virus (HBV)- and HCV-related HCC patients, and performed a close analysis of the impact of diabetes on the postoperative recurrence of HCC in each group.

PATIENTS AND METHODS

Patients

A total of 150 patients were diagnosed with primary HCC and underwent surgical treatment in Kanazawa University Hospital between June 1987 and May 2004. Of these patients, 90 were analyzed who had HBV or HCV infection and underwent curative resection.

HCCs were detected by imaging modalities such as ultrasound scan, dynamic CT scan, MR imaging, and abdominal arteriography. The diagnosis of HCC was made by typical hypervascular tumor staining on angiography in addition to using typical findings, which showed hyperattenuation areas in the early phase and hypoattenuation in the late phase on dynamic CT (15).

All resected tumors were examined pathologically for the degree of differentiation of HCC, vascular invasion, and persistence of tumor in the surgical stump. Pathological degree of differentiation of HCC was assessed according to the general rules for the clinical and pathologic study of primary liver cancer (16).

Treatment and Follow-Up

In selecting surgery as a treatment option for HCC, we considered the following criteria: (a) good general condition of the patient whose Karnofsky performance status was over 80, (b) primary HCC, (c) Child-Pugh classification A or B, (d) the number of HCC was solitary and no CT, MRI, or angiographic evidence of vascular invasion or distant metastasis. Curative resection was defined as complete excision of the tumor with tumor-free surgical margins and no local recurrence at the surgical margin within 6 months after surgery.

Patients were followed postoperatively on an outpatient basis by abdominal ultrasound, dynamic CT, or MRI at 3-month intervals for at least 60 months. Recurrence was diagnosed by dynamic CT or MRI, and the date of recurrence was defined as the date of examination when the recurrence of HCC was noted. In patients with recurrent HCC, the recurrence-free period was defined as the time between the date of surgery and the date of recurrence. We confirmed the date of the patient's last visit to our hospital and checked the status of HCC using each patient's medical record.

Laboratory and Virologic Testing

Blood samples were tested for hepatitis B surface antigen (HBs-Ag) and hepatitis C virus antibody (HCV-Ab) by commercial immunoassays (Fuji Rebio, Tokyo, Japan). Serum AFP level was measured by enzyme immunoassay (AxSYM AFP, Abbott Japan, Tokyo, Japan). Diabetes was diagnosed according to the American Diabetes Association criteria for type II diabetes (17) and the severity of liver disease (stage of fibrosis) was evaluated according to the criteria of Desmet *et al.* (18).

Statistical Analysis

Between-group differences were assessed by univariate analysis with Student's t-test for numerical data and the $\chi 2$ test with Yates' correction (or Fisher's exact test where appropriate) for nominal data. Overall survival and recurrence-free survival was examined using the method of Kaplan-Meier, and differences were assessed by the log-rank test. Impact factors for the recurrence of HCC after hepatic resection were analyzed by univariate and multivariate analysis using Cox proportional hazards model. Seventeen variables were analyzed, consisting of age, gender, etiology, body mass index (BMI), prevalence of alcohol abuse, diabetes, hemoglobin A1c (HbA1c), liver fibrosis degree, Child-Pugh classification, platelet count, alanine aminotranseferase (ALT), T-bil, Alb, AFP, tumor size, tumor differentiation degree, and the presence of vascular invasion. $P < 0.05$ was considered statistically significant.

RESULTS

Comparison of Baseline Characteristics

Of the 90 patients (75 men and 15 women, with a mean age of 61.0 yr) who were followed and analyzed, 30 were diagnosed as having coexistence of diabetes, and 60 had no diabetes. The characteristics of the patients in both groups

Table 1. Characteristics of Patients

Characteristic	Patients With Diabetes (N = 30)	Patients Without Diabetes (N = 60)	*P* Value
Median age (yr)	62.0	60.6	0.453
Gender (male/female)	24/6	51/9	0.560
Etiology (HBV/HCV/ HBV + HCV)	8/22/0	17/40/3	0.438
Body mass index (kg/m^2)	23.5	22.73	0.316
Alcohol abuse (+/−)	13/17	27/33	0.881
HbA1c (%)	6.4	4.8	<0.001
HOMA-IR	4.1	3.3	0.399
Platelet count ($\times 10^4/\mu$L)	12.2	13.5	0.284
ALT (IU/L)	69.8	56.4	0.318
Total bilirubin (mg/dL)	0.9	0.8	0.510
Albumin (g/dL)	4.1	4.2	0.341
AFP (ng/mL)	417	395	0.931
Fibrosis (F1/F2/F3/F4)	0/4/4/22	4/5/5/46	0.732
Inflammatory grading (A1/A2/A3)	11/17/2	24/33/3	0.385
Child-Pugh grade (A/B/C)	24/6/0	53/7/0	0.294
Tumor size (mm)	34.3	29.8	0.359
Diff. degree (wel/mod/por)*	11/12/7	20/19/21	0.264
Vascular invasion (+/−)	11/19	20/40	0.757
Date of operation (1987–1995/1995–2000/ 2001–2004)	8/14/8	13/30/17	0.538

*Histological degree of HCC: wel = well differentiated; mod = moderately differentiated; por = poorly differentiated.

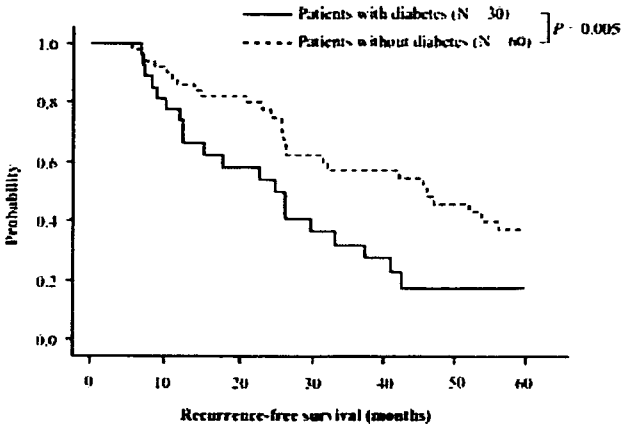

Figure 1. Kaplan-Meier curves for recurrence-free survival in the groups of patients with and without diabetes.

are shown in Table 1. No significant differences were noted between the two groups in age, gender, HBV or HCV infection rate, BMI, or prevalence of alcohol abuse. HbA1c was significantly higher, at 6.4%, in the diabetic group than in the nondiabetic group with an HbA1c of 4.8% ($P < 0.001$). There were no significant differences in platelet count, ALT, T-bil, Alb, AFP, or Child-Pugh classification between the two groups. In addition, no significant differences were observed in the liver fibrosis degree, or in the size, degree of differentiation, and presence of microscopic vascular invasion of resected HCC. Homeostasis model assessment-insulin resistance (HOMA-IR) of the patients with diabetes, which was high compared with that of Japanese healthy subjects (19), was higher than that of the patients without diabetes, although it was not statistically significant.

Impact of Diabetes on Recurrence After Surgical Treatment of HCC

Next, the diabetic and nondiabetic groups were compared for the rate of HCC recurrence after surgical treatment. HCC recurred after surgical treatment in 49 patients, consisting of 22 diabetic patients (73.3%) and 27 nondiabetic patients (45.0%). The mean time to recurrence was 32.8 months (range 8–60 months) and the median time to recurrence was 29.4 months.

Figure 1 shows the Kaplan-Meier curves for recurrence-free survival of the patients with and without diabetes. The recurrence-free survival rates 1, 2, 3, 4, and 5 yr after surgical treatment were 77.8%, 55.6%, 36.0%, 16.7%, and 16.7%, respectively, in the diabetic patient group, and 89.5%, 80.4%, 56.8%, 45.0%, and 36.6%, respectively, in the nondiabetic patient group; all rates except 1 yr were significantly lower in the diabetic patient group ($P = 0.155$, $P = 0.010$, $P = 0.009$, $P = 0.002$, and $P = 0.005$).

To further examine the degree of contribution of diabetes to the postoperative recurrence of HCC, we performed univariate and multivariate analysis. Univariate analysis identified the following variables as factors significantly contributing to HCC recurrence after surgical treatment: presence of diabetes ($P = 0.003$), high HbA1c level ($P < 0.001$), and Child-Pugh classification B against A ($P < 0.001$) (Table 2). When we conducted multivariate analysis, we chose variables that had already pointed out a risk factor for HCC recurrence and the P value was lower than 0.1 in univariate analysis. As a result, multivariate analysis of these variables showed that the presence of diabetes (risk 2.9, 95% CI 1.5–5.4, $P < 0.001$) and Child-Pugh classification B against A (risk 3.6, 95% CI 1.7–7.7, $P = 0.001$) were significant factors contributing to HCC recurrence after surgical treatment (Table 3).

Table 2. Univariate Proportional Hazard Model for Recurrence of HCC After Surgical Treatment

Variable	Hazard Ratio	95% CI	*P* Value
Age (yr)	1.0	1.0–1.1	0.258
Gender (male)	0.5	0.3–1.1	0.104
Etiology (HCV)	1.0	0.5–1.8	0.965
Body mass index (>25 kg/m^2)	1.2	0.6–2.3	0.554
Alcohol abuse (+)	1.1	0.7–2.0	0.644
Diabetes (+)	2.4	1.3–4.2	0.003
HbA1c (%)	1.5	1.2–1.9	<0.001
Fibrosis (F4)	1.4	0.7–3.1	0.349
Child-Pugh grade (B)	3.1	1.6–6.0	<0.001
Platelet count ($\times 10^4/\mu$L)	1.0	0.9–1.0	0.465
ALT (IU/L)	1.0	1.0–1.1	0.717
Total bilirubin (mg/dL)	1.4	0.6–3.6	0.451
Albumin (g/dL)	1.4	0.8–2.4	0.225
AFP (>200 ng/mL)	1.0	0.5–1.8	0.906
Tumor size (>50 mm)	1.5	0.7–1.2	0.213
Diff. degree (P)	1.1	0.6–2.1	0.675
Vascular invasion (+)	1.2	0.4–1.7	0.490

Impact of Diabetes on Prognosis After Surgical Treatment of HCC

To examine the impact of diabetes on prognosis of HCC patients, we analyzed the overall survival rates after surgical treatment. Figure 2 shows the Kaplan-Meier curves for overall survival of the patients with and without diabetes after surgical treatment of HCC. The overall survival rates 1, 2, 3, 4, and 5 yr after surgical treatment were 100%, 88.9%, 75.0%, 63.6%, and 45.5%, respectively, in the diabetic patient group, and 100%, 98.1%, 88.9%, 85.7%, and 76.3%, respectively, in the nondiabetic patient group; the rates of more than 3 yr were significantly lower in the diabetic patient group ($P = 1.000$, $P = 0.073$, $P = 0.028$, $P = 0.039$, and $P = 0.005$).

Table 3. Multivariate Proportional Hazard Model for Recurrence of HCC After Surgical Treatment

Variable	Hazard Ratio	95% CI	*P* Value
Diabetes	2.9	1.5–5.5	<0.001
Fibrosis (F4)	1.9	0.8–4.5	0.148
Child-Pugh grade (B)	3.6	1.7–7.7	0.001
AFP(>200 ng/mL)	0.7	0.3–1.5	0.390
Diff. degree (P)	0.9	0.5–1.8	0.776
Vascular invasion (+)	2.0	1.0–4.0	0.061

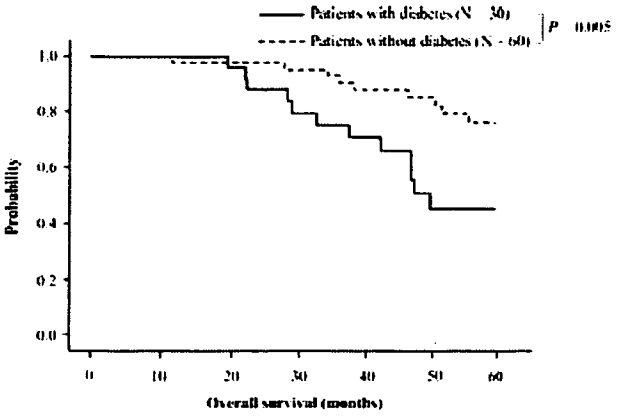

Figure 2. Kaplan-Meier curves for overall survival in the groups of patients with and without diabetes.

These curves indicated that overall survival rates were significantly lower in the diabetic patient group ($P = 0.005$).

Differential Impact of Diabetes on Prognosis After Surgical Treatment Between HBV- and HCV-Infected Patients

Next, we classified the HCC patients into HBV- and HCV-related HCC patients, and examined the impact of diabetes on recurrence-free and overall survival rates after surgical treatment. We divided all patients into 25 HBs-Ag (+), HCV-Ab (−) patients (with HBV-related HCC) and 62 HBs-Ag (−), HCV-Ab (+) patients (with HCV-related HCC), and further divided these two groups of patients into four groups according to the presence or absence of diabetes. In 62 patients who were HCV-Ab positive, 53 patients were also positive for HCVRNA. The other 9 patients were not examined for HCVRNA. The clinical profiles of these four groups of patients are shown in Table 4. Three HBs-Ag (+), HCV-Ab (+) patients, who were not complicated by diabetes, were excluded from the analysis. There were no significant differences between the groups of HBV-related HCC patients with and without diabetes in age, gender, BMI, prevalence of alcohol abuse, platelet count, ALT, total bilirubin, Child-Pugh classification, liver fibrosis degree, tumor size, tumor differentiation degree, or the presence of vascular invasion, except for Alb and AFP. Similarly, there were no significant differences between the groups of HCV-related HCC patients with and without diabetes. The HbA1c levels were higher in the groups of diabetic patients with HBV- or HCV-related HCC.

The Kaplan-Meier curves for recurrence-free survival in the groups of HBV-related HCC patients with and without diabetes are shown in Figure 3. The recurrence-free survival rates 1, 3, and 5 yr after surgical treatment were 85.7%, 57.1%, and 42.9%, respectively, in the diabetic patient group, and 76.5%, 46.7%, and 40.0%, respectively, in the nondiabetic patient group, showing no significant differences between the two groups ($P = 0.596$, $P = 0.670$, and $P = 0.827$). In the analysis of overall survival in the groups of HBV-related HCC patients with and without diabetes by the method of Kaplan-Meier, it indicated that there was no difference between the two groups ($P = 0.505$) (Fig. 4).

Figure 5 shows the Kaplan-Meier curves for recurrence-free survival in the groups of HCV-related HCC patients with and without diabetes. The recurrence-free survival rates 1, 2, 3, 4, and 5 yr after surgical treatment were 75.0%, 38.9%, 22.2%, 11.1%, and 11.1%, respectively, in the diabetic patient group, and 94.6%, 83.9%, 62.1%, 35.0%, and 29.2%, respectively, in the nondiabetic patient group, indicating that the recurrence-free survival rates were significantly lower in the diabetic patient group than in the nondiabetic patient group ($P = 0.030$, $P < 0.001$, $P < 0.001$, $P < 0.001$, and $P < 0.001$).

In the analysis of overall survival in the groups of HCV-related HCC patients with and without diabetes, the overall survival rates 1, 2, 3, 4, and 5 yr after surgical treatment were 100%, 88.9%, 76.5%, 64.7%, and 43.8%, respectively, in the diabetic patient group, and 100%, 100%, 96.7%, 92.5%, and

Table 4. Characteristics of Patients With HBV- or HCV-Related HCC

	Patients With HBV-Related HCC			Patients With HCV-Related HCC		
Characteristic	With Diabetes (N = 8)	Without Diabetes (N = 17)	*P* Value	With Diabetes (N = 22)	Without Diabetes (N = 40)	*P* Value
Median age (yr)	61.6	57.3	0.3	62.2	62.9	0.737
Gender (male/female)	7/1	13/4	0.5	17/5	35/5	0.302
Body mass index (kg/m^2)	23.4	23.2	0.9	23.5	22.7	0.348
Alcohol abuse (+/−)	2/6	6/11	0.6	11/11	21/19	0.853
HbA1c (%)	5.9	4.6	0.07	6.6	4.9	<0.001
Fibrosis (F1/F2/F3/F4)	0/0/2/6	3/2/0/12	0.8	0/4/2/16	1/3/5/31	0.680
Child-Pugh grade (A/B)	6/2/0	15/2	0.4	18/4	35/5	0.551
Platelet count (× $10^4/\mu$L)	11.5	13.9	0.3	12.4	13.5	0.520
ALT (IU/L)	31.8	42.3	0.6	84.2	63.5	0.233
Total bilirubin (mg/dL)	0.9	0.9	1.0	0.9	0.8	0.303
Albumin (g/dL)	3.8	4.2	0.02	4.2	4.2	0.983
AFP (ng/mL)	1,056	121	0.001	162	328	0.460
Tumor size (mm)	28.9	31.2	0.8	36.0	29.6	0.295
Diff.degree (W/M/P)	2/2/4	5/7/5	0.3	9/10/3	14/11/15	0.058
Vascular invasion (+/−)	1/7	4/13	0.5	12/10	15/25	0.548

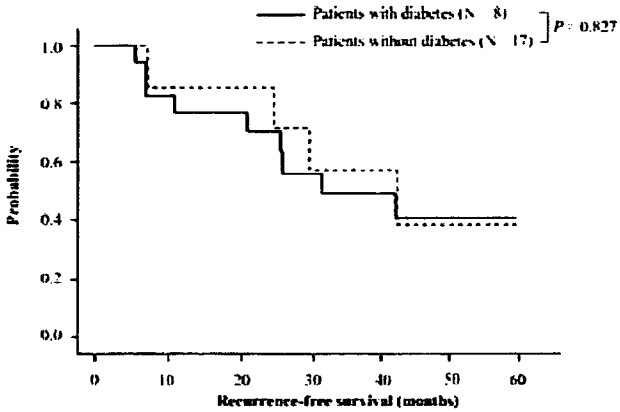

Figure 3. Kaplan-Meier curves for recurrence-free survival in HBV patients with diabetes and HBV patients without diabetes.

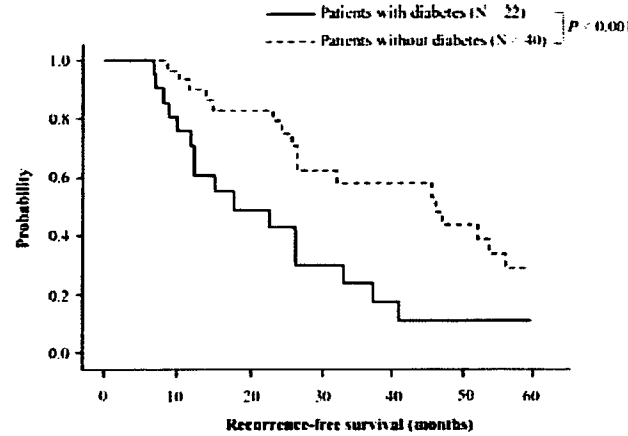

Figure 5. Kaplan-Meier curves for recurrence-free survival in HCV patients with diabetes and HCV patients without diabetes.

82.6%, respectively, in the nondiabetic patient group, indicating that the overall survival rates of more than 3 yr were significantly lower than in the nondiabetic patient group ($P = 1.000$, $P = 1.000$, $P = 0.035$, $P = 0.015$, $P = 0.004$) (Fig. 6).

Factors Associated With Recurrence-Free Survival After Surgical Treatment for HCC in Patients With Diabetes

Finally, we performed univariate and multivariate analyses to determine the variables that might affect the postoperative recurrence of HCC in the 30 HCC patients with diabetes, consisting of 17, 4, and 9 patients receiving insulin therapy, oral hypoglycemic drugs, and no treatment, respectively. Univariate analysis identified Child-Pugh classification B as a factor significantly contributing to the postoperative recurrence of HCC ($P < 0.001$) (Table 5). When we conducted multivariate analysis, we chose variables that had been already pointed out as a risk factor for HCC recurrence and whose P value was lower than 0.1 in univariate analysis. Multivariate analysis identified Child-Pugh classification B (risk 40.0, 95% CI 4.4-362.1, $P = 0.001$) and the presence of insulin therapy (risk 3.9, 95% CI 1.0-15.3, $P = 0.049$) as factors significantly contributing to the postoperative recurrence of HCC (Table 6). In multivariate analysis, both factors showed significant P value. Based on the results, we considered that both factors contribute to recurrence of HCC independently.

DISCUSSION

In the present study, univariate and multivariate analyses identified the presence of diabetes as a factor significantly contributing to the recurrence of HCC after surgical treatment. The results are consistent with the findings of Ikeda *et al.* (12). They analyzed a population of 64 HBV-related HCC patients and a larger population of 144 HCV-related HCC patients, but did not compare the postoperative recurrence rate between the two populations. In our study, 25 and 62 patients with HBV- and HCV-related HCC, respectively, were included, similar to the proportion of such patients in the study by Ikeda *et al.* (12), presumably leading to similar results. On the other hand, none of the variables that have been reported to contribute to the postoperative recurrence of HCC, such as liver fibrosis degree, Alb level, AFP level, tumor differentiation degree,

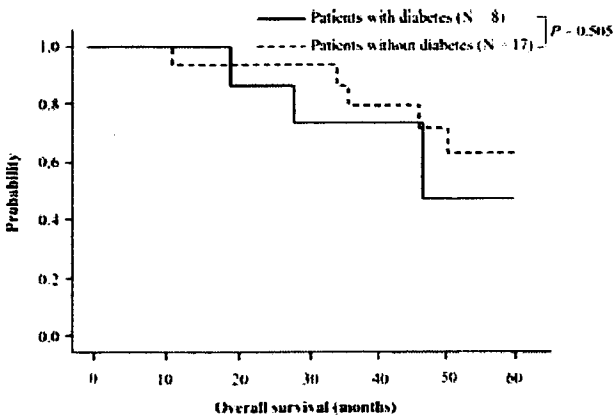

Figure 4. Kaplan-Meier curves for overall survival in HBV patients with diabetes and HBV patients without diabetes.

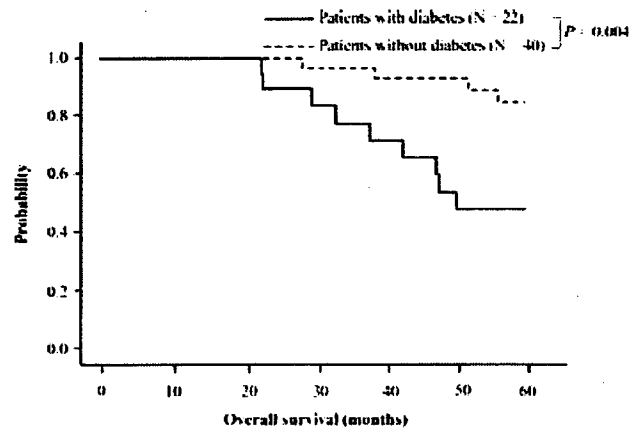

Figure 6. Kaplan-Meier curves for overall survival in HCV patients with diabetes and HCV patients without diabetes.

Table 5. Univariate Proportional Hazard Model for Recurrence of HCC After Surgical Treatment in Diabetic Patients

Variable	Hazard Ratio	95% CI	*P* Value
Age (yr)	1.0	1.0–1.1	0.534
Gender (male)	0.8	0.3–2.2	0.681
Etiology (HCV)	0.5	0.2–1.4	0.183
Body mass index (>25 kg/m^2)	1.0	0.4–2.6	0.938
Alcohol abuse (+)	1.0	0.4–2.3	0.972
HbA1c (%)	1.4	1.0–1.9	0.059
Fibrosis (F4)	1.2	0.5–3.3	0.651
Child-Pugh grade (A/B)	11.8	3.2–43.9	<0.001
Platelet count ($\times 10^4/\mu$L)	1.0	0.9-1.1	0.548
ALT(IU/L)	1.0	1.0-1.0	0.699
Total bilirubin (mg/dL)	0.6	0.2–2.5	0.506
Albumin (g/dL)	1.2	0.5–2.9	0.689
AFP (>200 ng/dL)	0.9	0.4–2.3	0.899
Tumor size (>50 mm)	0.8	0.7-1.2	0.668
Diff degree (W/M/P)	0.9	0.4–2.0	0.723
Vascular invasion	0.9	0.4–2.2	0.843
Insulin therapy	2.5	1.0–6.6	0.058

and the presence of vascular invasion, were identified as significant factors. This is probably because of our criteria for surgical treatment and that patients who recurred within 6 months after surgery were excluded from the study, resulting in the inclusion of only a population with little variation in these variables.

Next, groups of patients with HBV- and HCV-related HCC were separately examined for the impact of diabetes on the recurrence of HCC after surgical treatment. No significant differences in the recurrence-free survival rates determined by the Kaplan-Meier curve were noted between the HBV-related HCC patient groups with and without diabetes, which was similar to the results reported by Poon *et al.* (13), Toyoda *et al.* (14), and Huo *et al.* (20). In contrast, the recurrence-free survival rate was significantly lower in the group of HCV-related HCC patients with diabetes than in the group of HCV-related HCC patients without diabetes. From the above findings, we concluded that the coexistence of diabetes was a factor contributing to the recurrence of HCC after surgical treatment in HCV-related HCC patients, and that the results of analysis of all HCC patients reflected those in the HCV-related HCC patients. In addition, the results of the analysis for the prognosis of HCV-related HCC patients after surgical treatment showed that the overall survival rate was significantly lower in the diabetic patient group than in the nondiabetic group. These results suggest that more frequent recurrence may contribute to shorter survival in HCV-related HCC patients with diabetes.

Table 6. Multivariate Proportional Hazard Model for Recurrence of HCC After Surgical Treatment in Diabetic Patients

Variable	Hazard Ratio	95% CI	*P* Value
Insulin therapy (+)	3.9	1.0–15.3	0.049
Fibrosis (F4)	2.2	0.5–9.8	0.306
Child-Pugh grade (B)	40.0	4.4–362.1	0.001
AFP (>200 ng/mL)	2.1	0.5–8.8	0.289
Diff degree (P)	0.6	0.1–2.8	0.542
Vascular invasion (+)	1.7	0.4–7.6	0.513
Etiology (HCV)	2.0	0.3–12.2	0.460
HbA1c (%)	1.1	0.8–1.6	0.629

To our knowledge, only one study has examined the impact of diabetes on the recurrence of HCC after surgical treatment separately in HBV- and HCV-related HCC patients. Contrary to the results of this study, Huo *et al.* (20) have reported that diabetes is not a risk factor for the recurrence of HCV-related HCC. The clinical characteristics of HCC patients, such as the number of tumors, tumor diameter, and background liver histology, differed between their study and ours, and the presence or absence of vascular invasion and hepatic reserve indicated by Child-Pugh classification were unknown in their study, which makes direct comparison difficult, but partially accounts for the different results. Although, to date, no studies have reported that, as shown in this study, there is a possibility that diabetes differently affects the postoperative recurrence of HCC in the groups of patients with HBV- or HCV-related HCC.

This may be because of different mechanisms of carcinogenesis in the two groups (21). It appears that neither HBV nor HCV damages liver cells, but these viruses induce chronic inflammation in the liver, and facilitate mutations in liver cells, leading to their malignant transformation (22, 23). Our previous study using the microarray technique showed that the genes expressed in the liver differed markedly between HBV- and HCV-related liver disease patients (24). This genetic heterogeneity is considered to be associated with different modes of pathogenesis of HBV- and HCV-related HCC (25–28). Previous studies have shown that, in HCV-related HCC, chronic inflammation and oxidative stress are closely associated with hepatocellular death and regeneration (29–33). Highly insulin-resistant diabetics show increased peripheral lipolysis and hepatic accumulation of free fatty acids (34, 35). The β-oxidation of fatty acids in mitochondria is decreased in these patients, and they are under high oxidative stress. We also previously reported that the gene expression profile in the liver of diabetic patients shows increasing fibrogenic, angiogenic, tumorigenic, and stress responsive factors (36). Taken together, these observations suggest that the coexistence of diabetes promotes the progression of liver fibrosis and development of HCC in HCV-related liver disease (37). In contrast, in HBV-related liver disease, integration of the virus genome into the host DNA appears to induce HCC (38–40). Therefore, in such a mechanism of carcinogenesis, the coexistence of diabetes may have little synergistic effect.

Finally, we examined variables that might contribute to the recurrence of HCC after surgical treatment in HCC patients with diabetes. Since insulin therapy is often administered to diabetic patients who have difficulty in controlling blood sugar, or who have advanced liver disease, this factor may be involved in the recurrence of HCC in those under insulin therapy. Therefore, we included HbA1c, liver fibrosis degree,

and Child-Pugh classification together with insulin therapy in multivariate analysis. As a result, multivariate analysis identified Child-Pugh classification B and insulin therapy as significant factors contributing to the postoperative recurrence of HCC. These findings suggest that insulin therapy and Child-Pugh classification B are independent risk factors for postoperative recurrence.

The mechanism by which insulin promotes HCC recurrence is unknown. However, the results of this study are consistent with the report that insulin acts as a tumor growth factor *in vitro* (41). In animal models, insulin has been shown to be a promoter of colonic carcinogenesis (42). Although there has been much debate about the use of insulin and the risk of cancer development, no consensus has been reached (43–45). A recent study has indicated that insulin therapy is a risk factor for the postoperative recurrence of colorectal cancer (46). These findings show the possibility that insulin therapy promotes HCC recurrence after surgical treatment. It should be discussed how to use insulin therapy in HCC patients with diabetes in the future.

There is a limitation to our study, because our study is retrospective and on a not so large population. However, the results of the present study suggest that diabetes is a risk factor for the recurrence of HCV-related HCC and decreases the overall survival rates after surgical treatment. HCV-related HCC patients with diabetes should be closely followed for post-treatment recurrence, and blood sugar control may also be important to reduce the rate of recurrence. However, since the use of insulin to treat diabetes in HCC patients may promote tumor recurrence, treatment methods for blood sugar control require further evaluation.

STUDY HIGHLIGHTS

What Is Current Knowledge

- Diabetes accumulates liver fibrosis for chronic hepatitis C.
- Diabetes is a risk factor for hepatocellular carcinoma (HCC).
- HCC has high recurrence rate after curative surgery.

What Is New Here

- Hepatitis C virus (HCV) related patients with diabetes have a higher possibility of HCC recurrence.
- HCV-related patients with diabetes have poorer prognosis.
- Controlling of blood sugar may reduce HCC recurrence.
- Insulin therapy may accumulate HCC recurrence.

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REFERENCES

1. Bosch FX, Ribes J, Borras J. Epidemiology of primary liver cancer. Semin Liver Dis 1999;19:271–85.
2. El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 1999;340:745–50.
3. Heathcote EJ. Prevention of hepatitis C virus-related hepatocellular carcinoma. Gastroenterology 2004;127:S294–302.
4. Yamamoto J, Kosuge T, Takayama T, et al. Recurrence of hepatocellular carcinoma after surgery. Br J Surg 1996;83:1219–22.
5. Poon RT, Fan ST, Lo CM, et al. Intrahepatic recurrence after curative resection of hepatocellular carcinoma: Long-term results of treatment and prognostic factors. Ann Surg 1999;229:216–22.
6. Adachi E, Maeda T, Matsumata T, et al. Risk factors for intrahepatic recurrence in human small hepatocellular carcinoma. Gastroenterology 1995;108:768–75.
7. Ikeda K, Saitoh S, Tsubota A, et al. Risk factors for tumor recurrence and prognosis after curative resection of hepatocellular carcinoma. Cancer 1993;71:19–25.
8. Predictive factors for long term prognosis after partial hepatectomy for patients with hepatocellular carcinoma in Japan. The Liver Cancer Study Group of Japan. Cancer 1994;74:2772–80.
9. Koike Y, Shiratori Y, Sato S, et al. Risk factors for recurring hepatocellular carcinoma differ according to infected hepatitis virus–an analysis of 236 consecutive patients with a single lesion. Hepatology 2000;32:1216–23.
10. El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. Gastroenterology 2004;126:460–8.
11. Davila JA, Morgan RO, Shaib Y, et al. Diabetes increases the risk of hepatocellular carcinoma in the United States: A population based case control study. Gut 2005;54:533–39.
12. Ikeda Y, Shimada M, Hasegawa H, et al. Prognosis of hepatocellular carcinoma with diabetes mellitus after hepatic resection. Hepatology 1998;27:1567–71.
13. Poon RT, Fan ST, Wong J. Does diabetes mellitus influence the perioperative outcome or long term prognosis after resection of hepatocellular carcinoma? Am J Gastroenterol 2002;97:1480–8.
14. Toyoda H, Kumada T, Nakano S, et al. Impact of diabetes mellitus on the prognosis of patients with hepatocellular carcinoma. Cancer 2001;91:957–63.
15. Araki T, Itai Y, Furui S, et al. Dynamic CT densitometry of hepatic tumors. AJR Am J Roentgenol 1980;135:1037–43.
16. Japan. LCSGo. Classification of primary liver cancer, English 2nd Ed. Tokyo: Kanehara & Co., Ltd., 1997.
17. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183–97.
18. Desmet VJ, Gerber M, Hoofnagle JH, et al. Classification of chronic hepatitis: Diagnosis, grading and staging. Hepatology 1994;19:1513–20.
19. Katsuki A, Sumida Y, Gabazza E, et al. Homeostasis model assessment is a reliable indicator of insulin resistance during follow-up of patients with type 2 diabetes. Diabetes Care 2001;24:362–5.
20. Huo TI, Wu JC, Lui WY, et al. Diabetes mellitus is a recurrence-independent risk factor in patients with hepatitis B virus-related hepatocellular carcinoma undergoing resection. Eur J Gastroenterol Hepatol 2003;15:1203–8.
21. Szabo E, Paska C, Kaposi Novak P, et al. Similarities and differences in hepatitis B and C virus induced hepatocarcinogenesis. Pathol Oncol Res 2004;10:5–11.

22. Brechot C. Pathogenesis of hepatitis B virus-related hepatocellular carcinoma: Old and new paradigms. Gastroenterology 2004;127:S56–61.
23. Liang TJ, Heller T. Pathogenesis of hepatitis C-associated hepatocellular carcinoma. Gastroenterology 2004;127:S62–71.
24. Honda M, Kaneko S, Kawai H, et al. Differential gene expression between chronic hepatitis B and C hepatic lesion. Gastroenterology 2001;120:955–66.
25. Edamoto Y, Hara A, Biernat W, et al. Alterations of RB1, p53 and Wnt pathways in hepatocellular carcinomas associated with hepatitis C, hepatitis B and alcoholic liver cirrhosis. Int J Cancer 2003;106:334–41.
26. Iizuka N, Oka M, Yamada-Okabe H, et al. Differential gene expression in distinct virologic types of hepatocellular carcinoma: Association with liver cirrhosis. Oncogene 2003;22:3007–14.
27. Laurent-Puig P, Legoix P, Bluteau O, et al. Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis. Gastroenterology 2001;120:1763–73.
28. Thorgeirsson SS, Grisham JW. Molecular pathogenesis of human hepatocellular carcinoma. Nat Genet 2002;31:339–46.
29. Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med 2001;345:41–52.
30. Sung VM, Shimodaira S, Doughty AL, et al. Establishment of B-cell lymphoma cell lines persistently infected with hepatitis C virus in vivo and in vitro: The apoptotic effects of virus infection. J Virol 2003;77:2134–46.
31. Guo JT, Zhou H, Liu C, et al. Apoptosis and regeneration of hepatocytes during recovery from transient hepadnavirus infections. J Virol 2000;74:1495–505.
32. Felsher DW, Bishop JM. Reversible tumorigenesis by MYC in hematopoietic lineages. Mol Cell 1999;4:199–207.
33. Shiratori Y, Shiina S, Teratani T, et al. Interferon therapy after tumor ablation improves prognosis in patients with hepatocellular carcinoma associated with hepatitis C virus. Ann Intern Med 2003;138:299–306.
34. Pessayre D, Berson A, Fromenty B, et al. Mitochondria in steatohepatitis. Semin Liver Dis 2001;21:57–69.
35. Chitturi S, Farrell GC. Etiopathogenesis of nonalcoholic steatohepatitis. Semin Liver Dis 2001;21:27–41.
36. Takamura T, Sakurai M, Ota T, et al. Genes for systemic vascular complications are differentially expressed in the livers of type 2 diabetic patients. Diabetologia 2004;47:638–47.
37. Fong DG, Nehra V, Lindor KD, et al. Metabolic and nutritional considerations in nonalcoholic fatty liver. Hepatology 2000;32:3–10.
38. Brechot C, Pourcel C, Louise A, et al. Presence of integrated hepatitis B virus DNA sequences in cellular DNA of human hepatocellular carcinoma. Nature 1980;286:533–5.
39. Kim CM, Koike K, Saito I, et al. HBx gene of hepatitis B virus induces liver cancer in transgenic mice. Nature 1991;351:317–20.
40. Bressac B, Galvin KM, Liang TJ, et al. Abnormal structure and expression of p53 gene in human hepatocellular carcinoma. Proc Natl Acad Sci U S A 1990;87:1973–7.
41. Giovannucci E. Nutrition, insulin, insulin-like growth factors and cancer. Horm Metab Res 2003;35:694–704.
42. Tran TT, Medline A, Bruce WR. Insulin promotion of colon tumors in rats. Cancer Epidemiol Biomarkers Prev 1996;5:1013–5.
43. Swerdlow AJ, Laing SP, Qiao Z, et al. Cancer incidence and mortality in patients with insulin-treated diabetes: A UK cohort study. Br J Cancer 2005;92:2070–5.
44. Schiel R, Muller UA, Braun A, et al. Risk of malignancies in patients with insulin-treated diabetes mellitus – results of a population-based trial with 10-year follow-up (JEVIN). Eur J Med Res 2005;10:339–44.
45. Chuang TY, Lewis DA, Spandau DF. Decreased incidence of nonmelanoma skin cancer in patients with type 2 diabetes mellitus using insulin: A pilot study. Br J Dermatol 2005;153:552–7.
46. Yang YX, Hennessy S, Lewis JD. Insulin therapy and colorectal cancer risk among type 2 diabetes mellitus patients. Gastroenterology 2004;127:1044–50.

CONFLICT OF INTEREST

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Intrahepatic interleukin-8 production during disease progression of chronic hepatitis C

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Abstract

The current study was designed to investigate the contribution of chemokines to the pathogenesis of chronic hepatitis C and hepatocellular carcinoma (HCC) by measuring the production of IL-8, monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1 alpha (MIP-1α). A solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) was established to quantitate serum concentrations of the chemokines. Expression of chemokines in liver tissues was evaluated immunohistochemically using specific monoclonal antibodies. As the severity of chronic hepatitis escalated, serum IL-8 levels increased progressively. Moreover, in the hepatocellular carcinoma (HCC) patients, IL-8 concentrations were positively correlated with the macroscopic staging of HCC, and inversely correlated with the duration of the survival periods. The results demonstrate that IL-8 production may be augmented upon the malignant transformation of hepatocytes in chronic hepatitis C.

Keywords: Interleukin-8; Monocyte chemoattractant protein-1; Macrophage inflammatory protein-1 alpha; Sandwich enzyme-linked immunosorbent assay; Chronic hepatitis C; Hepatocellular carcinoma

1. Introduction

Chemokines are known not only to mediate the recruitment of inflammatory cells such as neutrophils or lymphocytes, but also to regulate the balance of helper T cells (Th1/Th2) as well as the activation of antigen-presenting dendritic cells, and thus to be deeply involved in immune responses. Moreover, chemokine-mediated cellular responses are known to be involved in neovascularization nd fibrosis, and since chemokines have growth factor activity, their association with malignant transformation has been suggested [1,2].

Recent findings that the core and nonstructural 5A (NS5A) proteins of hepatitis C virus (HCV) induce the expression of interleukin (IL)-8 gene *in vitro* have suggested that chemokines may be

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involved in the progression of chronic hepatitis (CH) and the development of liver cirrhosis (LC) and hepatocellular carcinoma (HCC) during the course of persistent HCV infection [3]. Thus, in this study we examined a possible correlation of serum concentrations of three chemokines, IL-8, monocyte chemoattractant protein-1 (MCP-1) or macrophage inflammatory protein-1 alpha (MIP-1α), detected by a sandwich enzyme-linked immunosorbent assay (ELISA) system with the severity of chronic hepatitis C. The results suggest that IL-8 production is enhanced progressively with escalating severity of liver disease and the development of HCC.

2. Materials and methods

2.1. Patients

The patients in this study included 30 cases of CH, 29 cases of LC and 30 cases of HCC, who had been attending in Kanazawa University Hospital from April, 1999 to April, 2000. Participants eligible for the study were anti-HCV antibody negative, between 20 and 80 years of age. All the patients were positive for anti-HCV antibody and serum HCV RNA was quantitated with the Amplicore HCV Monitor, version 2. In addition, 17 patients without chronic liver disease and also negative for anti-HCV antibody were enrolled as controls. All studied patients were negative for both hepatitis B surface antigen (HBsAg), HIV and alcoholic liver disease. In order to exclude the effects of inflammation other than liver diseases in our analyses, control subjects were selected with white blood cell (WBC) counts and C-reactive protein (CRP) values within normal range. There were significant differences in age, platelet count, alanine transaminase (ALT) activity and hepaplastin test (HPT) values among the four groups, i.e., CH, LC, HCC and control. These findings were considered to reflect differences in the pathological states among the groups (Table 1). This study was approved by the local ethics committee, and patients gave consent for the use of samples in these experiments.

2.2. Sandwich ELISA for IL-8, MCP-1 and MIP-1α

Serum concentrations of IL-8, MCP-1 and MIP-1α were determined by sandwich ELISA [4,5]. Each well in 96-well plates was coated with 100 μl of either anti-IL-8, anti-MCP-1 or anti-MIP-1α monoclonal antibody overnight at 4 °C. The wells were then treated with blocking solution (1% BSA-PBS) for 1 h at 37 °C. Serum samples were diluted with Tween-PBS containing 0.5% BSA and 100 μl of the samples were added to the wells and incubated at overnight 4 °C. Then, 100 μl of rabbit polyclonal antibodies against each of the chemokines (1 μg/ml) was added to the wells and the plates were incubated for 2 h at 37 °C. Thereafter, alkaline phosphatase conjugated anti-rabbit IgG was added to the wells and the plates were incubated for 2 h at 37 °C. Finally, 1 M diethanolamine (pH 9.8) containing 1 mg/ml *p*-nitrophenyl phosphate was added and the optical density of each well at 405 nm was measured using a microplate reader.

2.3. Criteria for clinical and pathological study

Serum chemokine concentrations were compared with the severity of chronic hepatitis C, macroscopic stages of HCC and the survival periods of the patients. Pathological classification of HCC was performed using general criteria for the clinical and pathological study of primary HCC [6].

2.4. Immunohistochemistry

Paraffin embedded sections of liver tissues were immunostained with mouse monoclonal IgG antibody against IL-8 at dilutions of 1:20 as described previously [7,8]. Then, horseradish peroxidase-labeled anti-mouse IgG

Table 1
Clinical characteristics of patients studied

	CH ($n = 30$)	LC ($n = 29$)	HCC ($n = 30$)	Control ($n = 17$)	*P*
Age (year)	48.8 ± 11.1	58.0 ± 8.4	66.6 ± 6.0	58.6 ± 15.6	<0.01*
Sex (M/F)	23/7	18/11	18/12	12/5	NS[a]
WBC (/μl)	5050 ± 1560	4360 ± 1470	3940 ± 1630	5760 ± 1850	NS*
Plt ($\times 10^4$/μl)	17.3 ± 5.1	9.9 ± 4.7	10.3 ± 5.1	20.7 ± 6.8	<0.01*
CRP (mg/dl)	0.12 ± 0.14	0.20 ± 0.45	0.80 ± 1.60	0.30 ± 0.56	NS*
ALT (IU/l)	100.0 ± 82.8	76.6 ± 52.8	83.7 ± 104.6	25.0 ± 19.0	<0.05*
HPT (%)	79.8 ± 11.4	70.1 ± 15.6	60.3 ± 14.6	92.5 ± 32.8	<0.01*

Note. Results are expressed as means ± SD.

Abbreviations: WBC, white blood cell; Plt, platelet; CRP, C-reactive protein; ALT, alanine transaminase; HPT, hepaplastin test; CH, chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma; NS, not significant.

[a] Fisher's exact test.

* Kruskal–Wallis test.

(Vector Laboratories, Inc., Burlingame, CA), was added and incubated. Immunocomplexes were detected with diaminobenzidine (Sigma Chemical Co, St. Louis, MO).

2.5. Statistical analysis

Differences between groups were analyzed for statistical significance using one-way ANOVA and the Mann–Whitney U test. Qualitative variables were compared by means of Fisher's exact test. Factors significantly associated with the progression of liver disease were determined by multivariate logistic regression analysis. All tests were two-tailed, and a P-value of <0.05 was considered statistically significant.

3. Results

3.1. Serum chemokine levels in patients with chronic hepatitis C

Correlation of serum chemokine levels with the severity of chronic liver disease was examined in patients with chronic hepatitis C (Fig. 1). The detection limits of our ELISA systems for IL-8, MCP-1 and MIP-1α were 10, 40 and 10 pg/ml, respectively. Serum IL-8 levels were elevated progressively as the disease severity increased escalated: control group, 17.43 ± 1.11 pg/ml; CH group, 18.75 ± 2.32 pg/ml; LC group, 32.12 ± 3.80 pg/ml; and HCC group 49.13 ± 11.03 pg/ml ($P < 0.01$). In contrast, there was no correlation between serum MCP-1 concentrations and the severity of chronic hepatitis C: control group, 209.56 ± 26.33 pg/ml; CH group, 219.22 ± 54.55 pg/ml; LC group, 192.75 ± 59.52 pg/ml; and HCC group, 302.67 ± 44.52 pg/ml ($P = 0.057$). In addition, serum MIP-1α levels did not correlate with disease severity: control group, 21.26 ± 9.26 pg/ml; CH group, 27.83 ± 14.57 pg/ml; LC group, 17.99 ± 6.63 pg/ml; and HCC group, 28.37 ± 7.95 pg/ml ($P = 0.051$). The data suggest that IL-8 production may be induced in the process of disease progression in chronic HCV infection.

3.2. Serum IL-8 levels in patients with HCC classified according to the severity liver damage

Since serum IL-8 levels were high in the HCC group, as shown in Fig. 1, we examined whether there were any differences in serum IL-8 concentrations among patients with varying stages of HCC. Namely, we evaluated the correlation between serum IL-8 levels and the degree of liver damage, which reflects the hepatic reserve in HCC patients: liver damage A, 29.98 ± 6.59 pg/ml; liver damage B, 58.80 ± 35.37 pg/ml; and liver damage C, 81.54 ± 25.11 pg/ml. There was a tendency for increased values with the progression of liver damage, although this effect was not significant.

3.3. Serum IL-8 levels in patients with HCC classified according to macroscopic staging

A correlation between the macroscopic staging of HCC and serum IL-8 levels was examined (Fig. 2): stage I, 33.10 ± 10.79 pg/ml; stage II, 46.12 ± 20.93 pg/ml; stage III, 16.27 ± 5.36 pg/ml; stage IV-A, 41.25 ± 12.86 pg/ml; and stage IV-B, 153.20 ± 47.22 pg/ml. Serum IL-8 values of patients in stage IV-B were significantly higher than those of patients in other stages. Thus, patients with advanced HCC accompanying remote metastasis (stage IV-B) were found to show elevated IL-8 levels, compared with patients without remote metastasis. In addition, we observed the elevation of IL-8 following the detection of HCC bone metastasis in two cases of stage IV-B whose serial samples were preserved (Fig. 3), suggesting that serum levels of IL-8 may directly reflect the onset of HCC remote metastasis.

3.4. Serum IL-8 levels in patients with HCC classified according to survival periods

When a correlation between serum IL-8 levels and the survival periods of patients with HCC was evaluated, patients with poor prognosis gave significantly higher val-

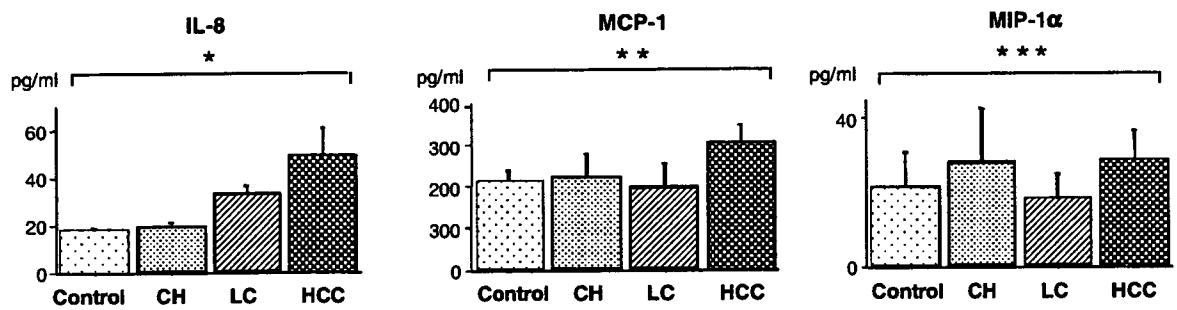

Fig. 1. Serum chemokine levels in patients with chronic hepatitis C, including 30 cases of chronic hepatitis (CH), 29 cases of liver cirrhosis (LC), 30 cases of hepatocellular carcinoma (HCC) and 17 controls. Serum IL-8 levels were elevated with the progression of disease: *F: 4.63, $P < 0.01$ when compared by analysis of ANOVA. Serum MCP-1 and MIP-1α concentrations were not correlated with disease severity: **F: 0.99, $P = 0.057$; ***F: 0.27, $P = 0.051$, respectively.

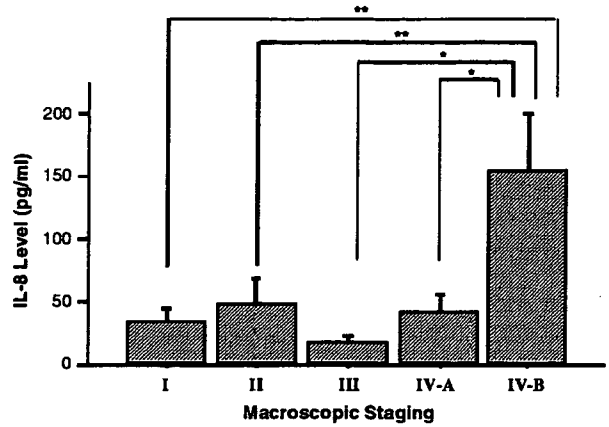

Fig. 2. Serum IL-8 levels in patients with HCC classified according to macroscopic staging. Stage I ($n = 3$) of macroscopic stage by the Liver Cancer Study Group of Japan; T1, N0, M0. Stage II ($n = 4$); T2, N0, M0. Stage III ($n = 7$); T3, N0, M0 or T1-3, N1, M0. Stage IV-A ($n = 12$); T4, N0-1, M0. Stage IV-B ($n = 4$); T1-4, N0-1, M1. In the HCC patients, IL-8 concentrations were positively correlated with macroscopic stages. F: 5.51, $P < 0.01$ when compared by analysis of ANOVA. $^{*}P < 0.01$ when compared by Mann–Whitney U test. $^{**}P < 0.05$ when compared by Mann–Whitney U test.

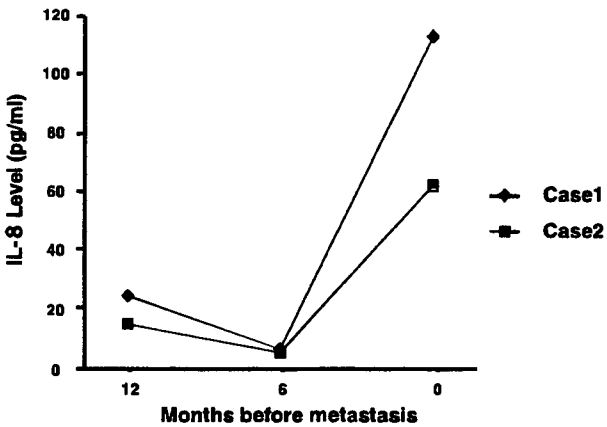

Fig. 3. Course of serum IL-8 levels in patients with HCC accompanying remote metastasis. Both cases 1 and 2 show the elevation of IL-8 following the detection of HCC bone metastasis.

ues (Fig. 4): over a 1-year survival period, 22.05 ± 5.90 pg/ml; over 3 months but less than 1 year, 64.34 ± 19.78 pg/ml; and less than 3 months, 132.72 ± 54.16 pg/ml. Furthermore, we performed multivariate logistic regression analysis between the prognosis of patients with HCC, and their ages, platelet counts, prothrombin times (PT), albumin levels, alpha-fetoprotein (AFP), IL-8 levels and the presence or absence of ascites (Table 2). The results indicated that AFP was not a factor that determined the prognosis, but IL-8 concentration was found to be an independent risk factor for a poor prognosis, as well as platelet count and serum albumin concentration elevated.

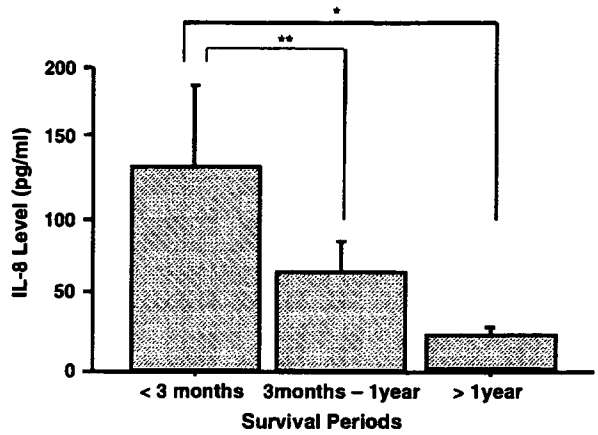

Fig. 4. Serum IL-8 levels in patients with HCC classified according to survival periods, <3 months ($n = 3$), 3 months–1 year ($n = 12$) and >1 year ($n = 16$). IL-8 concentrations were inversely correlated with the length of the survival periods. F: 6.40, $P < 0.01$ when compared by analysis of ANOVA. $^{*}P < 0.01$ when compared by Mann–Whitney U test. $^{**}P < 0.05$ when compared by Mann–Whitney U test.

3.5. IL-8 expression in liver cells

To identify IL-8 producing cells in the liver, an immunohistochemical analysis of liver tissues was performed. IL-8 was strongly stained in the cytoplasm of HCC cells, was weakly stained in the cytoplasm of some hepatocytes in LC, and was undetectable in hepatocytes from control tissue (Fig. 5). The data indicated that IL-8 is produce upon the malignant transformation of hepatocytes.

4. Discussion

The current study demonstrates that of the three chemokines, IL-8, MCP-1 and MIP-1α, determined by ELISA in patients with chronic hepatitis C, serum concentrations of IL-8 alone were increased, correlating with the progression of liver disease. Notably, the levels of IL-8 were significantly increased in patients with advanced HCC with remote metastasis and IL-8 levels were elevated in patients with poor prognoses. Interestingly, immunohistochemical analysis showed that IL-8 was detectable mainly in the cytoplasm of HCC cells. These findings suggest that the expression of IL-8 may be augmented upon the malignant transformation of hepatocytes during the course of chronic HCV infection.

IL-8 is known to be closely associated with pathological states of CH through the activation of inflammatory cells such as granulocytes or T lymphocytes. The levels of IL-8 were elevated in

Table 2
Characteristics of patients with HCC classified according to survival periods

	>3 months ($n = 3$)	3 months–1 year ($n = 12$)	<1 year($n = 16$)	Logistic regression	
				Regression coefficient	P^*
Age (year)	68.7 ± 3.8	68.3 ± 6.0	65.0 ± 6.3	3.067	0.2157
Plt ($\times 10^4/\mu l$)	9.3 ± 3.7	11.4 ± 6.1	9.6 ± 4.7	6.737	0.0344
PT (s)	14.3 ± 1.7	12.5 ± 1.5	12.2 ± 1.5	1.862	0.3942
Alb (mg/dl)	3.4 ± 1.0	3.4 ± 0.5	3.8 ± 0.9	9.013	0.0110
AFP (ng/ml)	57600 ± 57600	29300 ± 28900	252 ± 137	1.593	0.4509
IL-8 (pg/ml)	132.7 ± 54.2	64.3 ± 21.6	22.1 ± 5.9	10.196	0.0061
Ascites	2/3	2/12	2/16	0.003	0.9984

Note. Results are expressed as means ± SD.
Abbreviations: Plt, Platelet; PT, prothrombin time; Alb, albumin; AFP, alpha-fetoprotein; IL-8, interleukin-8.
* Kruskal–Wallis test.

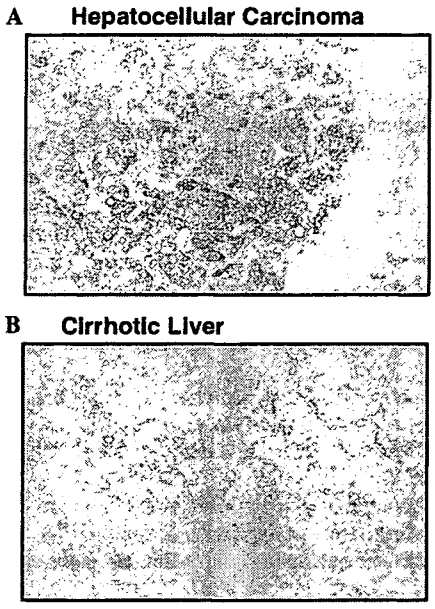

Fig. 5. Immunohistochemical analysis of liver tissues stained with the anti-IL-8 antibody. Five surgically resected HCC tissues were examined, in three cases IL-8 was strongly stained in the cytoplasm of HCC cells. A representative case of HCC stage II was shown in (A), whose serum IL-8 level was 28 pg/ml. IL-8 was weakly expressed in the cytoplasm of some hepatocytes in the cirrhotic liver (B) and was undetectable in hepatocytes of the control tissues (not shown). Original magnification 200×.

patients with acute and chronic liver damages, e.g., viral hepatitis, autoimmune hepatitis or alcohol-induced liver dysfunction [9–14]. Moreover, it has been reported that there is a correlation between IL-8 levels and the severity of liver disorders, including HCV infection [15–18]. Recently it has also been reported that the core and NS5A proteins of HCV induce the expression of the IL-8 gene [3], and that serum IL-8 levels in chronic hepatitis C patients are associated with resistance to interferon treatment [19], suggesting that IL-8 plays an important role in the maintenance of persistent infection with HCV. In the current study, serum IL-8 levels increased as the disease progressed from CH to LC and further to HCC, suggesting that the increase may be due not only to immune response against persistent HCV infection but to the development of HCC.

There has been no report, to our knowledge, investigating the possible correlation between serum MCP-1 or MIP-1α level and the pathology of chronic liver disease. The present results did not indicate a significant correlation between them. Therefore, we conclude that persistent HCV infection may not increase serum MCP-1 or MIP-1α levels.

Among the many chemokines, IL-8 level has been reported to show a tendency to increase during the progression of cancers of the stomach [20], pancreas [21], lung [22] and prostate [23]. In patients with HCC, IL-8 was shown to be expressed in the cytoplasm of hepatoma cells and in vascular endothelial cells of tumors [7,24,25], suggesting that the angiogenetic activity of IL-8 may contribute to the growth of HCC. In addition, we have observed that neither of the two IL-8 receptors, CXC chemokine receptor (CXCR) 1 or CXCR2, is detectable in HCC cell lines or tissues [5], suggesting that the growth promotion of HCC cells by IL-8 may be an indirect effect. IL-8 has the capacity to recruit various inflammatory cells that eventually produce proinflammatory cytokines including IL-1. Recently, we found that IL-1 enhances the production of CC chemokine ligand 3 (CCL3) which may interact with the CC chemokine receptor (CCR) 1 on HCC cells and contribute to tumor pro-

gression [5]. Moreover IL-8 levels were reported to be correlated with the growth of breast cancer cells having a high metastatic activity [26]. In line with these observations, our study similarly indicated that serum IL-8 levels were highly elevated in patients with HCC accompanied by remote metastasis (stage IV-B). Furthermore, we observed that serum IL-8 values rose following the detection of HCC bone metastasis in two cases. These findings suggest that IL-8 may promote the attachment and growth of cancer cells at extrahepatic sites. Moreover, IL-8 levels in cervical cancer tissues were shown to be correlated with the prognosis of patients [27]. Otherwise, IL-8 levels can simply correlate with overall tumor burden at advanced stages of HCC. Consistent with these observations, our study also showed that serum IL-8 levels increased significantly in patients with poor prognoses and whose survival periods were less than 1 year, as compared with patients with better prognoses. When we performed multivariate regression analyses of possible prognosis factors, IL-8, as well as platelet counts and albumin levels, was found to be a significant factor. These findings suggest that serum IL-8 levels can be a marker predicting the prognosis of patients with HCC.

This study indicates that IL-8 may be involved in the progression of chronic hepatitis C and the development of HCC. There is a report indicating the significant correlations of IL-8 levels with tumor size and disease stage in chronic hepatitis B as well [28], suggesting that IL-8 may be a useful biological marker of HCC invasiveness and a prognostic factor for HCC patients. The molecular biological mechanisms explaining these findings remain to be clarified in the future by using HCC cell lines or animal models.

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References

[1] F. Melchers, A.G. Rolink, C. Schaniel, The role of chemokines inregulating cell migration during humoral immune responses, Cell 99 (1999) 351–354.

[2] J.J. Campbell, E.C. Butcher, Chemokines in tissue-specific and microenvironment-specific lymphocyte homing, Curr. Opin. Immunol. 12 (2000) 336–341.

[3] S.J. Polyak, K.S. Khabar, D.M. Paschal, et al., Hepatitis C virus nonstructural 5 A protein induces interleukin-8, leading to partial inhibition of the interferon-induced antiviral response, J. Virol. 75 (2001) 6095–6106.

[4] H. Yokoyama, T. Wada, K. Furuichi, et al., Urinary levels of chemokines(MCAF/MCP-1, IL-8) reflect distinct disease activities and phases human IgA nephropathy, J. Leukoc. Biol. 63 (1998) 493–499.

[5] P. Lu, Y. Nakamoto, Y. Memoto-Sasaki, et al., Potential interaction between CCR1 and its ligand, CCL3, induced by Endogenously produced interleukin-1 in human. hepatoma, Am. J. Pathol. 162 (2003) 1249–1258.

[6] Liver cancer study group of Japan. The general rules for the clinical and pathological study of primary liver cancer, Jpn. J. Surg. 19 (1989) 98–129.

[7] A. Iguchi, I. Kitajima, M. Yamakuchi, et al., PEA3 and AP-1 are required for constitutive IL-8 gene expression in hepatoma cells, Biochem. Biophys. Res. Commun. 279 (2000) 166–179.

[8] Y. Takahashi, T. Kasahara, T. Sawai, et al., The participation of IL-8 in the synovial lesion at an early stage of rheumatoid arthritis, J. Exp. Med. 188 (1999) 75–87.

[9] D.B. Hill, Increased plasma interlukin-8 concentration in alcoholic hepatitis, Hepatology 18 (1993) 576–580.

[10] N. Sheron, G. Bird, J. Koskinas, et al., Circulation and tissue levels of the neutrophil chemotaxin interlukin-8 are elevated in severe acute alcoholic hepatitis, and tissue levels correlate with neutrophil infiltration, Hepatology 18 (1993) 41–46.

[11] J. Napoli, G.A. Bishop, G.W. McCaughan, Increased intrahepatic messenger RNA expression of interleukin 2, 6, and 8 in human cirrhosis, Gastroenterology 107 (1994) 789–798.

[12] J. Maggiore, F. Benedetti, M. Massa, P. Pignatti, A. Martini, Circulating levels of interlukin-6, interlukin-8, and tumor necrosis factor-alpha in children with autoimmune hepatitis, J. Pediatr. Gastroenterol. Nutr. 20 (1995) 23–27.

[13] A. Al-Wabel, B. al-Knawy, S. Raziuddin, Interleukin-8 and granulocytemacrophage colony-stimulating factor secretion in hepatocellular carcinoma and viral chronic active hepatitis, Clin. Immunol. Immunopathol. 74 (1995) 231–235.

[14] T. Masumoto, K. Ohkubo, K. Yamamoto, et al., Serum IL-8 levels and localization IL-8 in liver from patients with chronic viral hepatitis, Hepatogastroenterology 45 (1998) 630–634.

[15] P.J. Scheuer, P. Ashrafzadeh, S. Sherlok, D. Brown, G.M. Dusheiko, The pathology of hepatitis C, Hepatology 15 (1992) 567–571.

[16] G. Kaplanski, C. Franarier, M.J. Payan, P. Bongernd, J.M. Durand, Increased levels of soluble adhesion molecules in the serum of patients with hepatitis C, Dig. Dis. Sci. 42 (1997) 2277–2284.

[17] K. Shimoda, M.A. Begum, K. Shibuta, M. Mori, H.L. Bonkovsky, B.F. Banner, G.F. Barnard, Interleukin-8 and hIRH(SDFI-α/PBSF) mRNA expression and histological activity index in patients with chronic hepatitis C, Hepatology 28 (1998) 108–115.

[18] M.G. Neuman, J.P. Benhamou, I.M. Malkiewicz, et al., Cytokines as predictors for sustained response and as

markers for immunomodulation in patients with chronic hepatitis C, Clin. Biochem. 34 (2001) 173–182.
[19] S.J. Polkak, K.S.A. Khabar, M. Rezeiq, D.R. Gretch, Elevated levels of interlukin-8 in serum are associated with hepatitis C virus infection and resistance to interferon therapy, J. Virol. 75 (2001) 6209–6211.
[20] Y. Kitadai, K. Haruma, N. Mukaida, et al., Regulation of Disease-Progression genes in human gastric carcinoma cell by interleukin 8, Clin. Cancer Res. 6 (2000) 2735–2740.
[21] M. Miyamoto, Y. Shimizu, K. Okada, Y. Kashi, K. Higuchi, A. Watanabe, Effect of interleukin-8 on producing of tumor-associated substances and autocrine growth of human liver and pancreatic cancer cells, Cancer Immunol. Immunother. 47 (1998) 47–57.
[22] R.M. Striter, P.J. Polverini, D.A. Arenberg, et al., Role of C-X-C chemokines as regulators of angiogenesis in lung cancer, J. Leukoc. Biol. 57 (1995) 752–762.
[23] G.F. Greene, Y. Kitadai, C.A. Pettaway, A.C. Von Eschenbach, C.D. Bucana, I.J. Fidler, Correlation of metastasis-related gene expression with metastatic potential in human prostate carcinoma cells implanted in nude mice using an in situ messenger RNA hybridization technique, Am. J. Pathol. 150 (1997) 1571–1582.
[24] K.F. Yoong, S.C. Afford, R. Jones, et al., Expression and function of CXC and CC chemokines in human malignant liver tumor: a role for human monokine induced by gamma-interferon in lymphocyte recruitment to hepatocellular carcinoma, Hepatology 30 (1999) 100–111.
[25] J. Akiba, H. Yano, S. Ogasawara, K. Higake, M. Kojiro, Expression and function of interleukin-8 in human hepatocellular carcinoma, Int. J. Oncol. 18 (2000) 257–264.
[26] E.J. De Larco, R.K.B. Wuertz, A.K. Rosner, et al., A potential role for interleukin-8 in the metastatic phenotype of breast cartinoma cells, Am. J. Pathol. 158 (2001) 639–646.
[27] J. Fujimoto, H. Sakaguch, I. Aoki, T. Tamaya, Clinical implication of expression of interleukin 8 related to angiogenesis in uterine cervical cancer, Cancer Res. 60 (2000) 2632–2635.
[28] Y. Ren, R.T. Poon, H.T. Tsui, et al., Interleukin-8 levels in Patients with Hepatocellular carcinoma: correlation with Clinicopathological features and prognosis, Clin. Cancer Res. 9 (2003) 5996–6001.

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Impact of diabetes mellitus on prognosis of patients infected with hepatitis C virus

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Abstract

Diabetes is a risk factor for the progression of liver fibrosis and development of hepatocellular carcinoma in chronic hepatitis C. However, the impact of diabetes on the long-term prognosis and the synergistic interactions of various host factors for diabetes to the progression of liver fibrosis are unknown. In the present study, we examined the host factors associated with the progression of hepatitis C in 68 patients with a posttransfusion hepatitis (PTH) and analyzed the relationships. Multivariate analysis showed that age of PTH, being male, and type 2 diabetes mellitus were risk factors for the progression of liver fibrosis. By the Kaplan-Meier method, the cirrhosis-free survival rates after the onset of PTH were significantly lower in the diabetic group than in the nondiabetic group ($P < .01$). Diabetes also had a great impact on the long-term prognosis of chronic hepatitis C by reducing the time from PTH to the occurrence of hepatocellular carcinoma ($P < .01$) and to liver-related death ($P < .05$). Coexistence of obesity (body mass index ≥ 25 kg/m^2) or hypertriglyceridemia (≥ 150 mg/dL) with diabetes had a synergistic effect on liver fibrosis progression in patients with chronic hepatitis C. Thus, the treatment of diabetes, obesity, and hypertriglyceridemia may hold the key to improving the prognosis of chronic hepatitis.

1. Introduction

Chronic infection with hepatitis C virus (HCV) is the leading cause of liver damage. Persistent chronic liver damage eventually progresses from chronic hepatitis to cirrhosis and to hepatocellular carcinoma (HCC) [1-3]. Previous studies have reported that host factors contributing to the progression of chronic hepatitis C to liver fibrosis are age at onset [4,5], sex [5,6], race [7,8], alcohol consumption [9,10], smoking [11], hepatitis B virus coinfection [12,13], HIV coinfection [14,15], complication by hemochromatosis [16], nonalcoholic steatohepatitis [17], schistosomiasis [18] and human leukocyte antigen haplotypes [19].

On the other hand, recent studies have reported that in addition to these host-related factors, the development of diabetes or obesity as a complication is a risk factor for the progression of liver fibrosis and development of HCC in chronic hepatitis C [20-24]. In addition, insulin resistance has been reported frequently in chronic hepatitis C [25]. Recently, Fartoux et al [26] have reported that, through steatosis, insulin resistance is associated with liver fibrosis in chronic hepatitis. However, previous studies were mainly aimed at finding factors related to the degree of liver fibrosis in chronic hepatitis C. Therefore, no studies have sufficiently examined the effects of these factors associated with liver fibrosis on the long-term prognosis, that is, the development not only of cirrhosis and HCC from HCV infection but also of liver-related death. Moreover, synergistic interactions of these factors to the progression of liver fibrosis are still unknown.

In this study, we examined the effects of diabetes and the synergistic factors on the prognosis of HCV infection in patients with a clear onset of posttransfusion hepatitis (PTH).

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2. Methods

2.1. Patients

Fig. 1 shows the design of this study. Of the 839 patients who were admitted to Kanazawa University Hospital and diagnosed with chronic hepatitis C between January 1990 and April 2004, 87 were found to have developed PTH at a definite age on close history taking. These 87 patients were followed periodically for 2 to 46 years with a mean of 20.3 years from the time of the first examination to December 2004. Of these patients, 33 received interferon therapy during the follow-up; and 19 of them achieved a complete response with the disappearance of HCV. Of the 87 patients whose age at onset was known, 68 were included in the study, excluding the 19 patients with a complete response to interferon. Informed consent was obtained from each patient included in the study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

2.2. Diagnosis of HCV infection and laboratory testing

Blood samples were tested for hepatitis B surface antigen and anti-hepatitis C virus antibodies by commercial immunoassays (Fuji Rebio, Tokyo, Japan). Hepatitis C virus infection was diagnosed by positive serum anti-hepatitis C virus antibodies and liver biopsy histology. The stage of fibrosis was evaluated according to the criteria of Desmet et al [27]. At the first examination, fasting serum lipid levels (total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglycerides), glycated hemoglobin (HbA_{1c}), aspartate aminotransferase (AST), alanine aminotransferase (ALT), platelet count, total bilirubin, albumin, prothrombin time, and indocyanine green (ICG) excretion were measured.

2.3. Variables examined

In all 68 patients, the age at onset of PTH, cirrhosis, and HCC, and the age at liver-related death were examined. At the first examination, height, body weight, body mass index (BMI), and the presence or absence of complicating diabetes or hyperlipidemia were examined. *Posttransfusion hepatitis* was defined as hepatitis in which liver function tests showed serum ALT levels to be elevated to more than 2.5 times the reference range between 1 week and 6 months after transfusion. Cirrhosis was diagnosed by histopathologic examination of liver biopsy specimens in 16 of 68 patients. In the remaining patients, cirrhosis was diagnosed by a combination of clinical features of portal hypertension (splenomegaly, ascites, and esophageal varices), biochemical evidence of hepatic failure (percentage of prothrombin time <70%, total bilirubin >2.5 mg/dL, albumin <3.5 g/dL), and abdominal ultrasound and computed tomographic (CT) findings. Hepatocellular carcinomas were detected by imaging modalities such as ultrasound scanning, dynamic CT scanning, magnetic resonance imaging, and abdominal arteriography. Hepatocellular carcinoma was diagnosed by angiographic demonstration of typical hypervascular tumor staining, as well as by typical findings on dynamic CT, such as hyperattenuation areas in the early phase and hypoattenuation areas in the late phase [28]. Liver-*related death* was defined as that associated with liver failure, rupture of esophageal varices, or HCC. To exclude diabetes secondary to cirrhosis, *type 2 diabetes mellitus* was defined as that with a fasting blood glucose level of 126 mg/dL or higher, or with a 2-hour blood glucose level of 200 mg/dL or higher in a 75-g oral glucose tolerance test and an *insulinogenic index*, which is defined as (insulin at 30 min − fasting insulin)/(glucose at 30 min − fasting glucose), of less than 0.4. *Obesity* was defined as a BMI of 25 kg/m^2 or higher, which is defined by the Japan Society for the Study of Obesity, at the first examination. *Hypertriglyceridemia* was defined as a fasting triglyceride level of 150 mg/dL or higher at the first examination. *Hypo–high-density lipoprotein (HDL) cholesterolemia* was defined as a fasting HDL cholesterol level of 40 mg/dL or lower at the first examination.

2.4. Statistical analysis

All serial data were expressed as means ± standard deviations. To identify variables influencing the disease-free survival rate in the period from the onset of PTH to the diagnosis of cirrhosis (*freedom from disease* refers to the absence of a diagnosis of cirrhosis or HCC up to the end of the follow-up or the nonoccurrence of liver-related death), the possibility of type 2 diabetes mellitus, obesity, and hyperlipidemia (hypercholesterolemia, hypertriglyceridemia) being involved was examined by regression analysis using a Cox proportional hazard model. Results of regression analysis were considered significant at $P < .05$ for a given hazard ratio with a 95% confidence interval (CI). Student t test was used to compare initial blood test results between the type 2 diabetes mellitus and nondiabetes groups. Disease-free survival rates in the period from the onset of PTH to the diagnosis of cirrhosis or HCC and to liver-related death were estimated by the Kaplan-Meier method. The influence of type 2 diabetes mellitus on the prognosis of chronic hepatitis C was investigated using the Breslow-Gehan-Wilcoxon

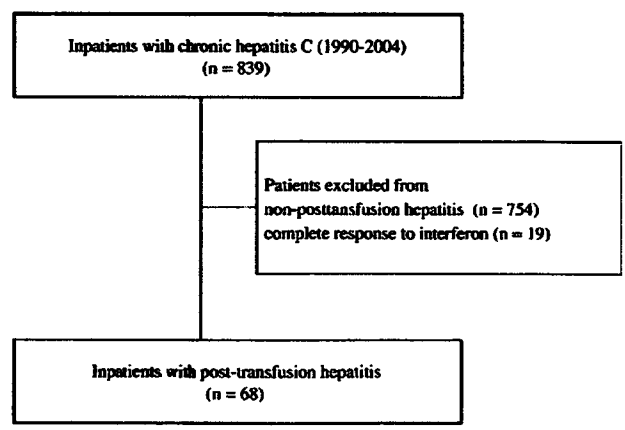

Fig. 1. Study design.

Table 1
Patient characteristics

Sex (male/female)	49/19
Age at onset of PTH (y)	34.6 ± 14.7
IFN therapy (+/−)	14/54
Type 2 diabetes mellitus (+/−)	40/28
BMI (kg/m^2)	23.2 ± 3.5
Obesity (+/−)	17/51
Hypercholesterolemia (+/−)	4/64
Hypertriglyceridemia (+/−/ND)	11/56/1
Hypo-HDL cholesterolemia (+/−/ND)	23/33/12
Alcohol 80 g/d (+/−)	11/57
Diagnosis of liver cirrhosis (+/−)	42/26
Diagnosis of HCC (+/−)	26/42
Occurrence of liver-related death (+/−)	22/46

IFN indicates interferon; ND, not determined.

method. Patients who were diagnosed as being complicated by cirrhosis or HCC at the first examination were analyzed on the assumption that the period from the onset of PTH to the first examination is the disease-free period.

3. Results

3.1. Study population

As shown in Fig. 1, 68 patients with chronic hepatitis C were finally analyzed. The patient characteristics of this group are shown in Table 1. The 68 patients consisted of 49 men and 19 women, with a mean age of 34.6 years at the onset of PTH. Of these patients, 40 were diagnosed as having diabetes as a complication in the period from the onset of PTH to this study; and 28 were not complicated by diabetes. Of 40 patients diagnosed as having diabetes, 31 patients were diagnosed for liver cirrhosis. In the patients, 25 of 31 had been diagnosed for diabetes before they were diagnosed for liver cirrhosis. The mean BMI at the first examination was 23.2 kg/m^2. When obesity was defined as a BMI of 25 kg/m^2 or higher, 17 of the 68 patients were obese. Four patients had hypercholesterolemia, 11 patients had hypertriglyceridemia, and 23 patients had hypo-HDL cholesterolemia. Of the 68 patients, 42 were diagnosed with cirrhosis; and 26 were complicated by HCC. Liver-related death occurred in 22 of the 68 patients between the onset of PTH and the present study. The overall median duration of disease progression to cirrhosis and HCC was 20 and 22 years, respectively.

Table 2
Factors influencing the progression from PTH to cirrhosis

Variables	Hazard ratio	95% CI	P
Age at onset of PTH ≥35 y	4.691	2.408-9.140	.001
Sex male	1.269	0.652-2.472	.483
Hypertension	1.378	0.632-2.472	.420
Type 2 diabetes mellitus	2.906	1.377-6.131	.005
Fasting plasma glucose	1.005	1.001-1.009	.007
HOMA-IR	1.065	0.962-1.179	.228
HbA_{1c} (%)	1.211	1.083-1.354	.001
Obesity	2.693	1.371-5.292	.004
BMI	1.106	1.010-5.476	.030
Hypercholesterolemia	2.728	0.408-1.735	.088
Hypertriglyceridemia	2.641	1.274-5.476	.009
Low-HDL cholesterolemia	0.842	0.408-1.735	.640
AST ≥80 IU/L	1.181	0.625-2.225	.608
ALT ≥80 IU/L	0.713	0.354-1.437	.345
Alcohol ≥80 g/d	1.087	0.519-2.275	.825

HOMA-IR indicates homeostasis model assessment of insulin resistance.

Table 3
Factors influencing the progression from PTH to cirrhosis

	Multivariate analysis		
Variables	Hazard ratio	95% CI	P
Age at onset of PTH ≥35 y	24.542	6.329-95.172	.001
Sex male	8.264	1.962-33.333	.004
Type 2 diabetes mellitus	8.395	2.234-31.541	.002
Obesity	2.168	0.809-5.814	.124
Hypertriglyceridemia	0.257	0.065-1.019	.053
AST ≥80 IU/L	1.473	0.439-4.939	.530
ALT ≥80 IU/L	0.419	0.115-1.528	.188
Alcohol >80 g/d	1.124	0.360-3.512	.841

3.2. Variables associated with progression of liver fibrosis in patients with chronic hepatitis C

Host factors having influence on liver fibrosis during the transition period from PTH to cirrhosis were evaluated by univariate and multivariate analysis. By univariate analysis, the following factors were identified as significantly contributing to the progression of liver fibrosis: onset of PTH at age 35 years or older, type 2 diabetes mellitus as a complication, high fasting plasma glucose, high HbA_{1c}, obesity (BMI ≥25 kg/m^2), high BMI, and hypertriglyceridemia (Table 2). By multivariate analysis, the following factors were identified as significantly contributing to the progression of liver fibrosis: onset of PTH at age 35 years or older, being male, and type 2 diabetes mellitus as a complication (Table 3).

3.3. Diabetes as a risk factor for progression of liver fibrosis

Disease-free survival rates in the period from PTH to cirrhosis in the diabetic and nondiabetic groups were estimated by the Kaplan-Meier method. Posttransfusion hepatitis progressed to cirrhosis in a total of 42 patients, of whom 30 (71.4%) were complicated by diabetes but 12 (28.6%) were not. Table 4 shows disease-free survival rates. The disease-free survival rates 10, 20, and 30 years after the onset of PTH were significantly lower in the diabetic group, at 85.0%, 50.0%, and 25.0%, respectively, than in the nondiabetic group, at 100%, 95.5%, and 43.6%, respectively ($P < .01$) (Table 4A).

Because HbA_{1c} was identified as a factor contributing to liver fibrosis by univariate analysis, the diabetic group was divided into a group with poor glycemic control (HbA_{1c} ≥7.0%) and a group with good glycemic control (HbA_{1c} <7.0%); and the disease-free survival rate was estimated by

Table 4
Disease-free survival rates from PTH to cirrhosis

		10 y	20 y	30 y
Disease-free survival rates for cirrhosis				
A:	DM(+) (n = 40)	85.0%	50.0%	25.0%
	DM(−) (n = 28)	100%	95.5%	43.6%*
B:	DM(+) HbA_{1c} ≥7.0 (n = 24)	95.8%	54.2%	33.3%
	DM(+) HbA_{1c} <7.0 (n = 15)	64.3%	35.7%	7.1%**
C:	DM(+) obesity(+) (n = 27)	72.4%	36.4%	18.2%
	DM(+) obesity(−) (n = 13)	94.2%	72.4%	31.2%*
D:	DM(+) TG(+) (n = 31)	57.1%	14.3%	0.0%
	DM(−) TG(−) (n = 7)	90.3%	50.8%	25.8%*

TG indicates hypertriglyceridemia.
* $P < .01$.
** $P < .05$.

the Kaplan-Meier method (Table 4B). The disease-free survival rates 10, 20, and 30 years after the onset of PTH were significantly lower in the group with poor glycemic control, at 64.3%, 35.7%, and 7.1%, respectively, than in the group with good blood glucose control, at 95.8%, 54.2%, and 33.3%, respectively ($P < .05$).

3.4. Synergistic effect of obesity or hypertriglyceridemia for liver fibrosis progression

Similar to glycemic control, obesity was identified as a significant factor contributing to liver fibrosis progression by univariate analysis. Therefore, disease-free survival rates for a combination of diabetes and obesity were estimated by the Kaplan-Meier method adjusted by sex and onset of PTH. The disease-free survival rates 10, 20, and 30 years after the onset of PTH were significantly lower in the obese diabetic group, at 72.7%, 36.4%, and 18.2%, respectively, than in the nonobese diabetic group, at 94.2%, 72.4%, and 31.2%, respectively ($P < .01$) (Table 4C).

In the same way, the disease-free survival rates were estimated by the Kaplan-Meier method in patients with a combination of diabetes and hypertriglyceridemia, which had been identified by univariate analysis as significant factors contributing to liver fibrosis progression. The disease-free survival rates 10, 20, and 30 years after the onset of PTH were significantly lower in the hypertriglyceridemic diabetic group, at 57.1%, 14.3%, and 0%, respectively, than in the nonhypertriglyceridemic diabetic group, at 90.3%, 50.8%, and 25.8%, respectively ($P < .01$) (Table 4D).

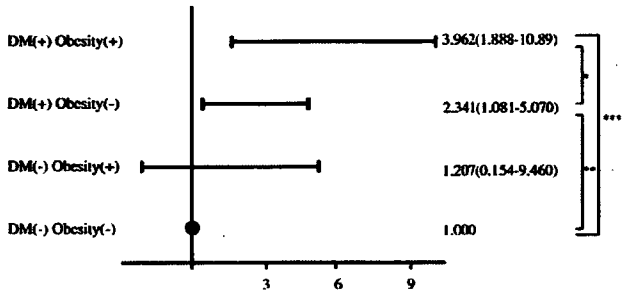

Fig. 2. Hazard ratio of diabetes complicated with obesity for progression to cirrhosis. *P = .013, **P = .031, and ***P = .007. DM indicates diabetes mellitus.

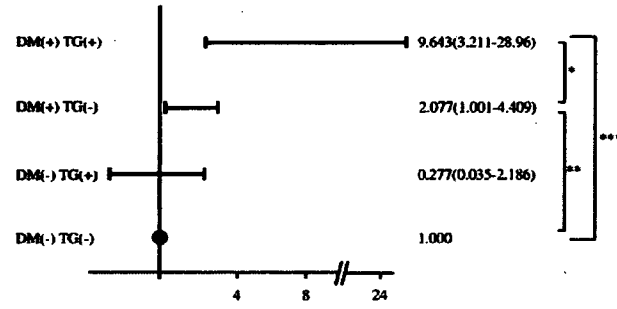

Fig. 3. Hazard ratio of diabetes complicated with hypertriglyceridemia for progression to cirrhosis. *P = .005, **P = .050, ***P = .001. TG indicates hypertriglyceridemia.

Combinations of obesity and diabetes or hypertriglyceridemia and diabetes as risk factors for progression to cirrhosis were analyzed using the Cox proportional hazard model adjusted by sex and onset of PTH. When the risk of nonobese nondiabetic patients was assumed to be 1, the hazard ratio of the nonobese diabetic patients was 2.341 (95% CI, 1.081-5.070; P = .031); and the hazard ratio of the obese diabetic patients was 3.962 (95% CI, 1.888-10.89; P = .007) (Fig. 2). When the risk of nonhypertriglyceridemic nondiabetic patients was assumed to be 1, the hazard ratio of the nonhypertriglyceridemic diabetic patients was 2.077 (95% CI, 1.001-4.409; P = .001); and the hazard ratio of the hypertriglyceridemic diabetic patients was 9.643 (95% CI, 3.211-28.96; P = .001) (Fig. 3).

3.5. Diabetes as a risk factor for HCC and liver-related death

In 26 of the 68 patients, HCC developed between the onset of PTH and the present study. We examined the influence of diabetes as a complication on the development of HCC from posttransfusion chronic hepatitis C. The disease-free survival rates 10, 20, and 30 years after the onset of PTH were significantly lower in the diabetic group, at 92.5%, 66.4%, and 40.9%, respectively, than in the nondiabetic group, at 100%, 95.5%, and 81.6%, respectively ($P < .01$) (Table 5A).

Liver-related death occurred in 22 of the 68 patients between the onset of PTH and the present study. We

Table 5
Disease-free survival rates from PTH to HCC and liver-related death

	10 y	20 y	30 y
A: Disease-free survival rates for HCC			
DM(+)	92.5%	66.4%	40.9%
DM(−)	100%	95.5%	81.6%*
B: Disease-free survival rates for liver-related death			
DM(+)	100%	84.1%	49.9%
DM(−)	100%	95.5%	75.8%**

* $P < .01$.
** $P < .05$.

examined the influence of diabetes as a complication on liver-related death after PTH. The survival rates 10, 20, and 30 years after the onset of PTH were significantly lower in the diabetic group, at 100%, 84.1% and 49.9%, respectively, than in the nondiabetic group, at 100%, 95.5% and 75.8%, respectively ($P < .05$) (Table 5B).

4. Discussion

In this study, we retrospectively examined the impact of diabetes as a complication on the natural course of chronic hepatitis C after HCV infection in 68 patients whose age at onset of PTH was known. The effects of diabetes on the long-term prognosis in the patients with HCV infection have not been well characterized because glucose intolerance including diabetes occurs when the liver disease is severe; and therefore, it is difficult to analyze the relationship. The liver is a key organ in glucose homeostasis. In the fasting state, normoglycemia is maintained by hepatic gluconeogenesis. Insulin suppresses hepatic glucose output by inhibiting gluconeogenesis and glycogenolysis. On the other hand, hepatic glucose uptake is generally considered to be passive and independent of insulin action. These are reasons why secondary diabetes due to severe hepatic diseases, such as hepatic failure or liver cirrhosis, is often characterized with relatively lower fasting plasma glucose levels due to the impaired hepatic reserve for gluconeogenesis and postprandial hyperglycemia due to the absolute reduction of liver mass. In contrast, type 2 diabetes mellitus is characterized by the impaired action of insulin to inhibit gluconeogenesis in the liver in the fasting state [29] and impaired early-phase insulin secretion after glucose challenge. In the present study, we focused on primary (type 2 diabetes mellitus) diabetes to examine whether it affects the prognosis of chronic hepatitis C or not. To clarify it, we defined *type 2 diabetes mellitus* as the disease showing high fasting glucose and impaired early-phase secretion of insulin (an insulinogenic index of less than 0.4). According to the criteria, 40 patients were diagnosed for diabetes. Thirty-one of 40 were diagnosed for liver cirrhosis. In the patients, 25 of 31 had been diagnosed for diabetes before they were diagnosed for liver cirrhosis. Therefore, in most cases (about 80%) in the present study, it does not mean that diabetes was caused by severe liver disease.

By univariate analysis, the following factors were identified as contributing to the progression of liver fibrosis after the onset of PTH: onset of PTH at age 35 years or older, type 2 diabetes mellitus a complication, high fasting blood glucose, high HbA_{1c}, high BMI, and hypertriglyceridemia. Many researchers have reported a close relationship between the progression of chronic hepatitis C and the age at onset of HCV infection [4,5]: an older age at infection is considered to be associated with its faster progression. This was in agreement with the finding of this study that the age of 35 years or older was a risk factor for the progression of liver fibrosis in PTH.

Recently, much has been elucidated about the relationship between type 2 diabetes mellitus as a complication and HCV infection [20-22,30-34], including HCV infection itself as a risk for the development of diabetes [30,32,33]. Type 2 diabetes mellitus has also been reported to have an impact on the promotion of liver cirrhosis in chronic hepatitis C [34,35] and has been suspected of not only being a risk for the development of HCC in chronic hepatitis C, but also of being involved in the development of HCC without hepatitis B virus or HCV infection [36,37]. In addition, it has been reported that diabetes accelerates the rate of recurrence of HCC in patients with surgical treatment [38]. In a cross-sectional study of liver biopsy specimens, Monto et al [35] examined the relationship between diabetes and liver fibrosis in terms of the degree of liver fibrosis at the time of liver biopsy. Considering the period from the onset of PTH to the diagnosis of cirrhosis, we estimated the disease-free survival rate by the Kaplan-Meier method and found that diabetes as a complication was a risk factor contributing to the progression of PTH to cirrhosis, which was consistent with the findings of Monto et al. In addition, in this study, the diabetic group with poor glycemic control ($HbA_{1c} \geq 7.0\%$) had a significantly faster progression to cirrhosis than the group with good glycemic control ($HbA_{1c} < 7.0\%$), suggesting the importance of strict glycemic control in patients with chronic hepatitis C complicated by diabetes in delaying its progression to cirrhosis.

Similar to diabetes, obesity was identified by univariate analysis as a risk factor contributing to the progression from the onset of PTH to cirrhosis. In accordance with our results, many researchers reported that obesity was a factor involved in the progression of liver fibrosis [23,24]. In addition, this study showed that hypertriglyceridemia was a factor contributing to the progression of liver fibrosis. To date, no studies have reported a relationship between hypertriglyceridemia and the progression of chronic hepatitis C, leaving the mechanism of hypertriglyceridemia in promoting liver fibrosis unclear. However, hypertriglyceridemia may be associated with insulin resistance as a pathologic state common to diabetes and obesity, which is potentially related to liver fibrosis. Recently, Fartoux et al [26] compared the homeostasis model assessment of insulin resistance and serum insulin levels with liver steatosis and fibrosis, and reported that insulin resistance is a risk factor for steatosis in liver tissue and that high blood insulin levels contribute to the progression of fibrosis through steatosis. In addition, we have experimentally demonstrated that insulin resistance accelerates not only steatosis, but also inflammation and fibrosis, in the liver of a dietary rat model of nonalcoholic steatohepatitis and that therapy focusing on insulin resistance ameliorates the entire pathologic spectrum of steatohepatitis [39].

On the other hand, multivariate analysis identified age at onset of PTH ≥35 years, being male, and type 2 diabetes mellitus as significant factors contributing to the progression of liver fibrosis, but did not identify obesity or hypertriglyceridemia as an independent factor. This is probably because

many of the patients studied were complicated by these diseases. Indeed, diabetic patients are known to be frequently complicated by obesity or hypertriglyceridemia; but the effects of these diseases complicating diabetes on the progression of liver fibrosis have not been elucidated. Therefore, in this study, we examined the impact of obesity or hypertriglyceridemia complicating diabetes on liver fibrosis by comparing the duration of progression of PTH to cirrhosis between the diabetic groups with or without obesity or hypertriglyceridemia. The comparison showed that PTH progressed to cirrhosis significantly faster in the complicated group than in the noncomplicated group. The rates of risk for progression to cirrhosis were 1.692 and 4.643 times higher in diabetic patients complicated with obesity and hypertriglyceridemia, respectively, compared with those in patients with diabetes alone. These results suggest that control of body weight and blood triglyceride in addition to blood glucose is more effective to prevent the progression of liver fibrosis in patients with chronic hepatitis C.

Regarding the impact of diabetes on long-term prognosis of patients with chronic hepatitis C, we examined the temporal influence of diabetes on the occurrence of HCC and liver-related death. The results indicate that diabetes as a complication has a great impact on the long-term prognosis of chronic hepatitis C by reducing the time from PTH to the occurrence of HCC and to liver-related death. Consistent with our results, recent studies have reported that complication of diabetes in chronic hepatitis C is a risk factor for the development of HCC [36,37] and is a prognosis-determining factor after hepatectomy for HCC [40,41]. The conclusions from this study are limited because this nonprospective study could not accurately determine the age at onset of diabetes, cirrhosis, or HCC. However, taken together with previous reports, the results of the present study suggest that the treatment of diabetes, obesity, and hypertriglyceridemia holds the key to improving the prognosis of chronic hepatitis C.

References

[1] Kiyosawa K, Sodeyama T, Furuta S, et al. Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. Hepatology 1990;12:671-5.

[2] Tong MJ, el-Farra NS, Reikes AR, Co RL. Clinical outcomes after transfusion-associated hepatitis C. N Engl J Med 1995;332:1463-6.

[3] Niederau C, Lange S, Nawrocki M, et al. Prognosis of chronic hepatitis C: results of a large, prospective cohort study. Hepatology 1998;28: 1687-95.

[4] Wiese M, Berr F, Oesen U, et al. Low frequency of cirrhosis in a hepatitis C (genotype 1b) single-source outbreak in Germany: a 20-year multicenter study. Hepatology 2000;32:91-6.

[5] Poynard T, Ratziu V, Albrecht J, et al. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis C. J Hepatol 2001;34:730-9.

[6] Bissell DM. Sex and hepatic fibrosis. Hepatology 1999;29:988-9.

[7] Wiley TE, Brown J, Chan J. Hepatitis C infection in African Americans: its natural history and histological progression. Am J Gastroenterol 2002;97:700-6.

[8] Harris DR, Gonin R, Seeff LB, et al. The relationship of acute transfusion-associated hepatitis to the development of cirrhosis in the presence of alcohol abuse. Ann Intern Med 2001;134:120-4.

[9] Ostapowicz G, Watson KJ, Desmond PV, et al. Role of alcohol in the progression of liver disease caused by hepatitis C virus infection. Hepatology 1998;27:1730-5.

[10] Wiley TE, McCarthy M, Layden TJ, et al. Impact of alcohol on the histological and clinical progression of hepatitis C infection. Hepatology 1998;28:805-9.

[11] Corrao G, Lepore AR, Arico S, et al. The effect of drinking coffee and smoking cigarettes on the risk of cirrhosis associated with alcohol consumption. A case-control study. Provincial Group for the Study of Chronic Liver Disease. Eur J Epidemiol 1994;10:657-64.

[12] Tsai JF, Jeng JE, Tsai JH, et al. Independent and additive effect modification of hepatitis C and B viruses infection on the development of chronic hepatitis. J Hepatol 1996;24:271-6.

[13] Pontisso P, Gerotto M, Alberti A, et al. Coinfection by hepatitis B virus and hepatitis C virus. Antivir Ther 1998;3:137-42.

[14] Benhamou Y, Bochet M, Vidaud M, et al. Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. The Multivirc Group. Hepatology 1999;30:1054-8.

[15] Ragni MV, Belle SH. Impact of human immunodeficiency virus infection on progression to end-stage liver disease in individuals with hemophilia and hepatitis C virus infection. J Infect Dis 2001;183: 1112-5.

[16] Smith BC, Gorve J, Bassendine MF, et al. Heterozygosity for hereditary hemochromatosis is associated with more fibrosis in chronic hepatitis C. Hepatology 1998;27:1695-9.

[17] Matteoni CA, Younossi ZM, McCullough AJ, et al. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116:1413-9.

[18] Kamal S, Madwar M, Rasenack JW, et al. Clinical, virological and histopathological features: long-term follow-up in patients with chronic hepatitis C co-infected with *S. mansoni*. Liver 2000;20: 281-9.

[19] Kuzushita N, Hayashi N, Kaneshige T, et al. Influence of HLA haplotypes on the clinical courses of individuals infected with hepatitis C virus. Hepatology 1998;27:240-4.

[20] Hourigan LF, Macdonald GA, Powell EE, et al. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. Hepatology 1999;29:1215-9.

[21] El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. Gastroenterology 2004;126:460-8.

[22] Mason AL, Lau JY, Guo L, et al. Association of diabetes mellitus and chronic hepatitis C virus infection. Hepatology 1999;29:328-33.

[23] Hu KQ, Kyulo NL, Runyon BA, et al. Overweight and obesity, hepatic steatosis, and progression of chronic hepatitis C: a retrospective study on a large cohort of patients in the United States. J Hepatol 2004;40: 147-54.

[24] Hickman IJ, Powell EE, Jonsson JR, et al. In overweight patients with chronic hepatitis C, circulating insulin is associated with hepatic fibrosis: implications for therapy. J Hepatol 2003;39: 1042-8.

[25] Narita R, Abe S, Otsuki M, et al. Insulin resistance and insulin secretion in chronic hepatitis C virus infection. J Hepatol 2004;41: 132-8.

[26] Fartoux L, Poujol-Robert A, Serfaty L, et al. Insulin resistance is a cause of steatosis and fibrosis progression in chronic hepatitis C. Gut 2005;54:1003-8.

[27] Desmet VJ, Gerber M, Scheuer PJ, et al. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994;19: 1513-20.

[28] Araki T, Itai Y, Tasaka A, et al. Dynamic CT densitometry of hepatic tumors. AJR Am J Roentgenol 1980;135:1037-43.

[29] Misu H, Takamura T, Matsuzawa N, et al. Genes involved in oxidative phosphorylation are coordinately upregulated with fasting