rhosis may be secondary to either hepatic parenchymal cell damage or to portal-systemic shunting[9-12]. The rate at which insulin is degraded in the liver is reduced in patients with liver cirrhosis[11,12]. Moreover, despite peripheral hyperinsulinemia, insulin levels in the portal and hepatic veins are decreased in cirrhotic patients with portal systemic shunting[9,10]. However, hyperinsulinemia is also seen in patients with chronic hepatitis C virus (HCV) infection who do not show both severe hepatic parenchymal cell damage and portal-systemic shunting[6,8,13-16], indicating that increased hepatic insulin resistance is another factor related to hyperinsulinemia in patients with liver disease, particularly in HCV-related chronic liver disease[8,13,17-21].

PATHOGENESIS OF INSULIN RESISTANCE IN PATIENTS WITH CHRONIC HEPATITIS C

Insulin resistance parallels the liver fibrosis stage[22-26] and is associated with a reduced level of sustained virological response (SVR) to pegylated interferon and ribavirin[27-30]. Thus, insulin resistance is involved in the disease progression and success of treatment and it is important to understand the pathogenesis of insulin resistance in patients with chronic hepatitis C.

Changes in serum levels of leptin, adiponectin, tumor necrosis factor-alpha and interleukin-6 are known to be associated with the development of insulin resistance[31-36]. However, in patients with chronic hepatitis C, changes in these cytokines are not always correlated with insulin resistance[37-39]. On the other hand, insulin resistance is increased in the HCV core cDNA-transfected hepatoma cell lines and mice[8,40] and serum levels of HCV core protein are associated with the development of insulin resistance in patients with chronic hepatitis C[14,41]. Furthermore, insulin resistance is correlated with HCV viral kinetics[42,43] and is improved by clearance of HCV by interferon therapy[44-47]. These findings suggest that HCV *per se* is an important factor for the development of insulin resistance.

Recently, the relationship between HCV genotype and insulin resistance has been revealed. HCV genotypes 1, 3 and 4 associated with more severe insulin resistance[24,42,48]. In human hepatoma cell lines, HCV genotype 1 up-regulates suppressor of cytokine signaling (SOCS) 3 and causes ubiquitination of insulin receptor substrate (IRS)1/2, which subsequently suppresses insulin-induced phosphorylation of the p85 subunit of phosphatidylinositol 3-kinase and Akt and reduces glucose uptake (Figure 1)[8]. These changes are not seen in hepatoma cell lines infected with HCV genotype 2, suggesting that IRS1/2 degradation through up-regulation of SOCS3 is a genotype-specific mechanism[49]. In agreement with these results of basic research, hepatic expression of SOCS3 is higher in patients with HCV genotype 1 than in those with genotype 2 and increased hepatic expression of SOCS3 is correlated with poor response to antiviral treatment[50,51]. Two further mechanisms are reported in HCV genotype 1: activation of

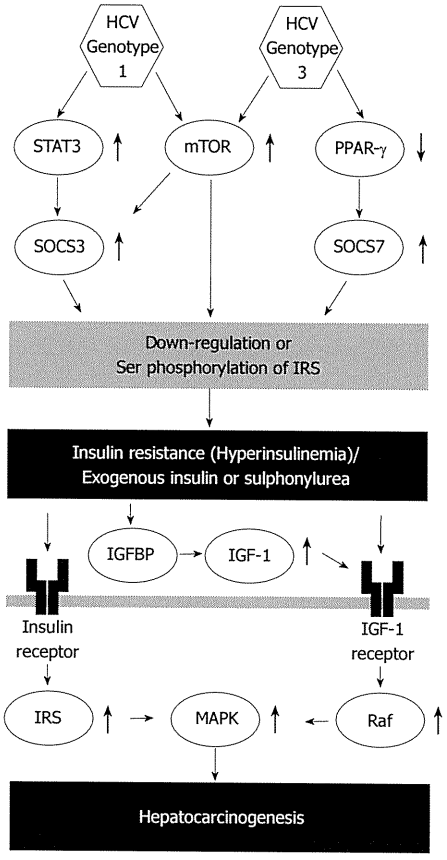

Figure 1 Scheme for HCV genotype difference in the molecular pathogenesis of insulin resistance and hepatocarcinogenesis. HCV: Hepatitis C virus; STAT: Signal transducer and activator of transcription; SOCS: Suppressor of cytokine signaling; mTOR: Mammalian target of rapamycin; PPAR: Peroxisome proliferator-activated receptor; IGFBP: Insulin-like growth factor binding protein; IGF: Insulin-like growth factor; IRS: Insulin receptor substrate; MAPK: Mitogen-activated protein kinase.

the mammalian target of rapamycin[52] and up-regulation of serine phosphorylation of IRS1(Figure 1)[43]. In addition, amino acid substitutions in the core region of HCV genotype 1b [Gln70 (His70) and/or Met91] have recently been reported as significant predictors of severe insulin resistance[53,54]. Although the underlying molecular mechanisms remain unclear, these findings indicate a unique molecular pathogenesis for insulin resistance in HCV genotype 1.

HCV genotype 3 also causes down-regulation of IRS1; however, the molecular pathogenesis differs from that of HCV genotype 1. HCV genotype 3 promotes down-regulation of IRS1 by up-regulating SOCS7 but not SOCS3 (Figure 1)[52]. SOCS-7 mRNA expression is independent of signal transducer and activator of transcription 3 and is modulated by peroxisome proliferator-activated receptor gamma activity (Figure 1)[52,55]. HCV genotype 4 is the most common variant in the Middle East and Africa and is increasing in prevalence in Western countries[56]. Infection with HCV genotype 4 is associated with a high prevalence of hepatic steatosis and obesity; however, the impact of

adiponectin on insulin resistance remains controversial[57,58] and specific mechanisms of insulin resistance in HCV genotype 4 infection also remain unclear.

Besides direct association of HCV with intracellular insulin signaling, hepatic steatosis is associated with increased BMI and insulin resistance and HOMA index is reported to be a predictor of SVR in patients with HCV non 3 genotypes[27,59-62]. In patients with HCV genotype 3, hepatic steatosis directly correlates with circulating and hepatic viral load, which is mediated by an impaired very-low-density lipoprotein assembly and secretion and by an up-regulation of the sterol depending protein signaling pathway, which regulates de novo lipogenesis and inhibits mitochondrial fatty acid b-oxidation[63,64].

CHANGES IN PANCREATIC BETA CELLS IN PATIENTS WITH LIVER DISEASE

A decrease in islet mass and/or beta-cell dysfunction is a pathogenesis for type 2 DM[65,66]. In patients with chronic liver disease, impairment of insulin secretion is also reported[11,67]; however, insulin resistance/hyperinsulinemia is also characteristic in such patients[8,13,17-21] and it therefore remains unclear whether the pathogenesis of hepatogenous DM is same as that of type 2 DM.

Pancreatic islet hypertrophy is reported in surgical biopsy tissue of patients with liver cirrhosis[68]. Islet hypertrophy and hyperplasia are also reported in thioacetamide-treated rats[69] and in HCV-core transgenic mice[40]. Moreover, Takei *et al* reported that islets in patients with cirrhosis show higher proliferation and lower apoptosis compare to those in patients with no chronic liver disease[70]. These findings suggest that hyperinsulinemia in cirrhotic patients may be caused by an adaptive response of the pancreatic beta cells to increased insulin resistance.

Although cross-talk between the pancreas and liver is an important issue in the development of insulin resistance, little is known about this relationship. Further studies regarding morphological and pathological changes of pancreatic alpha-or beta cells are required to characterize the pathogenesis of insulin resistance in patients with liver disease.

CAUSES OF DEATH IN DIABETIC PATIENTS WITH LIVER DISEASE

The prevalence of DM in patients with chronic liver disease is reportedly 18%-71%[18,20,71-73]. DM leads to several complications including cardiovascular disease. Generally, the therapeutic strategy in DM is to reduce the incidence of cardiovascular disease and to prevent a subsequent decrease in quality of life and improve prognosis. However, hepatogenous DM is less often associated with a positive family history, retinopathy and cardiovascular diseases[18,74-76]. In fact, major causes of death in cirrhotic patients with DM relate to liver disease or its complications, such as chronic liver failure, hepatocellular carcinoma (HCC) and gastrointestinal hemorrhage[18,19,77-79]. Therefore, the management of DM in patients with liver cirrhosis should aim to reduce such hepatic complications and to improve prognosis. Because the incidence of HCC has been well demonstrated to relate to DM[80], a major target in the management of DM should be to reduce the incidence of HCC in patients with liver cirrhosis.

ASSESSMENT OF DM IN PATIENTS WITH LIVER DISEASE

Plasma glucose and hemoglobin A1c (HbA1c) are generally used for routine assessment and management of patients with type 2 DM, whereas there is less information regarding the association between these markers and HCC incidence or prognosis in patients with liver cirrhosis. HbA1c level in patients with HCC is higher than in patients with liver cirrhosis or in control subjects[81]. In patients with liver cirrhosis, however, HbA1c does not properly represent glycemic control status in cirrhotic patients because of the short lifespan of erythrocytes caused by hypersplenism[82-86]. These data indicate that assessment and management of hepatogenous DM using HbA1c is inaccurate, although poor glucose control is associated with HCC incidence.

Strict control of blood glucose levels may improve survival in HCV patients. In patients with HCV-related liver cirrhosis, the prognosis for patients with hyperglycemia (fasting plasma glucose $\geqslant$ 7.0 mmol/L; 126 mg/dL) was worse than for those with normoglycemia[19]. Therefore, fasting plasma glucose < 7.0 mmol/L (126 mg/dL) appears to be meaningful in hepatogenous DM.

Fasting serum insulin and homeostasis model assessment of insulin resistance (HOMA-IR) are also used as markers of glucose tolerance. In patients with HCV infection, HCC development is associated with increased fasting serum insulin level and by HOMA-IR[87]. Moreover, HCC recurrence has also been demonstrated to be related to HOMA-IR[88,89]. In addition, prognosis is worse in HCC patients with increased fasting serum insulin level or HOMA-IR[90]. These data suggest that the assessment of insulin is also meaningful in patients with liver cirrhosis. Taken together, fasting plasma glucose and either serum insulin or HOMA-IR are candidate markers for the assessment of hepatogenous insulin resistance/DM. However, further studies are required to clarify the utility of these markers and their target values in terms of complications induced by liver cirrhosis including HCC or prognosis.

IMPACT OF ANTI-DIABETIC AGENTS IN PATIENTS WITH LIVER DISEASE

Exogenous insulin and sulphonylureas

Despite the recognition of this potential link between insulin resistance and life-threatening complications including HCC, there is no common therapeutic strategy for

Table 1 Effects of anti-diabetic agents in patients with chronic liver disease

Anti-diabetic agent	Subjects	Outcome	Reference
Exogenous insulin or sulphonylurea	Patients with liver cirrhosis or HCC	Increased HCC risk	[100]
Exogenous insulin or sulphonylurea	Patients with chronic hepatitis C	Increased HCC risk	[101]
Exogenous insulin	Chronic viral hepatitis patients who had undergone curative resection for HCC	Increased risk of HCC recurrence	[102]
Metformin	Treatment-naïve female patients with HCV genotype 1-related chronic hepatitis and insulin resistance	Increased SVR rate	[16]
Metformin	Patients diabetes mellitus and liver cirrhosis or HCC	Decreased HCC risk	[101]
Metformin	Patients with liver cirrhosis or HCC	Decreased HCC risk	[112]
Pioglitazone	Chronic hepatitis C patients who had previously failed to respond to antiviral therapy	No increase in EVR rate	[115]
Pioglitazone	Treatment-naïve chronic hepatitis C patients with insulin resistance	Increased SVR rate	[116]

HCC; hepatocellular carcinoma, EVR; early virological response, SVR; sustained virological response.

insulin resistance in patients with chronic liver disease. Since insulin is a growth-promoting hormone with mitogenic effects[91], exogenous insulin and sulphonylureas, which increase serum insulin levels, are considered to enhance carcinogenesis. In fact, a large-scale cohort study has reported that exogenous insulin increases the risk of malignancies in patients with DM[92,93]. Exogenous insulin and sulphonylureas are known to promote breast cancer[94], colorectal cancer[95,96] and pancreatic cancer[95,97] in patients with DM. Recently, a possible link between anti-diabetic agents and the risk of cancer is noted in the consensus statement from the American Diabetes Association and the American Cancer Society[98].

An association between anti-diabetic agents and hepatocellular carcinoma (HCC) was first described in 1986 by Lawson *et al*[99]. In addition, we, along with others, have recently shown that use of exogenous insulin or sulphonylurea increases the development and recurrence of HCC in patients with chronic hepatitis C (Table 1)[80,100-102]. Exogenous insulin or second-generation sulphonylurea increases serum insulin levels. Since insulin has mitogenic and cell proliferative effects, these anti-diabetic agents could be a carcinogenic factor. Insulin binds to insulin receptors and activates the mitogenactivated protein kinase pathway[91,103]. Insulin also cross-reacts with insulin like growth factor (IGF)-1 receptor and activates the Raf cascade, leading to mitosis and cell proliferation[104]. Moreover, excess insulin binds to IGF-binding proteins, resulting in increased levels of free serum IGF-1 (Figure 1)[87,105-107]. Thus, hyperinsulinemia induced by use of exogenous insulin or sulphonylurea may enhance hepatocarcinogenesis through multiple pathways.

The association of exogenous insulin or second-generation sulphonylurea with HCC was more evident in females than in males[101]. Sex affects the development of HCC and females are less prone to HCC than males[108,109]; therefore, we assume that use of exogenous insulin or a 2nd-generation sulfonylurea may accelerate development of HCC mainly in patients who have negative factor for the development of HCC.

Metformin

Metformin is an oral biguanide with insulin-sensitizing effects. However, biguanides are reported to predispose patients with liver cirrhosis to lactic acidosis and are considered as a contraindication in this situation[110]. Recently, Romero-Gomez *et al* first reported that adding metformin to peginterferon and ribavirin is safe and improved insulin sensitivity in treatment-naïve patients with HCV genotype 1 infection and DM[16]. In an intent-to-treat analysis, no beneficial effects of metformin on SVR were seen; however, in female patients with insulin resistance, adding metformin to antiviral treatment doubled the SVR rate (58% *vs* 29%)[16]. Although the reason for this sex difference is still unclear, elevated estradiol-to-testosterone ratio is known to be associated with better response to metformin treatment[111], suggesting a possible association between sex hormones and metformin-induced high SVR rate. Donadon *et al* and our research group have reported that metformin reduced risk of HCC in patients with DM and chronic liver disease[101,112]. Metformin is also known to attenuate the response of cancer cells to insulin *in vitro*[113,114]. Thus, metformin has potential benefits as an insulin sensitizer for patients receiving antiviral treatment or those with liver cirrhosis(Table 1).

Pioglitazone

Pioglitazone is a thiazolidinedione with insulin-sensitizing effects. Recently, Overbeck *et al* reported that adding pioglitazone to pegylated interferon-alpha and ribavirin improves insulin resistance; however, none of the patients achieved a satisfactory virological response after 12 wk of treatment (Table 1)[115]. On the other hand, Khattab *et al* reported that pioglitazone improves sustained virological response to antiviral therapy in hepatitis C patients with insulin resistance (Table 1)[116]. The effect of pioglitazone on SVR therefore remains controversial; however, a difference in enrolled subjects may account for this discrepancy. The study by Overbeck *et al* enrolled patients with chronic hepatitis C who previously failed to respond to peginterferon plus ribavirin therapy[115], whereas the

study by Khattab *et al* enrolled naïve chronic hepatitis C patients with insulin resistance[116]. Thus, pioglitazone may not enhance the effect of antiviral therapy in intractable chronic hepatitis C. However, insulin resistance is reduced in both studies and pioglitazone may therefore be able to improve insulin resistance-related complications in patients with HCV infection. Further study will need to focus on the effects of pioglitazone, not only on antiviral treatment but also on the development of hepatic fibrosis, hepatocarcinogenesis and patient prognosis.

Dipeptidyl peptidase-4 inhibitors

Dipeptidyl peptidase (DPP)-4 inactivates incretin hormones including glucagon-like peptide-1 (GLP-1)[117,118], which enhances insulin secretion and reduces body weight[119,120]. DPP-4 inhibitors are therefore used as anti-diabetic agents[117,118]. DPP-4 is also known as CD26, an immune-regulation molecule expressed on T-cells[121], and transfection of a HCV non-structural genome region is reported to increase DPP-4 expression in a hepatoma cell line[122]. Treatment of HCV-infected patients with interferon decreases serum DPP-4 activity, which is related to interferon-induced immune activation[123]. Although changes in DPP-4 activity after interferon treatment may just represent indirect evidence, one would think that changes in DPP-4 activity could be involved in the pathogenesis of HCV-related insulin resistance.

Although changes in GLP-1 and DPP-4 remain unclear in hepatogenous insulin resistance, we previously investigated changes of these molecules in patients with HCV infection[124]. The serum level of the active GLP-1 in HCV-infected patients is significantly lower than that in hepatitis B virus-infected patients and healthy subjects. On the other hand, DPP-4 is up-regulated in the serum, ileum and liver of HCV-infected patients more than that of hepatitis B-infected patients and healthy subjects. Taken together, it seems that inactivation of GLP-1 through up-regulation of DPP-4 is a possible pathogenetic mechanism for HCV-related insulin resistance.

DPP-4 inhibitors are now available in the clinical setting and decrease plasma glucose levels as well as HbA1c levels with a low incidence of hypoglycemia in patients with type 2 diabetes mellitus[125,126]. Unlike other anti-diabetic agents, DPP-4 inhibitors are metabolized in the kidney and rarely cause hepatic dysfunction[127,128]. Moreover, GLP-1 analogs improve insulin sensitivity in insulin-resistant obese fa/fa Zucker rats[129] and DPP-4 inhibitors increase hepatic glucose uptake[130]. Thus, further study will be focus on the effects of DPP-4 inhibitors on HCV-related insulin resistance.

COFFEE CONSUMPTION

In various studies including a large prospective study, patients with HCV-related liver disease with a regular coffee consumption show a lower rate of disease progression such as hepatic fibrosis[131-133] and HCC[134-138]. Recently, it was also reported that more than 3 cups per day coffee drinkers are three times more likely to have a virological response to peginterferon plus ribavirin treatment than non-drinkers[139]. Since coffee consumption increases insulin sensitivity[140] and inhibits the development of non-alcoholic fatty liver disease in healthy subjects[141], coffee intake may be protective by mechanisms modulating insulin sensitivity and resulting in a reduced extent of liver steatosis in patients with HCV infection.

CONCLUSION

In this paper, we summarize the features of insulin resistance in relationship to chronic liver disease. Pathogenesis, assessment and cause of death in insulin resistance related to liver disease differ from those of lifestyle-related insulin resistance. Furthermore, exogenous insulin or sulfonylureas may be harmful because these agents may promote hepatocarcinogenesis. There is, therefore, a need for a unique therapeutic strategy for hepatogenous insulin resistance.

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Case Report

Development of intrahepatic cholangiocarcinoma after a 14-year follow-up of a patient with primary sclerosing cholangitis and ulcerative colitis

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Intrahepatic cholangiocarcinoma (ICC) is one of the life-threatening complications of primary sclerosing cholangitis (PSC). However, the incidence of ICC in Japanese PSC patients is low, and the association between the development of ICC and morbidity duration of PSC is largely unknown. Here, we describe a case of ICC that developed after a long-term follow-up of a patient with PSC and ulcerative colitis (UC). At the age of 10 years, the patient was first diagnosed with UC and its remission was achieved with systemic steroid therapy. Since then, he was routinely followed-up. At the age of 19 years, laboratory tests showed abnormalities in liver function parameters, and the patient was diagnosed with PSC. Although treatment with ursodeoxycholic acid improved the abnormalities in serum levels of biliary enzymes and no PSC-related symptoms were seen for 13 years, calculous cholecystitis frequently occurred in the patient since the age of 32 years. He developed ICC, which expressed some hepatic progenitor cell markers such as CD133, neural cell adhesion molecule, keratin 7, and keratin 19 at the age of 33 years. ICC was treated by curative partial hepatectomy and adjuvant chemotherapy with gemcitabine. Eight months later, however, the patient developed multiple metastases in the abdominal lymph nodes and lungs, and died 21 months after the onset of ICC. Here, we report a case of ICC that developed after a 14-year follow-up of a patient with PSC and UC.

Key words: cholangiocarcinoma, Japanese, long-term follow-up, primary sclerosing cholangitis, ulcerative colitis.

INTRODUCTION

PRIMARY SCLEROSING CHOLANGITIS (PSC) is a cholestatic liver disease characterized by multiple fibrotic strictures of the intra- and extrahepatic biliary tree.[1–3] Although PSC is generally slow progressive, it is refractory to therapy, and frequently results in advanced liver cirrhosis.[1–3] Intrahepatic cholangiocarcinoma (ICC) is the most feared complication of PSC and the occurrence of ICC leads to a poor prognosis for PSC patients.[4,5]

The prevalence of ICC in patients with PSC is reported to be approximately 7–15% in the USA.[2] While, a Japanese national survey disclosed the prevalence of ICC to be only 3.6% in PSC patients (14/391).[5] The survey also revealed two clinical characteristics for PSC-associated ICC in Japan. First, the average follow-up period between PSC and ICC diagnoses is relatively short (average period, 2.6 ± 3.5 years).[5] Second, PSC patients with inflammatory bowel disease (IBD) are less likely to develop ICC.[5] However, PSC-associated ICC is a rare disorder in Japan, and limited information is available about the clinical characteristics of this devastating disorder. Here, we report a case of ICC, which developed

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after a 14-year follow-up of PSC and ulcerative colitis (UC).

CASE REPORT

A 10-YEAR-OLD Japanese male visited Kurume University Hospital because of abdominal pain and hematochezia. The patient was diagnosed with UC by colonic biopsy. Since the patient had an allergic reaction to 5-aminosalicylic acid, remission of UC was achieved by systemic steroid therapy. After withdrawal of steroid therapy, exacerbation of UC occurred, and he was administered a continuous 5 mg/day of prednisolone (Fig. 1).

At the age of 19 years, laboratory tests of the patient showed abnormalities in liver function parameters with elevated serum levels of biliary enzymes, including alkaline phosphatase (ALP) (Fig. 1). Multiple strictures and dilatation of the intrahepatic bile ducts were seen on endoscopic retrograde cholangiography (ERC). His liver biopsy showed concentric periductal fibrosis (Fig. 2). Thus, the patient was diagnosed with PSC associated with UC. After treatment with 600 mg/day of ursodeoxycholic acid (UDCA), his serum levels of biliary enzymes were decreased (Fig. 1).

At the age of 25 years, the patient complained of abdominal pain and hematochezia. Colonoscopy revealed diffuse mucosal inflammation and ulcerations

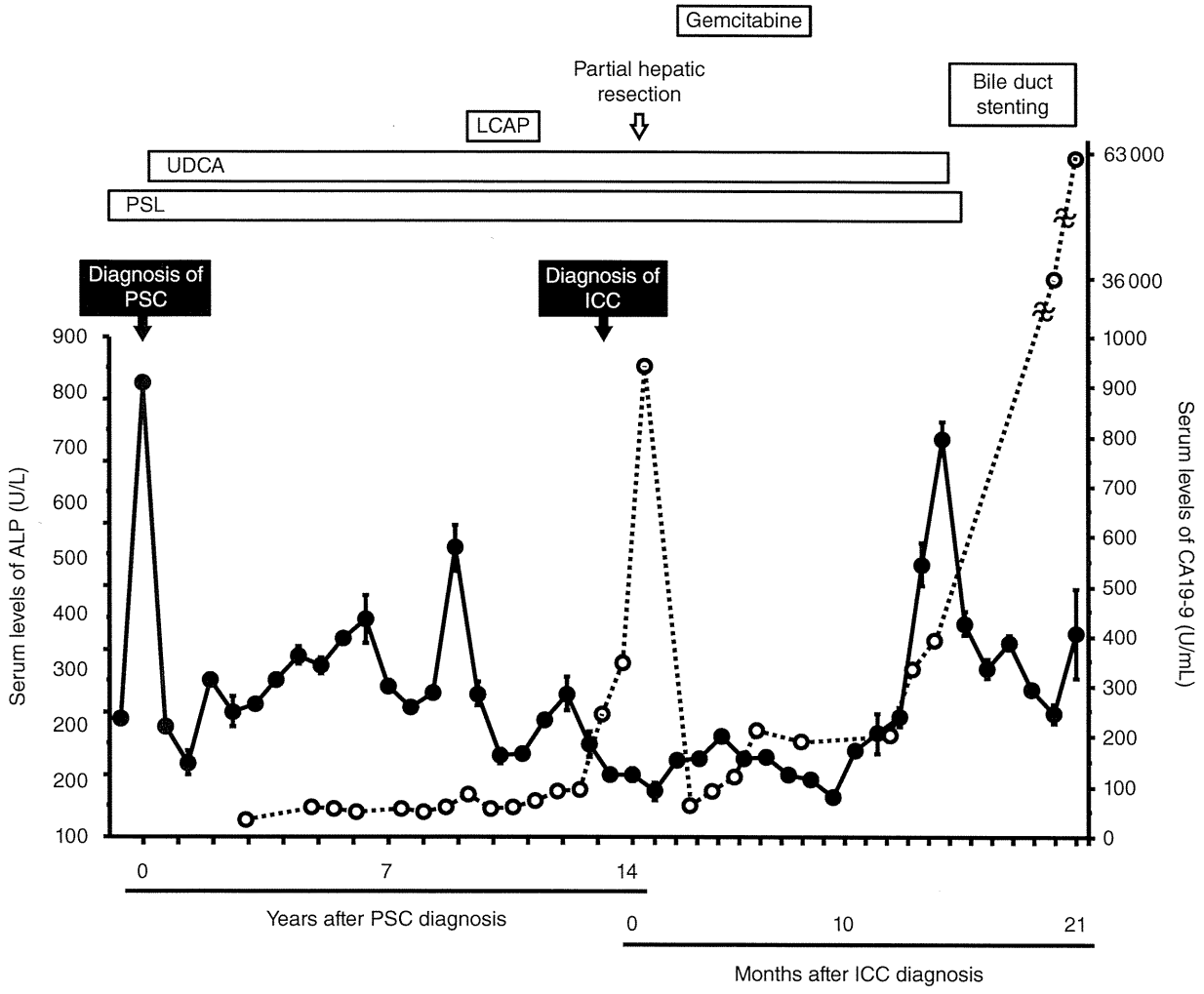

Figure 1 Clinical course and changes in serum alkaline phosphatase (ALP) and carbohydrate antigen 19–9 (CA19-9) levels. Serum levels of ALP (black circle) are shown as mean ± standard deviation (SD) of the all data measured in the indicated year or month, and serum levels of CA19-9 (white circle) are shown as absolute values. PSC, primary sclerosing cholangitis; ICC, intrahepatic cholangiocarcinoma; PSL, prednisolone; UDCA, ursodeoxycholic acid; LCAP, leukocytapheresis. (—●—): ALP; (···○···): CA19-9.

in the descending colon. Simultaneously, laboratory tests showed elevated serum levels of biliary enzymes, including ALP and total bilirubin levels, indicating exacerbation of both UC and PSC. Leukocytapheresis therapy was initiated and marked improvement in clinical symptoms and colonoscopic findings were noted (Fig. 1). Furthermore, serum levels of biliary enzymes decreased, as previously reported.[6]

At the age of 32 years, the patient complained of abdominal pain with no hematochezia. After evaluation of biochemical and diagnostic images, the patient was diagnosed with calculous cholecystitis. His abdominal pains and biochemical abnormalities were improved by a conservative therapy of total parenteral nutrition. Although brush cytology using ERC showed suspected adenocarcinoma, ICC was not detected by computed tomography (CT) and ERC (Fig. 3). Thereafter, frequent recurrence of calculous cholecystitis was observed.

When the patient was 33 years of age, a hepatic space-occupying lesion (SOL; diameter, 35 mm) was detected by the abdominal ultrasonography (Fig. 4a). His biochemical parameters are summarized in Table 1. His serum carbohydrate antigen 19–9 (CA 19–9) level was 72.6 U/mL. On CT, contrast enhancement was not seen in the hepatic SOL (Fig. 4b). The hepatic SOL showed an accumulation of fluoro-2-deoxyglucose on positron emission tomography (Fig. 4c). On the basis of these findings, the hepatic SOL was clinically diagnosed as ICC. Since bile duct histology demonstrated no malignancy under ERC, curative partial hepatectomy was performed. The pathological diagnosis of the cancerous lesion was ICC and that of non-cancerous lesion was non-cirrhotic PSC (Inuyama classification A1, F1) (Fig. 4d–f). No infiltration of IgG4-positive cells was found in his liver specimens (Fig. 4g). We also performed immunohistochemistry for hepatic progenitor cell (HPC) markers such as CD133, neural cell adhesion molecule (NCAM), keratin 7 (K7), and K19 of the resected tissue (Fig. 5a–h). Some of ICC cells were weak immunoreactive for CD133 (Fig. 5b) and NCAM (Fig. 5d), and most of the ICC cells were strong immunoreactive for K7 and K19 (Fig. 5f,h).

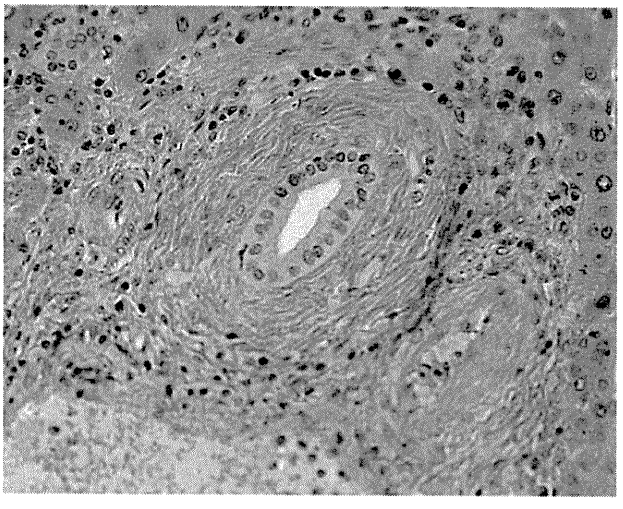

Figure 2 Histology of liver biopsy. Examination of liver biopsy specimen shows concentric periductal fibrosis, "onion-skin appearance". Original magnification × 200.

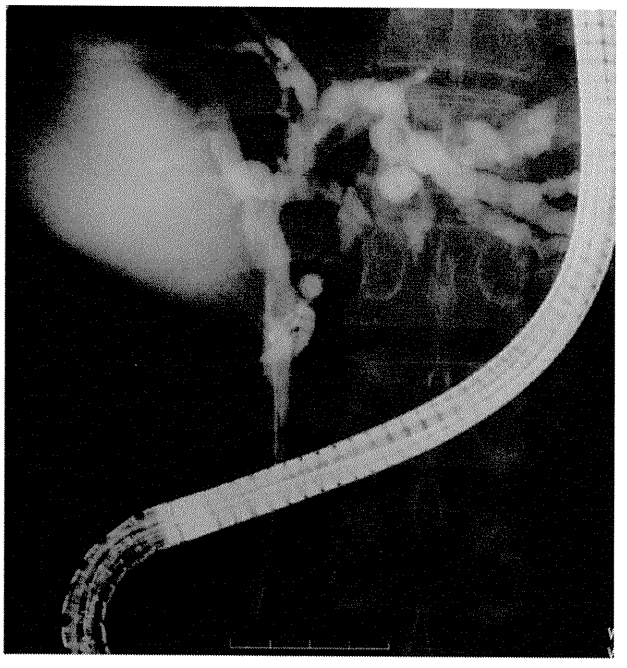

Figure 3 Endoscopic retrograde cholangiography shows multiple strictures and dilatation of the intrahepatic bile ducts. There are no findings indicating cholangiocarcinoma.

After partial hepatectomy, he was administered gemcitabine (1400 mg/day) every 2 weeks for 6 months as adjuvant chemotherapy. However, 2 months after termination of chemotherapy, he developed multiple metastases in the liver, abdominal lymph nodes, and lungs and died 21 months after the onset of ICC (Fig. 1).

DISCUSSION

THE CHARACTERISTICS OF ICC in patients with PSC are different depending on the geographical location.[2,3,5] In this report, we presented a case of ICC that developed after a 14-year follow-up of PSC and UC in Japan.

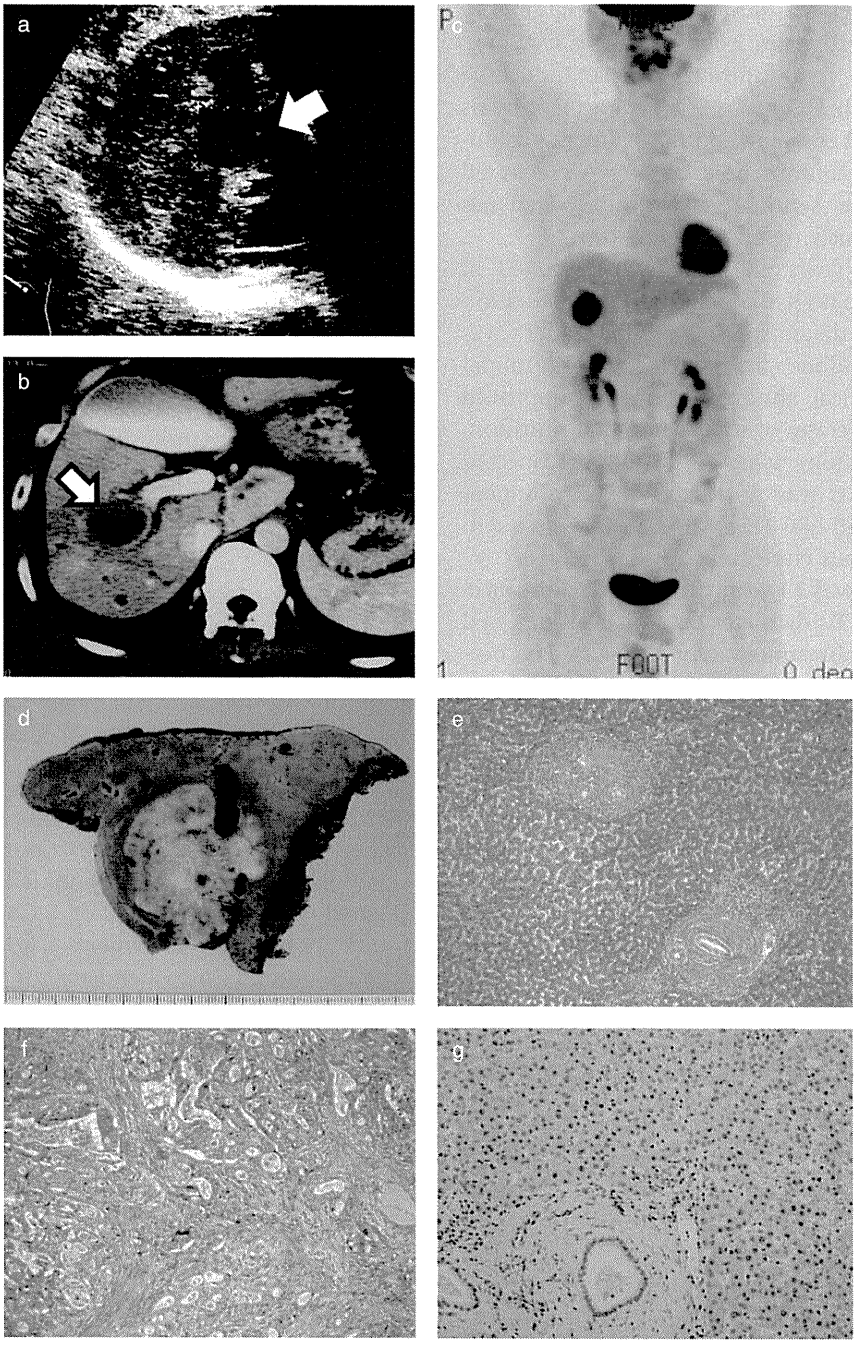

Figure 4 (a) Abdominal ultrasonography shows hypoechoic a space-occupying lesion (SOL; diameter, 35 mm) in the liver (arrow). (b) The hepatic SOL shows no contrast enhancement in the early phase of computed tomography (arrow). (c) The hepatic SOL shows accumulation of fluoro-2-deoxyglucose on positron emission tomography scan. (d) Macroscopic view shows a mass with a blurred border arising in a non-cirrhotic liver. (e) Liver histology demonstrates concentric periductal fibrosis, mild inflammation, and fibrous expansion of the portal area (Inuyama classification A1, F1). Hematoxylin-eosin staining. Original magnification × 20. (f) Tumor histology shows an adenocarcinoma showing small tubular, acinar or cord-like structures with a slit-like lumen. Hematoxylin-eosin staining. Original magnification × 100. (g) No infiltration of IgG4-positive cells is seen in the liver specimens. Immunostaining for IgG4. Original magnification × 100.

Table 1 Biochemical parameters measured at the onset of cholangiocarcinoma

Examination	Reference value	Value
Red blood cell ($\times 10^4$/mm^3)	430–570	567
Hemoglobin (g/dL)	14.0–18.0	15.7
White blood cell (/mm^3)	4000–9000	6000
Platelet ($\times 10^4$/mm^3)	13–36	29.6
Aspartate transaminase (U/L)	13–33	32
Alanine aminotransferase (U/L)	8–42	49
Lactate dehydrogenase (U/L)	119–229	161
Alkaline phosphatase (U/L)	115–359	286
γ-glutamyl transpeptidase (U/L)	10–47	101
Total protein (g/dL)	6.70–8.30	7.64
Albumin (g/dL)	4.00–5.00	3.90
Total bilirubin (mg/dL)	0.30–1.50	0.89
C-reactive protein (mg/dL)	<0.40	0.17
Total cholesterol (mg/dL)	128–220	190
Fasting blood glucose (mg/dL)	80–109	102
Hemoglobin A1c (%)	4.3–5.8	4.9
Prothrombin activity (%)	60–130	97
α-fetoprotein (ng/mL)	<8.7	6.1
Protein induced by vitamin K absence (mAU/mL)	<40	30
Carcinoembryonic antigen (ng/mL)	<5.0	3.6
Carbohydrate antigen 19–9 (U/mL)	<37.0	76.2
DuPan-2 (U/mL)	<150	63
S-pancreas-1 antigen (U/mL)	<30	25
Erastase-1 (ng/dL)	100–400	170
IgG4 (mg/dL)	4.8–105.0	39.9

The average period between PSC and ICC diagnoses are reported to be 2.3 years in Sweden.[7] Similarly, a Japanese national survey for PSC conducted in 2003 reported that a relatively short period between PSC and ICC diagnoses is a characteristic of PSC-associated ICC (average period, 2.6 ± 3.5 years).[5] However, the period between PSC and ICC diagnoses in our case was 14 years, which is more than five times longer than the average period in Japan. Although the reason for this discrepancy remains unclear, a possible reason is that the national survey was performed in hospitals specializing in gastroenterology. In the Japanese survey, 50% of ICC cases were diagnosed within a month after diagnosis of PSC, suggesting that about a half of the PSC patients were referred to medical specialists for examination of suspected ICC, and ICC might be already developed when these patients visited the hospitals. Another possible reason is UDCA administration. UDCA is known to inhibit taurocholate and taurolithocholate-induced growth of human cholangiocytes.[8] In addition, the incidence of ICC in PSC patients treated with UDCA is reported to be lower than that in PSC patients not treated with UDCA.[9] In our case, administration of UDCA from the early stage of PSC may have contributed to late onset of ICC.

Another feature of ICC in Japanese PSC patients is that the complication of IBD is a negative factor for the development of ICC. Although our patient had UC, the impact of IBD on the development of ICC is unclear. Recently, Melum *et al.* reported that the natural killer cell receptor G2D, which plays a crucial role in tumor surveillance by NK cells,[10] is involved in preventing development of ICC in patients with PSC.[11] On the other hand, steroids inhibit natural killer cell receptor G2D-mediated NK cell activity.[12] Since our patient had an allergic reaction to 5-aminosalicylic acid, the cumulative dose of steroids administration is more than 25 000 mg. Thus, IBD might have had an indirect effect on the development of ICC in our patient.

Even in transplanted patients with PSC-associated ICC, average tumor-free survival rate is only 30–35% in 3 years,[4] therefore, data on risk factors and effect of chemotherapy for ICC may provide important information on the clinical management of PSC patients. ICC is generally considered a late complication of advanced PSC-related liver cirrhosis.[13] However, histological examination in non-cancerous lesions showed mild inflammation and fibrous expansion of the portal area in our patient. Likewise, the prevalence of esophageal varices is low in PSC patients with ICC,[14] suggesting that ICC may not inevitably be a late complication of PSC; therefore, it may be necessary to be alert to the development of ICC in any stage of PSC.

Intrahepatic cholangiocarcinoma shows a variable cholangiocytic differentiation[15] and ICC with HPC phenotypes has been proposed recently.[16–19] In our case, some of the ICC cells were weak immunoreactive for CD133 and NCAM, and most of the ICC cells were strong immunoreactive for K7 and K19, suggesting that the origin of ICC is derived from HPCs. Even though curative partial hepatectomy was performed, intra- and extra-hepatic metastases and poor prognosis were seen in our case. HPC phenotype is reported to be an independent factor for worse prognosis of ICC patients.[20] Thus, poor prognosis could be related to the HPC phenotype in our case and immunohistochemistry for HPC markers may be important for selecting therapeutic strategy and predicting prognosis for ICC patients.

Concerning chemotherapy, gemcitabine was administered as adjuvant chemotherapy in our patient.

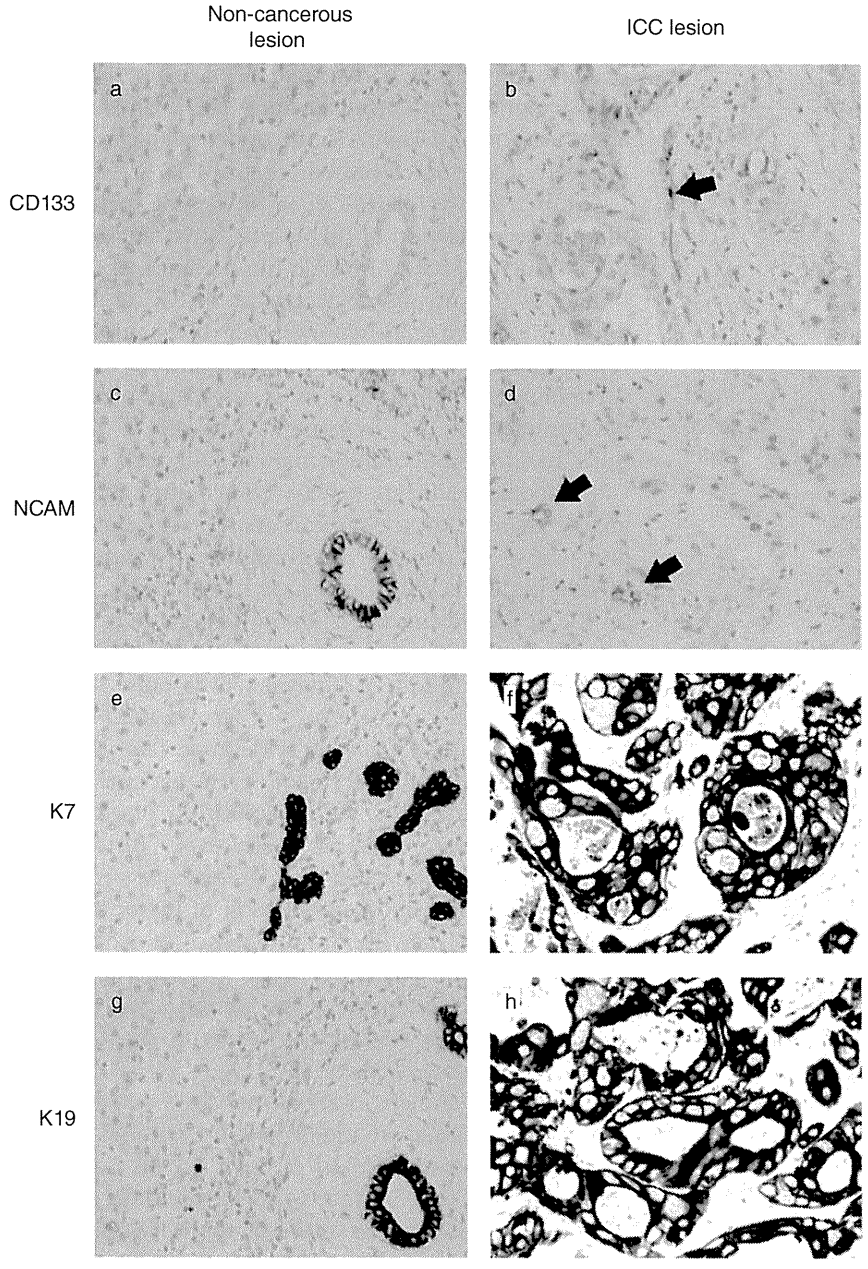

Figure 5 Immunostainig for CD133 (a and b), neural cell adhesion molecule (NCAM) (c and d), K7 (e and f), and K19 (g and h) of non-cancerous and Intrahepatic cholangiocarcinoma (ICC) lesions. Some ICC cells are weak immunoreactive for CD133 (b; arrow) and NCAM (d; arrow), and most of the ICC cells are strong immunoreactive for K7 (f) and K19 (g). Original magnification × 400. K, keratin.

However, multiple metastases occurred in the patient and he died 21 months after the onset of the ICC, suggesting that gemcitabine may not have had significant beneficial effects on the prognosis of our patient. Since there is no specific data on chemotherapy for PSC-associated ICC, it is hoped that ongoing study of molecular targeted therapy for epidermal growth factor receptors will result in a better prognosis for patients with PSC-associated ICC.[4] Thus, future studies will be focused on examining risk factors and chemotherapy for ICC in patients with PSC.

Here, we report a case of ICC that developed after a 14-year follow-up of a patient with PSC and UC in Japan. This report provides novel information on the association of development of ICC with morbidity duration of PSC and complication of IBD in Japan.

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CLINICAL STUDIES

Association of exogenous insulin or sulphonylurea treatment with an increased incidence of hepatoma in patients with hepatitis C virus infection

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Keywords
hepatitis C virus – hepatocellular carcinoma – diabetes mellitus – insulin – sulphonylurea

Abbreviations
ALP, alkaline phosphatase; ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; γ-GTP, γ-glutamyl transpeptidase; HbA1c, haemoglobin A1c; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HOMA-IR, homeostasis model assessment of insulin resistance; IGF, insulin-like growth factor; LDH, lactate dehydrogenase; OR, odds ratio.

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Abstract

Background: Diabetes mellitus is frequently seen in hepatitis C patients and is often treated with antidiabetic agents that increase serum insulin levels. Because insulin is a growth-promoting hormone, antidiabetic agents could pose a risk for hepatocellular carcinoma (HCC). *Aim:* The aim of this study was to investigate an association between antidiabetic therapies and the incidence of HCC in hepatitis C patients with diabetes mellitus. *Methods:* A nested case–control study was conducted. Participants were recruited from a cohort study, in which patients with hepatitis C were consecutively registered. Participants were assigned to an HCC group ($n = 138$) or a non-HCC group ($n = 103$). To identify independent factors, variables including use of antidiabetic agents were analysed by logistic regression analysis. *Results:* Besides ageing, being male, cirrhosis and hypoalbuminaemia, use of exogenous insulin and a second-generation sulphonylurea were significant independent factors associated with an incidence of HCC [odds ratio (OR) 2.969, 95% confidence interval (CI) 1.293–6.819, $P < 0.0103$ and OR 6.831, 95% CI 1.954–23.881, $P < 0.0026$ respectively). In stratified analyses, the impact of these antidiabetic agents was more evident in patients who were non-cirrhotic than in those who were cirrhotic. *Conclusions:* Exogenous insulin and a second-generation sulphonylurea were independent variables associated with an incidence of HCC in hepatitis C patients with diabetes mellitus. This association was evident in patients who were non-cirrhotic. To verify a causal relationship between these antidiabetic agents and the development of HCC, a prospective cohort study is required.

Type 2 diabetes mellitus is a common metabolic abnormality worldwide and is associated with various complications. For example, cardio-vascular complications are well known for diabetes mellitus (1), while recent epidemiological studies have shown that patients with type 2 diabetes mellitus are highly predisposed to cancer (2). Type 2 diabetes mellitus has been implicated in the development of cancer of the pharynx, oesophagus, colorectum, pancreas, cervix uteri, breast and prostate (2–5). In addition, type 2 diabetes mellitus is also known to be associated with the development of hepatocellular carcinoma (HCC) (6–8).

Type 2 diabetes mellitus is also frequently seen in patients with chronic hepatitis C virus (HCV) infection (9–11). Factors that are involved in the development of type 2 diabetes mellitus include not only life-style choices such as diet and exercise but also hepatic inflammation, fibrosis, steatosis, iron deposition and HCV core protein (10, 12–15). A high prevalence of HCC is seen in patients with HCV infection compared with patients with other

chronic liver diseases. Although the mechanism for HCV-related hepatocarcinogenesis is unclear, an association between type 2 diabetes mellitus and HCV infection could be responsible for the high prevalence of HCC in patients with HCV infection.

Hyperglycaemia is a common feature of type 2 diabetes mellitus. Hyperglycaemia increases oxidative stress, which causes oxidative DNA damage, an initial step in carcinogenesis (16). Moreover, hyperglycaemia is a factor leading to carcinogenesis because of immune suppression through the regulation of T-cell function (17). Thus, hyperglycaemia itself might stimulate hepatocarcinogenesis.

Hyperinsulinaemia combined with insulin resistance is another common feature of type 2 diabetes mellitus. Insulin is a growth-promoting hormone with mitogenic effects (18), and therefore could stimulate hepatocarcinogenesis. An increase in circulating insulin levels is also seen in diabetic patients treated with sulphonylureas or exogenous insulin. Indeed, exogenous insulin injection promotes colonic carcinogenesis in rats (19) and use of exogenous insulin significantly increases the risk of colorectal cancer among diabetic patients (20). Similarly, patients with type 2 diabetes exposed to sulphonylureas and exogenous insulin have a significantly increased risk of cancer-related mortality compared with patients exposed to metformin, a insulin sensitizer (21). Moreover, metformin reduces the risk of cancer in patients with type 2 diabetes (22). These findings suggest that pharmacologic effects of antidiabetic agents on circulating insulin levels play an important role in carcinogenesis. Despite the recognition of this potential link between type 2 diabetes mellitus and hepatocellular carcinoma, a role for antidiabetic agents in hepatocarcinogenesis has not been established.

Accordingly, in this study, we examined a possible association between antidiabetic therapies and an incidence of HCC in patients with HCV infection.

Methods

Study design and participants

A nested case–control study was conducted. Hepatitis C patients with diabetes mellitus were culled from the HCV-related diabetes mellitus study (HDMS), a hospital-based, prospective, multi-centre cohort. Patients with hepatitis C were consecutively recruited from three Japanese hospitals specialized for liver diseases (Kurume University Hospital, Nagata Hospital, and Chikugo City Hospital) from January 2004 to December 2008. Eligible participants were identified from all patients who were aged $\geq$40 years old, and had both a positive result for anti-HCV antibodies and a diagnosis of type 2 diabetes mellitus. The diagnosis of type 2 diabetes mellitus was based on the 2004 American Diabetes Association criteria (23) or use of any anti-diabetic agent. Participants in whom diabetes was diagnosed before age 30 with a positive result for pancreatic beta-cell autoantibodies (antiglutamic acid decarboxylase, anti-insulinoma-associated protein-2 or anti-islet-cell antibodies) were categorized as having type 1 diabetes mellitus and were excluded.

A total of 265 participants provided baseline data. The analysis was performed on the data of 241 participants, after excluding 24 participants because of unavailability of data on glucose metabolism, coincidence with other causes of liver disease such as chronic hepatitis B, autoimmune hepatitis, a metastatic liver tumour, or cholangiocellular carcinoma, taking corticosteroids or a history of pancreatitis or a pancreatic tumour. HCC was diagnosed by ultrasonic-guided biopsy, the non-invasive European Society of Study of the Liver criteria for the diagnosis of HCC or superparamagnetic iron oxide-enhanced magnetic resonance imaging (24, 25). All patients were classified into an HCC or a non-HCC group according to the incidence of HCC.

Informed consent for participation in this study was obtained from each participant. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in prior approval by the Ethics Committee of the Kurume University School of Medicine. None of the participants was institutionalized.

Measurements

Clinical data including age, sex and alcohol intake were collected at the first medical examination. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of the height in metres (kg/m^2).

The diagnosis of cirrhosis was based on a liver biopsy or the aspartate aminotransferase (AST) to platelet ratio index (APRI); serum AST level (U/L)/upper limit of normal AST (33 U/L) $\times$ 100/platelet count ($\times 10^4$/ml) (26). APRI is one of the models for predicting the stage of liver fibrosis. Patients with APRI values higher than 2.0 were diagnosed as having cirrhosis (26). In stratification analysis, the severity of liver disease was classified into non-cirrhosis or cirrhosis.

Venous blood samples were taken in the morning after a 12-h overnight fast. The following were measured using standard clinical methods: platelet count, plasma glucose, haemoglobin A1c (HbA1c), serum AST, alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), albumin and total bilirubin levels (Department of Clinical Laboratory, Kurume University Hospital, Nagata Hospital and Chikugo City Hospital).

Classification of antidiabetic agents

Antidiabetic agents were classified into exogenous insulin (any type of insulin preparation), second-generation sulphonylurea (gliclazide or glybenclamide), third-generation sulphonylurea (glimepiride), α-glucosidase inhibitor (acarbose, voglibose or miglitol), glinide (nateglinide or mitiglinide), biguanide (metformin) or

thiazolidine (pioglitazone) according to each characteristic of these agents.

Statistical analysis

All data are expressed as mean ± standard deviation. Comparisons between the two groups were performed using the Mann–Whitney *U*-test for continuous variables and univariate analysis for discrete variables. In order to evaluate the possible importance of variables, a univariate analysis was performed initially. The relevant variables with univariate *P* values < 0.1 were selected for inclusion in the initial step of logistic analysis. Then, logistic regression analysis was used to identify any independent variable that was related to the incidence of HCC. The variables analysed were age ($\geq$60 years old), sex, BMI ($\geq 25\ kg/m^2$), alcohol intake ($\geq$50 g/day), the incidence of cirrhosis, use of exogenous insulin, use of a second-generation sulphonylurea, use of biguanide, total bilirubin ($\geq$2 mg/dl), albumin (< 3.5 g/dl), AST ($\geq$40 U/L), ALT ($\geq$40 U/L), LDH ($\geq$230 U/L), ALP ($\geq$360 U/L), γ-GTP ($\geq$50 U/L), platelet count ($< 10 \times 10^4/\mu l$), fasting plasma glucose ($\geq$126 mg/dl) and HbA1c ($\geq$5.9%). Stratification analysis was conducted to examine the impact of use of exogenous insulin and a second-generation sulphonylurea on the incidence of HCC. Stratification factors were severity of liver disease (non-cirrhotic patients, cirrhotic patients), hypoalbuminaemia (≥ 3.5 g/dl or < 3.5 g/dl) and sex (male or female). All the statistical tests were two-sided, and a *P* value of < 0.05 was considered to be statistically significant.

Results

Comparison of the characteristics and use of antidiabetic agents between hepatocellular carcinoma and non-hepatocellular carcinoma groups in hepatitis C patients with diabetes mellitus

Age, prevalence of males, the incidence of cirrhosis and fasting plasma glucose were significantly higher in the HCC group compared with those in the non-HCC group (Table 1).

There were no significant differences in the HbA1c levels between two groups. However, use of anti-diabetic agents was more frequent in the HCC group than that in the non-HCC group ($P = 0.0030$; Table 1). In order to investigate an association between HCC and the pharmacologic effects of anti-diabetic agents, anti-diabetic agents were classified into seven subgroups according to the characteristics of each agent: exogenous insulin, second-generation sulphonylurea, third-generation sulphonylurea, α-glucosidase inhibitor, glinide, biguanide or thiazolidine. There were no significant differences in the use of a third-generation sulphonylurea, α-glucosidase inhibitor, glinide, biguanide or thiazolidine between HCC and non-HCC groups. However, use of exogenous insulin and a second-generation sulphonylurea were significantly more frequent in the HCC group than those in the non-HCC group ($P = 0.0020$ and $P = 0.0003$, respectively; Table 1).

Table 1. Comparison of characteristics and use of anti-diabetic agents between hepatocellular carcinoma and non-hepatocellular carcinoma groups in hepatitis C patients with diabetes mellitus

	HCC ($n = 138$)	Non-HCC ($n = 103$)	*P* value
Age (year)	68.8 ± 8.0	64.7 ± 10.3	0.0032
Male	103	60	0.0083
BMI (kg/m^2)	22.9 ± 3.2	22.8 ± 3.5	0.4595
Excess alcohol intake (≥50 g/day)	29	12	0.0590
Cirrhosis	101	34	< 0.0001
Total bilirubin (mg/dl)	1.37 ± 1.96	1.09 ± 1.46	0.1171
Albumin (g/dl)	3.39 ± 0.53	3.82 ± 0.56	< 0.0001
AST (U/L)	65.4 ± 33.0	55.4 ± 32.8	0.0027
ALT (U/L)	56.9 ± 32.6	56.4 ± 40.5	0.3534
LDH (U/L)	229.6 ± 70.1	214.7 ± 65.9	0.0286
ALP (U/L)	392.0 ± 213.3	343.0 ± 161.3	0.0547
γ-GTP (U/L)	130.6 ± 239.5	74.8 ± 80.4	0.0046
Platelet count ($\times 10^4/\mu l$)	10.5 ± 5.2	12.9 ± 5.2	0.0002
Fasting plasma glucose (mg/dl)	163.4 ± 70.1	146.1 ± 60.5	0.0230
HbA1c (%)	6.6 ± 1.1	7.1 ± 1.7	0.0905
Use of antidiabetic agents	98	53	0.0030
Exogenous insulin	43	14	0.0020
Second-generation sulphonylurea	26	4	0.0003
Third-generation sulphonylurea	22	20	0.4969
α-glucosidase inhibitor	25	15	0.4898
Glinide	8	11	0.2266
Biguanide	4	5	0.5024
Thiazolidine	1	4	0.1670

All data are expressed as mean ± standard deviation.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GTP, glutamyl transpeptidase; HCC, hepatocellular carcinoma; LDH, lactate dehydrogenase.

Variables associated with the incidence of hepatocellular carcinoma in hepatitis C patients with diabetes mellitus

In the univariate analysis, age ($\geq$60 years old), being male, cirrhosis, albumin (< 3.5 g/dl), AST ($\geq$40 U/L), LDH ($\geq$230 U/L), ALP ($\geq$360 U/L), platelet count ($< 10 \times 10^4/\mu l$) and HbA1c ($\geq$5.9%) were significant variables associated with the incidence of HCC (Table 2). In addition, use of exogenous insulin and a second-generation sulphonylurea were significant variables associated with the incidence of HCC [odds ratio (OR) 2.877, 95% confidence interval (CI) 1.474–5.617, $P < 0.0020$ and OR 5.746, 95% CI 1.938–17.038 respectively: Table 2).

In the logistic regression analysis, age ($\geq$60 years old), being male, cirrhosis and albumin (< 3.5 g/dl) were independent factors associated with a greater incidence of HCC (Table 2). Moreover, use of exogenous insulin

Table 2. Univariate and logistic regression analyses for the incidence of hepatocellular carcinoma in all patients ($n = 241$)

	Univariate analysis			Logistic regression analysis		
Variable	HR	95% CI	*P* value	HR	95% CI	*P* value
Age (≥60 years)	2.270	1.221–4.222	0.0096	2.781	1.231–6.283	0.0139
Male	2.109	1.219–3.649	0.0076	3.075	1.511–6.258	0.0019
BMI (≥25 kg/m^2)	1.085	0.559–2.105	0.8092			
Excess alcohol intake (≥50 g/day)	2.018	0.974–4.179	0.0588			
Cirrhosis	5.540	3.173–9.672	< 0.0001	3.366	1.465–7.731	0.0042
Total bilirubin (≥2 mg/dl)	2.537	0.801–8.028	0.1133			
Albumin (< 3.5 g/dl)	5.078	2.843–9.070	< 0.0001	3.008	1.373–6.591	0.0059
AST (≥40 U/L)	2.447	1.403–4.267	0.0016	1.690	0.797–3.582	0.1709
ALT (≥40 U/L)	1.441	0.856–2.427	0.1694			
LDH (≥230 U/L)	1.919	1.099–3.350	0.0220	0.936	0.436–2.008	0.8650
ALP (≥360 U/L)	1.912	1.100–3.325	0.0216	1.044	0.499–2.187	0.9084
γ-GTP (≥50 U/L)	1.565	0.929–2.637	0.0926			
Platelet count (< 10 × 10^4/μl)	2.000	1.177–3.398	0.0104	0.913	0.401–2.079	0.8285
Fasting plasma glucose (≥126 mg/dl)	1.559	0.9296–2.624	0.0945			
HbA1c (≥5.9%)	0.451	0.219–0.929	0.0308	0.654	0.268–1.596	0.3511
Use of exogenous insulin	2.877	1.474–5.617	0.0020	2.969	1.293–6.819	0.0103
Use of second-generation sulphonylurea	5.746	1.938–17.037	0.0016	6.831	1.954–23.881	0.0026
Use of third-generation sulphonylurea	0.787	0.404–1.535	0.4823			
Use of α-glucosidase inhibitor	1.298	0.646–2.609	0.4641			
Use of glinide	0.515	0.199–1.330	0.1702			
Use of biguanide	0.585	0.153–2.235	0.4332			
Use of thiazolidine	0.181	0.020–1.641	0.1285			

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; GTP, glutamyl transpeptidase; HR, hazard ratio; LDH, lactate dehydrogenase.

and a second-generation sulphonylurea were also identified as independent variables associated with an incidence of HCC (OR 2.969, 95% CI 1.293–6.819, $P = 0.0103$, OR 6.831, 95% CI 1.954–23.881, $P = 0.0026$ respectively; Table 2). Use of a second-generation sulphonylurea showed the highest OR among all the variables (Table 2).

Variables associated with the incidence of hepatocellular carcinoma in stratification analysis by severity of liver disease

All patients were stratified into two subgroups: a non-cirrhosis or a cirrhosis group. In patients with cirrhosis, age (≥60 years old) and albumin (< 3.5 g/dl) were identified as independent factors associated with the incidence of HCC in the logistic regression analysis (Table 3). However, use of any antidiabetic agent was not an independent factor associated with the incidence of HCC in patients with cirrhosis (Table 3).

In non-cirrhotic patients, not only being male but also use of exogenous insulin and a second-generation sulphonylurea were determined to be independent variables associated with a greater incidence of HCC (Table 3).

Variables associated with the incidence of hepatocellular carcinoma in stratification analysis by hypoalbuminaemia

All patients were stratified into two subgroups: ≥3.5 g/dl of albumin or < 3.5 g/dl of albumin group. In patients with < 3.5 g/dl of the albumin, being male and age (≥60 years old) were associated with the incidence of HCC in the logistic regression analysis (Table 4). Use of exogenous insulin or a second-generation sulphonylurea was not determined to be an independent variable, while use of biguanide was negatively associated with the incidence of HCC (Table 4).

In patients with ≥3.5 g/dl of albumin, not only being male and cirrhosis but also use of a second-generation sulphonylurea were determined to be independent variables associated with a greater incidence of HCC (Table 4). Use of a second-generation sulphonylurea showed the

Table 3. Logistic regression analysis for the incidence of hepatocellular carcinoma by stratification according to severity of liver disease

	Logistic regression analysis		
Variables	OR	95% CI	*P* value
Chronic hepatitis ($n = 107$)			
Male	6.150	1.705–22.185	0.0055
Use of exogenous insulin	4.142	1.072–16.007	0.0393
Use of second-generation sulphonylurea	4.822	0.963–24.144	0.0556
AST (≥40 U/L)	1.506	0.530–4.285	0.4423
γ-GTP (≥50 U/L)	2.234	0.759–6.578	0.1444
Albumin (< 3.5 g/dl)	3.632	0.940–14.031	0.0614
Cirrhosis ($n = 134$)			
Age (≥60 years)	3.357	1.335–8.440	0.0100
Albumin (< 3.5 g/dl)	2.402	1.061–5.436	0.0355

AST, aspartate aminotransferase; CI, confidence interval; GTP, glutamyl transpeptidase, OR, odds ratio.

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Table 4. Logistic regression analysis for the incidence of hepatocellular carcinoma by stratification according to hypoalbuminaemia

	Logistic regression analysis		
Variable	OR	95% CI	*P* value
≥3.5 g/dl of albumin ($n = 139$)			
Male	2.536	1.066–6.034	0.0353
Cirrhosis	2.830	1.096–7.308	0.0317
Use of exogenous insulin	2.557	0.973–6.718	0.0567
Use of second-generation sulphonylurea	5.195	1.338–20.171	0.0173
AST (≥40 U/L)	1.602	0.696–3.691	0.2682
LDH (≥230 U/L)	1.777	0.696–4.535	0.2289
Platelet count ($< 10 \times 10^4/\mu l$)	1.200	0.445–3.237	0.7183
< 3.5 g/dl of albumin ($n = 102$)			
Male	4.922	1.562–15.502	0.0065
Age (≥60 years)	3.357	2.178–28.454	0.0016
Use of biguanide	0.060	0.004–0.846	0.0371

AST, aspartate aminotransferase; CI, confidence interval; LDH, lactate dehydrogenase; OR, odds ratio.

highest OR in patients with ≥3.5 g/dl of albumin (OR 5.195, 95% CI 1.338–20.171, $P = 0.0173$; Table 4).

Variables associated with the incidence of hepatocellular carcinoma in stratification analysis by sex

All patients were stratified into two subgroups: a male or a female group. In male patients, age (≥60 years old) and albumin (< 3.5 g/dl) were identified as independent factors associated with the incidence of HCC in the logistic regression analysis (Table 5). Moreover, use of a second-generation sulphonylurea was also identified as an independent variable associated with a greater incidence of HCC (OR 4.267, 95% CI 1.046–17.412, $P = 0.0431$; Table 5).

In female patients, cirrhosis was identified as an independent factor associated with an incidence of HCC in the logistic regression analysis (Table 5). Use of a second-generation sulphonylurea was also identified as an independent variable associated with a higher incidence of HCC in female patients and its OR was higher than that in male patients (Table 5).

Discussion

We conducted a hospital-based nested case–control analysis in order to identify variables associated with an increasing incidence of HCC. Besides known variables such as ageing, being male, cirrhosis, and hypoalbuminaemia, we found that use of exogenous insulin and a second-generation sulphonylurea that increase circulating insulin levels were independent variables associated with a greater incidence of HCC. In addition, the impact of the use of exogenous insulin and a second-generation sulphonylurea was more evident in patients who did not have cirrhosis or showed ≥3.5 g/dl of albumin.

Table 5. Logistic regression analysis for the incidence of hepatocellular carcinoma by stratification according to sex

	Logistic regression analysis		
Variable	OR	95% CI	*P* value
Male ($n = 163$)			
Age (≥60 years)	2.807	1.114–7.073	0.0286
Cirrhosis	2.298	0.805–6.564	0.1201
Use of exogenous insulin	2.195	0.827–5.825	0.1143
Use of second-generation sulphonylurea	4.267	1.046–17.412	0.0431
AST (≥40 U/L)	1.665	0.678–4.087	0.2656
LDH (≥230 U/L)	1.094	0.434–2.756	0.8486
ALP (≥360 U/L)	1.482	0.605–3.630	0.3890
Platelet count ($< 10 \times 10^4/\mu l$)	0.717	0.249–2.067	0.5383
Albumin (< 3.5 g/dl)	3.516	1.299–9.518	0.0133
Total bilirubin (≥2 mg/dl)	0.986	0.095–10.199	0.9905
Female ($n = 78$)			
Cirrhosis	16.710	2.743–101.785	0.0023
Use of exogenous insulin	4.985	0.868–28.618	0.0716
Use of second-generation sulphonylurea	50.993	3.011–863.633	0.0065
HbA1c (≥5.9%)	0.472	0.083–2.682	0.3968
LDH (≥230 U/L)	0.753	0.169–3.366	0.7107
Platelet count ($< 10 \times 10^4/\mu l$)	1.800	0.393–8.247	0.4491
Albumin (< 3.5 g/dl)	1.740	0.348–8.700	0.4998

ALP, alkaline phosphatase; AST, aspartate aminotransferase; CI, confidence interval; GTP, glutamyl transpeptidase; LDH, lactate dehydrogenase; OR, odds ratio.

Use of antidiabetic agents was a variable associated with a greater incidence of HCC in this study. Thus, we found a possible association between antidiabetic agents and the risk of HCC. Among antidiabetic agents, use of exogenous insulin and a second-generation sulphonylurea were significant variables associated with the incidence of HCC. Hyperinsulinaemia combined with insulin resistance is considered as a promoter for hepatocarcinogenesis and tumour growth (27, 28). Because exogenous insulin and a second-generation sulphonylurea increase circulating insulin levels, we hypothesize that the use of these antidiabetic agents accelerates the development of HCC in patients with HCV infection. Use of a third-generation sulphonylurea or an α-glucosidase inhibitor was not a variable associated with the incidence of HCC in this study. A third-generation sulphonylurea is categorized as a sulphonylurea because it contains a sulphonylurea structure. However, a third-generation sulphonylurea is known to improve hyperinsulinaemia through extra-pancreatic effects (29). In fact, a preliminary study in our HDMS cohort showed that treatment of the third generation sulphonylurea causes a reduction of serum insulin levels in hepatitis C patients with diabetes mellitus (data not shown). α-glucosidase inhibitors reduce post-prandial glucose elevation by delaying the release of glucose from disaccharides and complexes of carbohydrates and do not promote insulin secretion