%), 65 (12%) and 43 (13%) of group A, B and C patients, respectively, were treated with HR. Furthermore, 147 (69%), 265 (49%) and 46 (15%) patients in groups A, B and C, respectively, were treated by LAT, including PEI, MCT and RFA, while 31 (15%), 196 (36%) and 213 (67%) patients in groups A, B and C, respectively, were treated with interventional radiology (IVR) including TACE and HAIC, systemic chemotherapy or radiotherapy. Six (3%), 18 (3%) and 17 (5%) patients in groups A, B and C, respectively, were followed up conservatively without any specific treatment for HCC because of hepatic failure or patient refusal of treatment for HCC.

Comparison between 1995 and 2000 and 2001–2006

Tables 2–4 summarize the comparison of groups A–C patients between January 1995 and December 2000, and January 2001 and December 2006. For group A, male : female ratio, age, background liver disease, cirrhosis, serum levels of prothrombin activity, total bilirubin, AFP and DCP were not different between the two periods, while serum albumin levels and the frequency of Child–Pugh class A were significantly higher in

Table 1 Clinical profile of 1074 patients with hepatocellular carcinoma

	Group A	Group B	Group C
Number of patients	211	544	319
Sex (M/F)	124/87	373/171	270/49
Age (median [range])	67 (49–86)	67 (16–88)	64 (29–87)
Background (HCV/HBV/HCV[−] and HBV[−])	179/18/14	454/54/36	214/59/46
Prothrombin activity (%; median [range])	81 (35–130)	79 (24–130)	83 (30–130)
Total bilirubin (mg/dL; median [range])	1.0 (0.3–3.3)	1.0 (0.2–12.5)	1.0 (0.1–20.0)
Albumin (g/dL; median [range])	3.6 (1.8–5.1)	3.5 (1.8–4.8)	3.5 (2.1–4.6)
Child–Pugh class (A/B or C)	141/70	363/181	228/91
Cirrhosis (yes/no)	174/37	427/117	213/106
AFP (ng/mL; median [range])	17 (1–195741)	39 (1–883828)	72 (1–2397149)
DCP (<100/≥100 mAU/mL)	173/38	371/173	110/209
Tumor size (mm; median [range])	18.0 (7–99)	24.0 (8–140)	50.0 (9–300)
Tumor number (1/2–3/≥4)	137/61/13	275/166/103	81/87/151
Vascular invasion (yes/no)	4/207	37/507	87/232
Extrahepatic metastasis (yes/no)	1/210	7/537	36/283
Milan criteria (met Milan/outside Milan)	192/19	374/170	84/235
Treatment (HR or LAT/IVR or supportive care)	174/37	330/214	89/230

HCV, hepatitis C virus; HBV, hepatitis B virus; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; HR, hepatic resection; LAT, locoregional ablative therapies; IVR, interventional.