DOI: 10.2169/internalmedicine.46.6419

lactate dehydrogenase (LDH, 331 U/L; normal, 119 to 229) and hyaluronic acid (59 ng/ml; normal, ≦50). Albumin was slightly low (3.49 g/dl; normal, 4.0 to 5.0). Indocyanine green retention (ICG R_{15}) was 55.3% (normal, ≦10), while ammonia (NH_3) was 135 μg/dl (normal, 12 to 66).

Abdominal ultrasonography showed evidence of chronic liver damage and steatosis, as well as iso- to hypoechoic nodule in segment VI (20×21 mm in diameter) (Fig. 1). Three smaller nodules were present (7×8 mm in segment VI, 6×8 mm in segment VI, and 10×10 mm in segment VII). Color Doppler ultrasonography revealed an artery branching from an artery at the margin of the largest nodule, then approaching the center of the nodule. No "spoke-wheel" pattern was seen (Fig. 2). Dynamic computed tomography (CT) detected only the largest nodule, which was isodense prior to contrast agent administration and in the delayed phase, showing mild enhancement at the early phase (Fig. 3A, B). Portal flow within the liver or in the portal tract approaching the hepatic hilum was not detected (Fig. 3C). The IVC communicated with the superior mesenteric vein (SMV) and the narrow splenic vein (SV). A normal IVC was not seen inferior to its connection with the SMV and SV; instead, two prominent veins likely to be the left IVC and the testicular vein drained into the left renal vein from below. The complexity of portal and systemic venous anatomy were shown clearly by three-dimensional (3D) CT (Fig. 4). Magnetic resonance imaging (MRI) showed the largest hepatic nodule to be hyperintense on both T1-weighted and T2-weighted images. Superparamagnetic iron oxide (SPIO)-enhanced MRI using Resovist as a

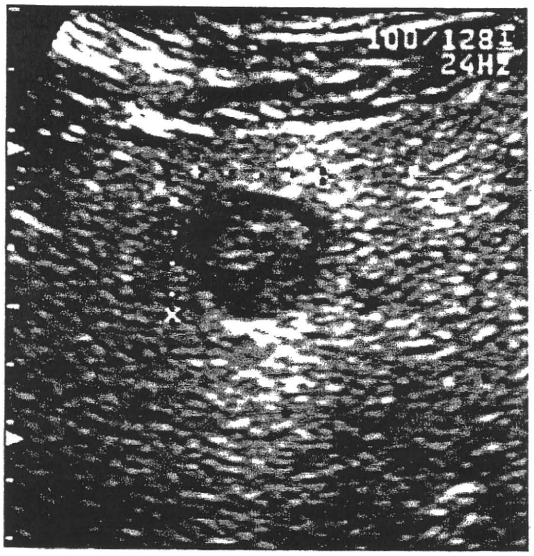

Figure 1. **Ultrasonogram showed an iso- to hypoechoic nodule in segment VI. This largest nodule measured 20×21 mm. The echo pattern in surrounding liver parenchyma demonstrated fatty liver and associated chronic damage.**

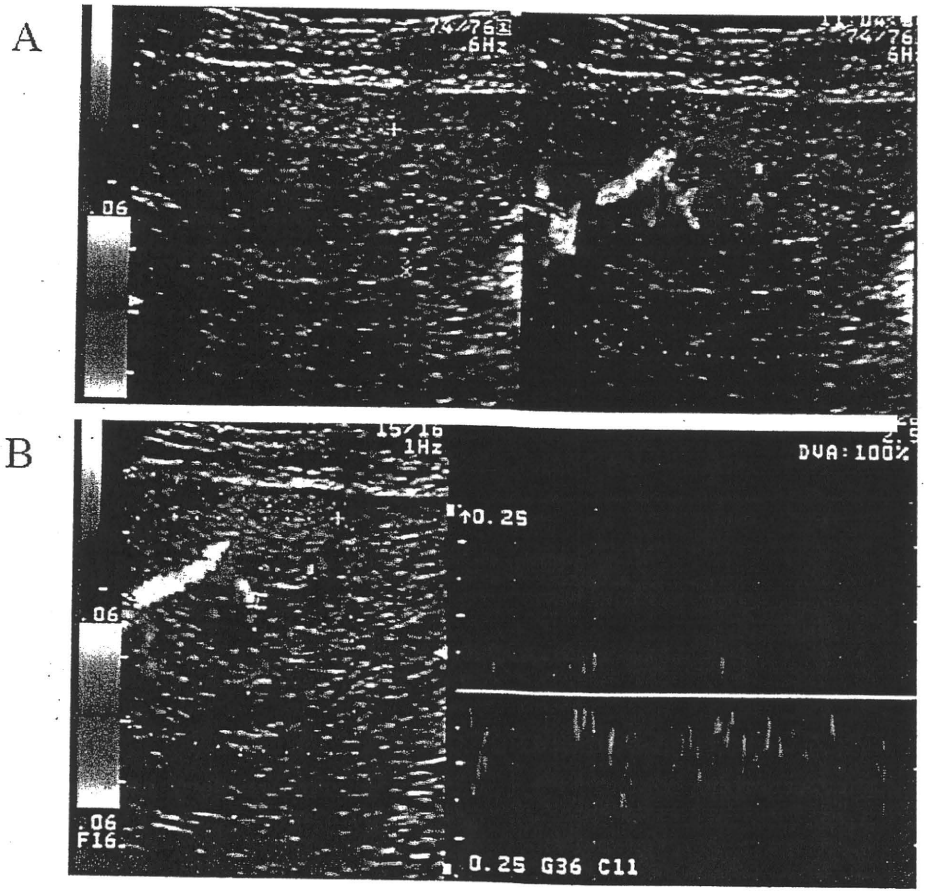

Figure 2. **Color Doppler examination. A: Color Doppler image showed an artery running along the margin of nodule, with a branch turning toward the center of the nodule. B: Fast Fourier transformation indistinctly demonstrated an arterial pulsatile wave in this branched artery.**

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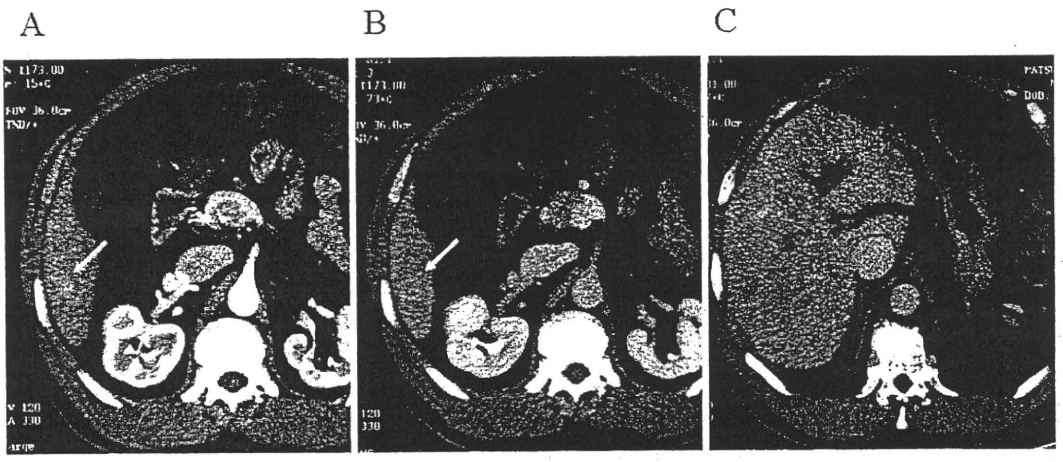

Figure 3. **Abdominal computed tomography. A: The largest nodule showed contrast enhancement in the early phase (arrow). B: The largest nodule appeared isodense in the delayed phase (arrow). C: No portal flow is shown in the liver (delayed phase).**

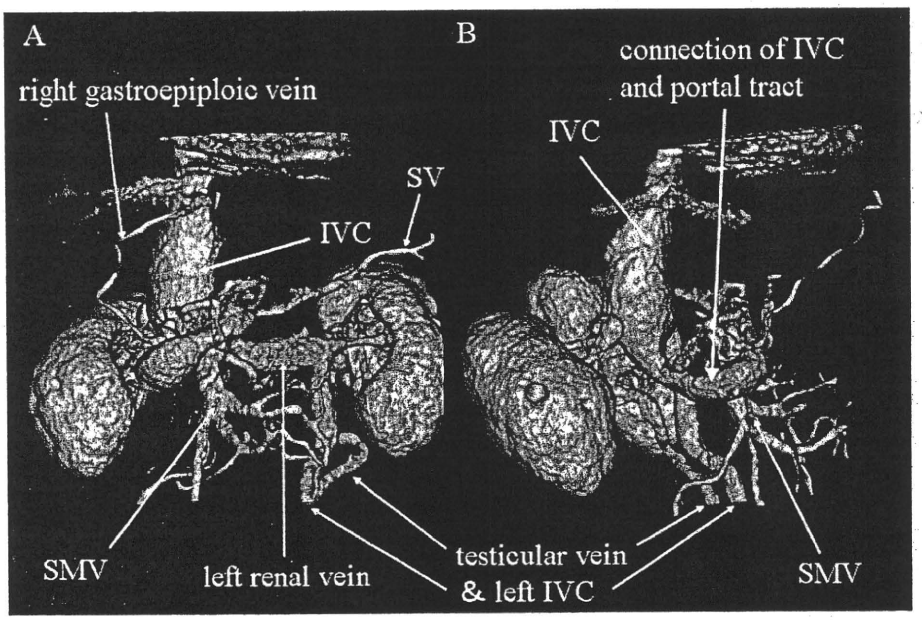

Figure 4. **Three-dimensional computed tomography of the venous system. The inferior vena cava (IVC) communicated with the superior mesenteric vein (SMV) and the splenic vein (SV). The IVC disappeared below the level of its connection with the SMV and SV. Two veins, apparently a left IVC and the testicular vein, flowed into the left renal vein. A: Anterior view. B: Right lateral view. IVC: the inferior vena cava, SMV: the superior mesenteric vein, SV: the splenic vein.**

contrast agent showed the largest nodule to be hyperintense relative to surrounding liver, indicating less accumulation of Resovist in the nodule (Fig. 5).

A biopsy specimen from the largest nodule consisted of tissue from the nodular lesion and also from surrounding liver (Fig. 6A). Histopathologically, the nodule showed mildly increased cellularity with distinct trabecular architecture, and occasionally a pseudoglandular pattern. Surrounding liver tissue showed mild fatty change. A specimen from a smaller nodule was similar to the trabecular areas of the larger nodule. Both nodules contained areas of fibrous scarring, with a few anomalous arterial vessels and biliary pseudoductules (Fig. 6B). Immunohistochemically, nodule tissue was strongly reactive to anti-CD34 along sinusoids, while surrounding liver tissue was not (Fig. 6C). Cells reactive to anti-CD68 along a nodule sinusoid were fewer than in surrounding liver (Fig. 6D). The nodules were diagnosed as showing the morphology of focal nodular hyperplasia (FNH). We decided to maintain outpatient clinical follow-up as opposed to treatment for tumors such as resection.

Discussion

We could not obtain any detailed early-life medical records concerning the patient's infection with malaria, splenectomy or shunt operation. Based upon radiologic findings

DOI: 10.2169/internalmedicine.46.6419

and any past history that the patient and his mother could remember, we concluded that somehow he was infected with malaria from his uncle, then underwent splenectomy as a treatment related to malaria. End-to-side portacaval shunting was carried out as treatment for portal hypertension consequent to malaria or to portal thrombosis after splenectomy. Antibodies characteristic of malarial infection were negative, and no organisms could be found in blood smears when the patient was admitted, most likely because the infection was remote.

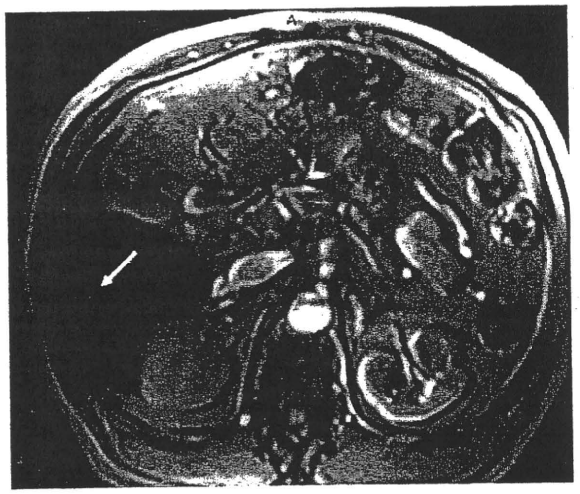

Figure 5. **Magnetic resonance imaging using superparamagnetic iron oxide (Resovist). This enhanced imaging showed the largest nodule to be hyperintense relative to surrounding liver (arrow).**

Paucity of intrahepatic portal blood flow resulted from the shunt operation at age 15. Images from 3D-CT suggested the absence of the IVC below its junction with the left renal vein was congenital. Lack of intrahepatic portal blood flow and radiologic configurations of the portal and systemic veins in the present patient resembled those of congenital absence of the portal vein (CAPV) type Ib (liver not perfuse with portal blood, while the SMV and SV form a confluence) (3, 4). Although our patient was not examined by angiography, 3D-CT clearly depicted the complexities of the abnormal abdominal vascular anatomy.

Biopsy specimens from nodular hepatic lesions in the present case showed marked hepatocytic hyperplasia with an area of fibrosis scarring containing anomalous vessels and showing marked sinusoidal capillarization as seen in FNH. Hirohashi et al demonstrated that FNH included capillarized sinusoids (5), while Tanaka et al showed that 15% of FNH cases showed fewer Kupffer cells in nodules than in surrounding liver tissue (6). Imaging findings in the largest nodule closely depicted the pathologic findings. Hypervascu-

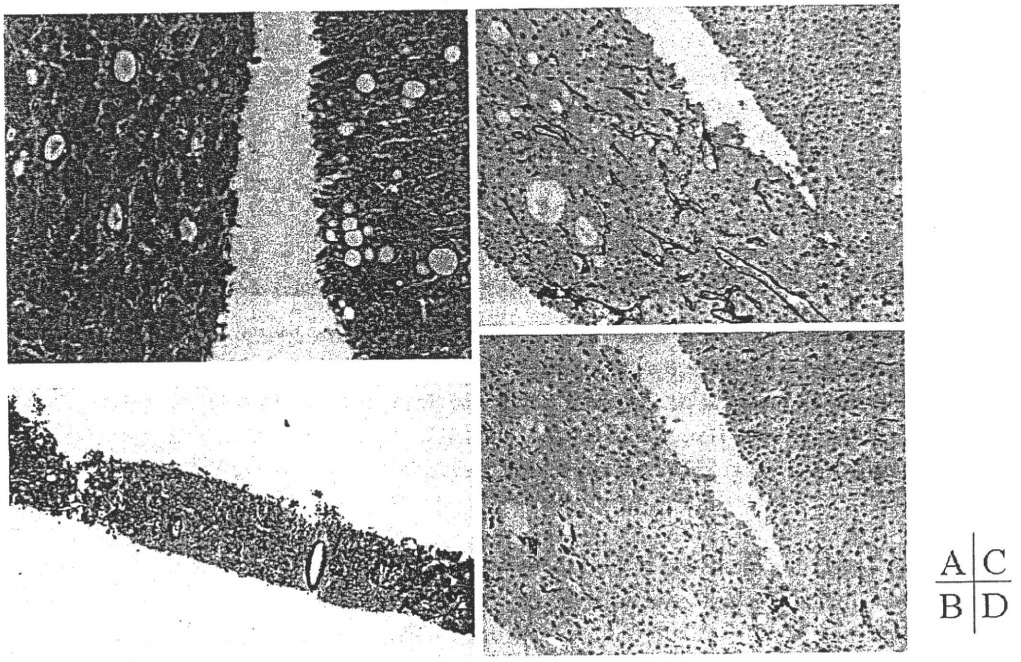

Figure 6. **Pathologic findings in biopsy specimens. A: A specimen from the largest nodule (20×21 mm, in segment IV) consisted of tissue from the nodule (left) and surrounding liver (right). Tissue from the nodule showed mildly increased cellularity, a distinct trabecular pattern, and occasional pseudoglands. The surrounding liver showed mild fatty change (hematoxylin and eosin stain, ×200). B: A specimen from a smaller nodule (7×8 mm, in segment IV) contained a fibrous scar with anomalous arterial blood vessels (Azan stain, ×100). C: Immunostaining with anti-CD34 showed a strongly positive reaction in sinusoidal endothelial cells (capillarization) in tissue from the largest nodule (left), exceeding the reactivity in the surrounding tissue (right)(×200). D: Immunostaining with anti-CD68 demonstrated fewer positive cells in the largest nodule (left) than in the surrounding liver (right)(×200).**

larity seen by dynamic CT corresponded to anomalous arterial vessels in a fibrous scar, as well as marked sinusoidal capillarization. Findings by SPIO-enhanced MRI are explained by a decrement of CD-68-positive cells, possibly Kupffer cells, along the sinusoids. Accordingly, the nodules were highly suggestive of multiple FNH lesions.

Various hyperplastic liver lesions have been associated with portal venous abnormalities, and frequently occurring in association with CAPV (7), a rare malformation seen mostly in children. Reviewing the literature concerning CAPV, De Gaetano et al found that 15 of 31 cases had hepatic tumors (7): hepatoblastoma in 1, FNH in 9, adenoma in 1, nodular regenerative hyperplasia (NRH) in 1, hemangioma in 1, and hepatocellular carcinoma (HCC) in 2. Additionally, 2 cases of CAPV with FNH (8, 9), 1 case with HCC (10), 3 cases with NRH (11, 12), and 3 cases with hyperplastic nodular hepatic lesions (13-15) recently were reported. Hemodynamic imbalance with insufficient blood supply and nonuniform arterial perfusion to the liver would appear likely to induce hyperplasia in livers of patients with CAPV (16, 17). In addition, the portal vein carries substances in splanchnic venous blood such as insulin, glucagon, epidermal growth factor and other hepatotrophic peptides. As these substances regulate hepatocytic function and development, diversion of portal flow may induce abnormalities by causing dysregulation (18, 19).

Alteration of portal and systemic venous structure thus would appear to be a likely cause of hyperplasia of hepatocytes in the present case, as in the occurrence of hyperplasic liver lesions in patients with CAPV. Grün et al reported that portacaval anastomosis in rats resulted in hyperestrogenemia and hypoandrogenemia, while the occurrence of FNH also has been observed (20). In the present case testosterone was in the normal range (5.30 ng/ml; normal, 2.07 to 7.61), while estradiol was elevated (55 pg/ml; normal, 15 to 35). Elevated estrogen in the systemic circulation after portacaval shunting may induce formation of hyperplastic nodular lesions in the liver. The observed association of FNH with oral contraceptive use (21), as well as estrogen receptor expression in mammalian hepatocytes (22) would support this speculation.

While the occurrence of hyperplastic liver lesions including FNH has been observed in animal model after experimental portacaval shunting (20, 23), few reports have described benign hyperplastic lesions in livers of a patient with portacaval anastomosis. FNH has been reported in nine patients with type I glycogen storage disease (GSD-I), while five of these nine patients had been treated with a portacaval shunt (24, 25). Takamura et al (24) suggested that portacaval shunts favored the development of FNH in livers of the patients with GSD-I, an inherited disorder frequently accompanied by hepatic tumors (mainly adenomas). Additionally, several studies suggested that portasystemic shunt was associated with a higher risk of developing HCC in the patients with cirrhosis (26, 27). From such previous literature portacaval anastomosis is considered to promote induction of tumor in the liver essentially inclined to develop liver tumor, for example cirrhotic liver or liver of patient with GSD-I. Webster et al (28) reported a hepatic adenoma that became greater after mesocaval shunt for portal venous obstruction, suggesting that portasystemic shunt promotes the development of hyperplastic lesion that already existed in the liver. The liver of present patient was not cirrhotic, Budd-Chiari syndrome and GSD-I were also excluded, and earlier abdominal ultrasonography had shown no nodular lesion in the liver. Thus, our case suggested that portasystemic shunt may produce a tumor in a liver with originally no tumor, and in the liver of a patient with no inclination to develop hepatic neoplasm. Previous malaria infection might have been assosiated with the occurrence of hyperplastic lesion in the present case. However, there is no report of hepatic tumor of a patient with malaria, only fibrosing necrotic nodule has been reported (29). One study suggested a negative correlation of malaria with HCC (30).

Our report appears to be the first to describe benign hyperplastic lesions in the human liver following portosystemic anastomosis, except for patients with GSD-I. This case suggests that iatrogenic changes in the portal and systemic venous systems can result in hyperestrogenemia and disappearance of intrahepatic portal flow to cause hyperplastic nodule formation in a previously normal liver. This case supports the theory that abnormality of intrahepatic blood flow initiates hyperplasia of hepatocytes which is often observed in the liver of patients with CAPV.

References

1. Hallett EB, Holton GW, Paterson JC, Schilling JA. Liver blood and following Eck fistula; liver blood flow before and after splenectomy. Surg Gynecol Obstet **95**: 401-406, 1952.
2. Blakemore AH, Lord JW. A non-suture method of blood vessel anastmosis: review of experimental study. Reports of clinical cases. Ann Surg **121**: 435-453, 1945.
3. Morgan G, Superina R. Congenital absence of the portal vein: two cases and a proposed classification system for portasystemic vascular anomalies. J Pediatr Surg **29**: 1239-1241, 1994.
4. Howard ER, Davenport M. Congenital extrahepatic portocaval shunts-the Abernethy malformation. J Pediatr Surg **32**: 494-497, 1997.
5. Hirohashi K, Yamamoto T, Tanaka S, et al. Immunohistochemical demonstration of sinusoidal capillarization in human benign liver tumors: distinction from neoangiogenesis. Hepatol Res **28**: 87-93, 2004.
6. Tanaka M, Nakashima O, Wada Y, Kage M, Kojiro M. Pathomorphological study of Kupffer cells in hepatocellular carcinoma and hyperplastic nodular lesions in the liver. Hepatology **24**: 807-812, 1996.
7. De Gaetano AM, Gui B, Macis G, Manfredi R, Di Stasi C. Congenital absence of the portal vein associated with focal nodular hyperplasia in the liver in an adult woman: imaging and review of the literature. Abdom Imaging **29**: 455-459, 2004.
8. Schmidt S, Saint-Paul MC, Anty R, Bruneton JN, Gugenheim J, Chevallier P. Multiple focal nodular hyperplasia of the liver asso-

ciated with congenital absence of the portal vein. Gastroenterol Clin Biol **30**: 310-313, 2006 (in French, Abstract in English).

9. Takagaki K, Kodaira M, Kuriyama S, et al. Congenital absence of the portal vein complicating hepatic tumors. Intern Med **43**: 194-198, 2004.

10. Pichon N, Maisonnette F, Pichon-Lefievre F, Valleix D, Pillegand B. Hepatocarcinoma with congenital agenesis of the portal vein. Jpn J Clin Oncol **33**: 314-316, 2003.

11. Tsuji K, Naoki K, Tachiyama Y, et al. A case of congenital absence of the portal vein. Hepatol Res **31**: 43-47, 2005.

12. Grazioli L, Alberti D, Olivetti L, et al. Congenital absence of portal vein with nodular regenerative hyperplasia of the liver. Eur Radiol **10**: 820-825, 2000.

13. Kanamori Y, Hashizume K, Kitano Y, Sugiyama M, Motoi T, Tange T. Congenital extrahepatic portocaval shunt (Abernethy type 2), huge liver mass, and patent ductus arteriosus-a case report of its rare clinical presentation in a young girl. J Pediatr Surg **38**: E15, 2003.

14. Tanaka Y, Takayanagi M, Shiratori Y, et al. Congenital absence of portal vein with multiple hyperplastic nodular lesions in the liver. J Gastroenterol **38**: 288-294, 2003.

15. Turkbey B, Karcaaltincaba M, Demir H, Akcoren Z, Yuce A, Haliloglu M. Multiple hyperplastic nodules in the liver with congenital absence of portal vein: MRI findings. Pediatr Radiol **36**: 445-448, 2006.

16. Wanless IR, Mawdsley C, Adams R. On the pathogenesis of focal nodular hyperplasia of the liver. Hepatology **5**: 1194-1200, 1985.

17. Kondo F. Benign nodular hepatocellular lesions caused by abnormal hepatic circulation: etiological analysis and introduction of a new concept. J Gastroenterol Hepatol **16**: 1319-1328, 2001.

18. Starzl TE, Francavilla A, Halgrimson CG, et al. The origin, hormonal nature, and action of hepatotrophic substances in portal venous blood. Surg Gynecol Obstet **137**: 179-199, 1973.

19. Francavilla A, Starzl TE, Porter K, et al. Screening for candidate hepatic growth factors by selective portal infusion after canine Eck's fistula. Hepatology **14**: 665-670, 1991.

20. Grün R, Grün M, Rolfs A, Kaffarnik H. Hyperestrogenemia following various shunt operations: on the role of estrogens in the development of focal nodular hyperplasia of the liver. Z Gastroenterol **27**: 378-382, 1989 (in German, Abstract in English).

21. Pain JA, Gimson AE, Williams R, Howard ER. Focal nodular hyperplasia of the liver: results of treatment and options in management. Gut **32**: 524-527, 1991.

22. Eisenfeld AJ, Aten R, Weinberger M, Haselbacher G, Halpern K, Krakoff L. Estrogen receptor in the mammalian liver. Science **191**: 862-865, 1976.

23. Rasenack U. Changes in the liver and brain after portacaval and modified portacaval end-to-side anastomosis: histology, autoradiography and clinical studies. Fortschr Med **99**: 107-112, 1981 (in German, Abstract in English).

24. Takamura M, Mugishima H, Oowada M, Harada K, Uchida T. Type Ia glycogen storage disease with focal nodular hyperplasia in siblings. Acta Paediatr Jpn **37**: 510-513, 1995.

25. Sakatoku H, Hirokawa Y, Inoue M, Kojima M, Yabana T, Sakurai M. Focal nodular hyperplasia in an adolescent with glycogen storage disease type I with mesocaval shunt operation in childhood: a case report and review of the literature. Acta Paediatr Jpn **38**: 172-175, 1996.

26. Bjorneboe M, Andersen JR, Christensen U, Skinhoj P, Jensen OM. Does a portal-systemic shunt increase the risk of primary hepatic carcinoma in cirrhosis of the liver? Scand J Gastroenterol **20**: 59-64, 1985.

27. Banares R, Nunez O, Escudero M, et al. Patients with cirrhosis and bare-stent TIPS may have increased risk of hepatocellular carcinoma. Hepatology **41**: 566-571, 2005.

28. Webster MW, Van Thiel DH, Bron KM, Barnes EL. Hepatic adenoma associated with portasystemic shunting in a young woman. Digestion **19**: 328-334, 1979.

29. Desai S, Prabhu SR, Shrividya S. Fibrosing necrotic nodule of the liver. Indian J Gastroenterol **14**: 23-24, 1995.

30. Lu SN, Lin TM, Chen CJ, et al. A case-control study of primary hepatocellular carcinoma in Taiwan. Cancer **62**: 2051-2055, 1988.

ONCOLOGY REPORTS 18: 545-552, 2007

Significance of glucose intolerance and SHIP2 expression in hepatocellular carcinoma patients with HCV infection

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Received January 30, 2007; Accepted May 7, 2007

Abstract. Glucose intolerance frequently is found in hepatocellular carcinoma (HCC) patients with hepatitis C virus (HCV) infection; however, the significance of glucose intolerance remains unclear. In addition, SH2 domain-containing inositol phosphatase (SHIP) 2 is a negative regulator of intracellular insulin signaling; however, changes in SHIP2 expression have not been investigated in HCC. To assess the significance of glucose intolerance, we analyzed 118 HCC patients with HCV infection. Twenty HCC specimens were used for immunoblotting and immunostaining for SHIP2. Patients were classified into two groups: a glucose intolerance group (n=39) and a normal glucose tolerance group (n=79). There was no significant difference in the disease-free survival (P=0.838) or long-term survival (P=0.091) between the groups. However, for males, the cumulative survival rate was significantly lower in the glucose intolerance group (n=22) than that in the normal glucose tolerance group (n=52) (P=0.036). In multivariate analysis, Child-Pugh class (P=0.0003) and glucose intolerance (P=0.036) were identified as statistically significant and independent prognostic factors in males. SHIP2 expression level decreased in HCC compared to that in nontumor tissues. In conclusion, this study is the first to demonstrate the significance of glucose intolerance in prognosis of male HCC patients and down-regulation of SHIP2 expression in HCC.

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Key words: hepatocellular carcinoma, hepatitis C virus, SH2 domain-containing inositol phosphatase 2, insulin resistance, prognosis

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world. The incidence of HCC has increased in Eastern Asia and Africa during the past several decades and has also increased in the US (1). This trend has been attributed to hepatitis C virus (HCV) infection. In many areas of the world, HCV infection accounts for more than half of the cases of HCC, and in Japan, ~75% of all HCC are associated with chronic liver disease and HCV infection (2). In order to prevent and treat this malignancy, it is important to understand the pathogenesis of HCC in patients with HCV infection.

Liver is one of the major organs regulating glucose metabolism and patients with chronic liver diseases frequently show glucose intolerance which is called hepatogenous diabetes (3). Although its pathogenesis involves various factors, insulin resistance and hyperinsulinemia are thought to play major roles (4,5). In chronic liver disease associated with HCV infection, the prevalence of glucose intolerance is higher than in other chronic liver diseases, including hepatitis B infection (6). We previously reported that down-regulation of insulin receptor substrate 1/2, central molecules for intracellular insulin signaling, was seen in livers from HCV core-transgenic mice as well as patients with HCV infection (7). In HCV hyperendemic areas, anti-HCV-positive subjects were nearly three-fold as likely as anti-HCV-negative subjects to develop diabetes mellitus with insulin resistance (8). Collectively, these findings suggest that HCV directly causes hepatic insulin resistance and subsequent hyperinsulinemia (9).

Glucose intolerance is frequently observed in HCC patients with chronic liver disease. In addition, glucose intolerance has been suggested as a potential risk factor for the incidence of HCC. Several large population-based cohort studies showed that the incidence of HCC was increased 2-4-fold in patients with diabetes mellitus (10,11). However, it is unclear how glucose intolerance is linked to the incidence of HCC in patients. Moreover, it is unclear whether the presence of glucose intolerance has an impact on the prognosis in HCC patients with HCV infection.

Insulin is known as one of the most important factors not only for a variety of metabolic pathways, but also for cell growth (12). Insulin stimulates activation of the tyrosine kinase activity of the insulin receptor and subsequently causes the phosphorylation of insulin receptor substrate families. Upon tyrosine phosphorylation, these proteins interact with signaling molecules through their Src homology 2 (SH2) domains (13), resulting in a diverse series of signaling pathways, including activation of phosphatidylinositol 3-kinase (PI3K) and Akt cascade (14), and Ras and mitogen-activated protein (MAP) kinase cascade (15). These cascades regulate cell proliferation, differentiation, and apoptosis. Changes in these insulin signaling cascades are involved in cell growth.

SH2-containing inositol phosphatase (SHIP)-2 plays an important role in the negative regulation of insulin sensitivity (16,17). In insulin signaling, PI3K produces phosphatidylinositol 3,4,5-trisphosphate (PIP3) from phosphatidylinositol 3,4-bisphosphate (PIP2) (14). PIP3 mediates insulin signals to downstream molecules including Akt (18). SHIP2 hydrolyses the PI3K product PIP3 to PIP2, leading to decreased level of this phospholipid and, simultaneously, reduced activation of PI3K and Akt signaling cascade (19). In addition, SHIP2 causes down-regulation of Ras and MAPK signaling cascade (20). Thus, SHIP2 suppresses cell growth through regulating intracellular insulin sensitivity. However, changes in expression of SHIP2 in patients with HCC have not been investigated.

The aim of this study was to evaluate the long-term impact of glucose intolerance and to examine the expression of SHIP2 in HCC patients with HCV infection.

Materials and methods

Materials. All reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan) unless otherwise indicated.

Patients. Between January 1994 and December 2000, 330 Japanese patients with HCV infection at the Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine were diagnosed with HCC. These patients had a single tumor ≤5 cm, and three or fewer tumors each ≤3 cm. HCC with HCV infection was defined as HCC with positive hepatitis C virus antibody and negative hepatitis B virus surface antigen. Among these patients, 120 patients were randomly selected and their blood samples analyzed. Blood samples were obtained from each patient on admission and stored at -20˚C for later analysis. Plasma glucose levels were measured by a glucose oxidase method. Serum insulin levels were measured using a sandwich enzyme immunoassay kit (Eiken Chemical, Tokyo, Japan).

Insulin resistance was calculated on the basis of fasting levels of plasma glucose and insulin, according to the homeostasis model assessment (HOMA) method. The formulas for the HOMA model are: Insulin resistance (HOMA-IR) = fasting glucose (mg/dl) x fasting insulin (μU/ml)/405. All patients were classified either into the glucose intolerance group or the normal glucose tolerance group based on the presence of hyperinsulinemia and/or insulin resistance. The glucose intolerance group was defined by fasting insulin level ≥15 μU/ml and/or HOMA-IR value ≥3, and the normal glucose tolerance group was defined by fasting insulin level <15 μU/ml and/or HOMA-IR value <3 according to previous studies (21,22). We enrolled 120 patients in this study, however, 2 patients were excluded because of type 1 diabetes mellitus. The remaining 118 patients were retrospectively examined.

The diagnosis of HCC was histologically confirmed by needle biopsy under ultrasonographic guidance in 78 of 118 patients. In the remaining 40 patients, the diagnosis of HCC was made based on the findings of typical radiological features on ultrasonography, contrast enhanced dynamic computed tomography, magnetic resonance imaging, and hepatic angiography along with elevated alpha-fetoprotein levels. The pretreatment hepatic functional reserve was determined using the Child-Pugh scoring system (23). Tumor staging of HCC was determined using the tumor node metastasis (TNM) classification (24). The measurement of tumor size was based on the largest dimension observed on ultrasonography and computed tomography. Alcohol drinking was defined as an average daily consumption of an amount exceeding 60 g per day of pure ethanol over a period of >5 years based on the report of Donato *et al* (25). Body mass index (BMI) was calculated as body weight in kg divided by the square of height in meters (kg/m^2). The study protocol was approved by the institutional review board, and informed consent for participation in the study was obtained from each subject and conformed to the guidelines of 1995 Declaration of Helsinki.

Treatment and follow-up. As treatment for HCC, 22 patients underwent hepatic resection, 80 patients received percutaneous ethanol injection therapy, microwave coagulation therapy or percutaneous radiofrequency ablation therapy, and 16 received transcatheter arterial chemoembolization. No patient underwent liver transplantation.

After initial treatment, the condition of each patient was followed carefully. Serum biochemistries, alpha-fetoprotein levels, and Child-Pugh score were measured and ultrasonography was performed monthly. Contrast enhanced dynamic computed tomography was performed every 3 months until 6 months post-treatment and every 6 months beyond 6 months post-treatment. Magnetic resonance imaging was performed as a supplemental examination. The closing date of this study was December 2004 or the time of the patient's death. Follow-up ranged from 12 to 128 months (median, 57 months). During follow-up, 74 patients died of HCC. No patient died of complications of cirrhosis or diabetes.

Measurement of HCV core. Serum HCV core levels were evaluated using a newly developed HCV core antigen enzyme linked immunosorbent assay test system (Ortho-clinical Diagnostics K.K., Tokyo, Japan) as previously described (26). This assay has high stability and reproducibility under all conditions and the detection limit is 50 fmol/l.

Human HCC tissues. Tumor and nontumor tissues were obtained from 20 patients with HCC who underwent partial hepatectomy [15 men and 5 women, mean age 67.7±6.7 years (range, 53-76 years)]. Tissue sections were stained with hematoxylin-eosin, and each HCC was histologically graded into one of three categories: well-, moderately- or

poorly-differentiated, according to criteria proposed by the Liver Study Group of Japan (27). The tumors included one well-differentiated, 18 moderately differentiated and one poorly differentiated HCCs. All sections were examined by immunostaining and four tissues from men were used for immunoblotting.

Immunoblotting. Tumor and nontumor tissues were obtained from patients with HCC who underwent partial hepatectomy, and were homogenized on ice in RIPA buffer (150 mmol/l NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 50 mmol/l/Tris-HCl, pH 7.5) containing 100 ng/phenylmethylsulfonyl fluoride, 4 μg/ml aprotinin, 2 μg/ml leupeptin, 1 μg/ml pepstatin, 10 μg/ml antipain, 10 μg/ml soybean trypsin inhibitor, and 2 mmol/l ethylenediaminetetraacetic acid. Then, sodium dodecyl sulfate-polyacrylamide gel electrophoresis sample buffer was added and immediately boiled for 5 min. An equal amount of protein (50 μg) of tumor or nontumor liver homogenates was applied to each lane and was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 7.5% acryl amide gels. The resolved proteins were transferred to polyvinylidene difluoride membranes (Amersham International, Buckinghamshire, UK). The membranes were incubated with an anti-human SHIP2 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:100 (vol/vol) with PBS at room temperature for 1 h, and then incubated with a horseradish peroxidase-conjugated goat anti-mouse IgG (Nichirei, Tokyo, Japan) diluted 1:1000 at room temperature for 1 h. After several washes, the membranes were incubated with chemiluminescence reagents (ECL-kit; Amersham International) for 1 min and immediately exposed on radiograph film.

Immunohistochemistry. Tumor and nontumor tissues were obtained from patients with HCC who underwent partial hepatectomy. Paraffin-embedded sections (3-μm) were deparaffinized and incubated in 0.2% (vol/vol) hydrogen peroxide in methanol for 15 min to inhibit endogenous peroxidase activity. To prevent nonspecific binding, sections were incubated with protein block serum-free (Dako, Kyoto, Japan) at room temperature for 30 min. Sections were then incubated with an anti-human SHIP2 polyclonal antibody (Santa Cruz Biotechnology) diluted 1:50 (vol/vol) with phosphate-buffered saline (pH 7.4, 130 mmol/l NaCl, 2 mmol/l NaH_2PO_4, and 7 mmol/l Na_2HPO_4, PBS) overnight at 4°C. Sections were washed 3 times for 5 min in PBS and incubated with a horseradish peroxidase-conjugated anti-goat antibody (Nichirei) diluted 1:50 (vol/vol) with PBS at RT for 1 h. After several washes with PBS, peroxidase activity was visualized using 3,3'-diaminobenzidine-tetrahydrochloride. Finally, sections were counterstained with hematoxylin.

Quantitation of SHIP2 expression. Immunoblotting and immunostaining for SHIP2 were quantified as previously described (28). Briefly, immunoblotting intensity and immunostaining intensity were quantified by measuring pixel intensities by an investigator without any information regarding the group assignment of the patients. This analysis was carried out on a Macintosh computer (PowerBook G4; Apple Computer, Cupertino, CA) using the public domain NIH Image-J program for Mac OS X (developed at the National Institutes of Health and available by anonymous FTP from http://rsb.info.nih.gov/ij/download.html).

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 the Chi-square test or the Fisher exact test for discrete variables. Cumulative overall survival and disease-free survival were estimated by the Kaplan-Meier method. Any significant differences in cumulative overall survival or disease-free survival rates were determined using the log-rank test. A Cox proportional hazard model was used for univariate and multivariate analysis to identify any independent variables that are related to cumulative overall survival. The variables analyzed were age at HCC diagnosis, gender, body mass index, alcohol drinking, Child-Pugh class, glucose intolerance, alpha-fetoprotein, tumor stage, and maximal tumor size. All P-values were two-tailed, and a level of <0.05 was considered to be statistically significant. Statistical analysis was performed using Stat View software (version 5.0: SAS Institute Inc., Cary, NC).

Results

Patient characteristics. A total of 118 patients were enrolled. The mean age was 66.9±6.8 years (range, 50-87 years). The mean BMI value was 22.6±2.6 (range, 14.7-32.3). Twenty-seven patients (23%), were considered alcohol drinkers. Among the 118, 79 patients (67%) were classified as the normal glucose tolerance group while 39 patients (33%) were classified as the glucose intolerance group. This classification was based on analysis of blood samples obtained at initial admission. Of the 39, 14 (36%) had already been diagnosed as having diabetes mellitus. The 14 had been treated as follows: 2 with insulin, 8 with oral hypoglycemics, and the rest were managed using diet only. The baseline clinical characteristics of both the glucose intolerance and normal glucose tolerance groups are summarized in Table I. BMI values (P=0.0008), bilirubin levels (P=0.048) and HCV core levels (P=0.049) in the glucose intolerance group were significantly higher compared to those of the normal glucose tolerance group. Albumin levels in the glucose intolerance group were significantly lower than that of the normal glucose tolerance group (P=0.007).

Disease-free survival rate and survival rate based on glucose intolerance. The comparison of the disease-free survival rate based on glucose intolerance was not significantly different between the groups (Fig. 1A) (P=0.838) nor was cumulative survival rate (Fig. 1B) (P=0.091). The 1-, 3-, and 5-year cumulative survival rates were 100, 64.1, and 42%, respectively, in the glucose intolerance group, and 97.4, 78.2, and 57.2%, respectively, in the normal glucose tolerance group.

Univariate and multivariate analysis in all patients. Cox proportional hazard regression analysis was performed to determine which of the 9 variables were independently associated with cumulative overall survival. The results of

univariate analysis are shown in Table II. Child-Pugh class [hazard ratio (HR): 2.81, 95% confidence interval (CI): 1.74-4.54, P<0.0001], and tumor stage (HR: 1.75, 95% CI: 1.09-2.82, P=0.021) were found to be significant factors affecting survival. In multivariate analysis, Child-Pugh class (HR: 3.37, 95% CI: 1.95-5.82, P<0.0001) was identified as an independent prognostic factor (Table II).

The difference in glucose intolerance between male and female. This study included 74 males and 44 females. Among patients who were glucose intolerant, there was no significant difference in the number of males and females [22 males (29.7%) vs. 17 females (38.6%); P=0.482]. There was also no significant difference in plasma glucose levels between males and females (87.6±29.8 vs. 92.8±39.6; P=0.264). However, the mean fasting insulin level in males (15.2±23.63 μU/ml) was significantly higher than in females (15.1±13.8 μU/ml) (P=0.045). Thus, glucose intolerance in males was more severe than that in females. We evaluated further the ability of glucose intolerance to influence long-term outcomes in male patients with HCC.

Male patient characteristics. Baseline clinical characteristics of male patients are summarized in Table III. Although BMI value in the glucose intolerance group was significantly higher compared to those in the normal glucose tolerance group (P=0.009), the mean value of BMI was within normal range. There were no significant differences in other clinical characteristics.

Table II. Univariate and multivariate analyses of survival for hepatocellular carcinoma by Cox proportional hazard model.

Variable	HR	95% CI	P-value
Univariate analysis			
Gender	1.12	0.70-1.80	0.637
Age	1.26	0.78-2.02	0.341
BMI	1.16	0.66-2.05	0.609
Alcohol drinking	1.32	0.77-2.27	0.319
Child-Pugh class[a]	2.81	1.74-4.54	<0.0001
Glucose intolerance	1.53	0.93-2.50	0.093
AFP	0.92	0.58-1.46	0.725
Tumor stage[b]	1.75	1.09-2.82	0.021
Maximal tumor size	0.97	0.48-1.95	0.932
Multivariate analysis			
Child-Pugh class[a]	3.37	1.95-5.82	<0.0001

BMI, body mass index; AFP, alpha-fetoprotein. [a]Child-Pugh scoring system (23). [b]TNM classification (24).

Disease-free survival rate and survival rate based on glucose intolerance in male patients. Disease-free survival rates were not significantly different between the two groups (Fig. 2A) (P=0.378). The 1-, 2-, and 3-year disease-free survival rates were 45.5, 27.3, and 18.2%, respectively, in the glucose intolerance group and 61.5, 32.7, and 26.9%, respectively, in

Table I. Comparison of patient characteristics based on glucose intolerance.

	Glucose intolerance group (n=39)	Normal glucose tolerance group (n=79)	P-value
Gender (male/female)	22/17	52/27	0.320
Age (yrs.)	67.7±7.0	66.5±6.8	0.339
BMI	23.8±2.6	22.1±2.4	0.0008
Alcohol drinking (+/-)	9/30	18/61	0.972
Child-Pugh class (A/B/C)[a]	25/13/1	60/18/1	0.392
AST (IU/l)	79.4±31.9	76.0±35.6	0.355
Bilirubin (mg/dl)	1.2±0.4	1.0±0.4	0.048
Albumin (g/dl)	3.4±0.4	3.6±0.4	0.007
AFP (ng/ml)	224.4±992.0	414.8±2363.2	0.567
HCV core	5031.3±5335.1	3235.1±3798.7	0.049
Tumor stage (I/II)[b]	23/16	54/25	0.423
Maximal tumor size (mm)	20.0±8.1	21.0±10.3	0.777
(≤30 / >30 to ≤50)	36/3	66/13	0.258

Data are expressed by mean ± SD. BMI, body mass index; AST, glutamine oxaloacetic transaminase; AFP, alpha-fetoprotein; HCV, hepatitis C virus. [a]Child-Pugh scoring system (21). [b]TNM classification (22).

Table III. Comparison of the characteristics of male patients based on glucose intolerance.

	Glucose intolerance group (n=22)	Normal glucose tolerance group (n=52)	P-value
Age (yrs.)	65.6±6.9	66.7±6.8	0.586
BMI	23.4±2.2	21.7±2.4	0.009
Alcohol drinking (+/-)	9/13	18/34	0.609
Child-Pugh class (A/B/C)[a]	15/6/1	44/8/0	0.133
AST (IU/l)	82.0±32.5	77.0±36.8	0.303
Bilirubin (mg/dl)	1.1±0.4	1.0±0.3	0.052
Albumin (g/dl)	3.5±0.4	3.7±0.4	0.079
AFP (ng/ml)	337.4±1321.0	183.9±554.2	0.404
Tumor stage (I/II)[b]	12/10	33/19	0.647
Maximal tumor size (mm)	21.1±8.6	21.0±10.3	0.776
(≤30 / >30 to ≤50)	20/2	44/8	0.725

Data are expressed by mean ± SD. BMI, body mass index; AST, glutamine oxaloacetic transaminase; AFP, alpha-fetoprotein. [a]Child-Pugh scoring system (23). [b]TNM classification (24).

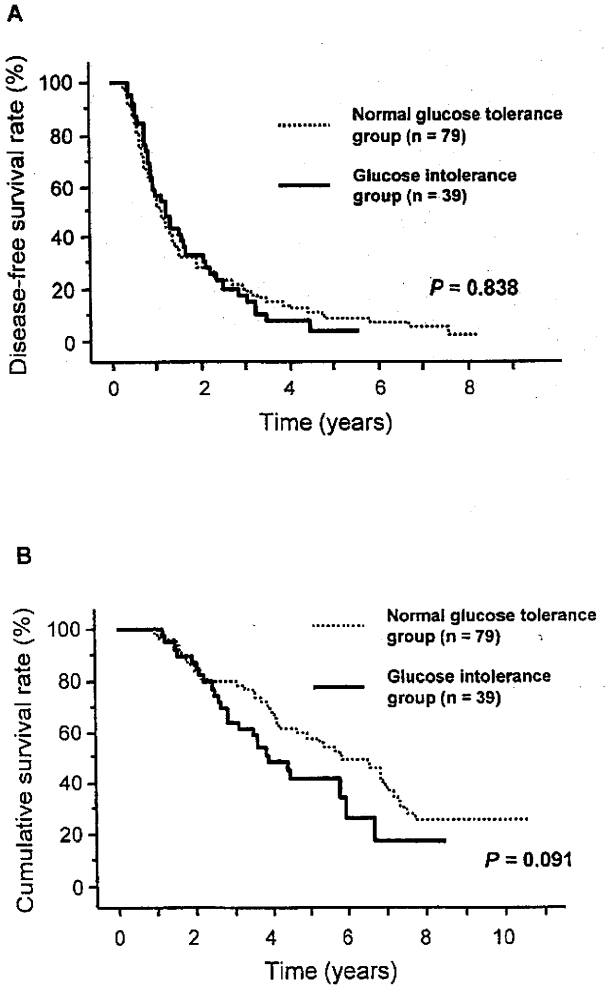

Figure 1. Comparison of disease-free survival rate (A) and cumulative survival rate (B) for all HCC patients between the normal glucose tolerance and the glucose intolerance groups.

Table IV. Univariate and multivariate analyses of survival for hepatocellular carcinoma in males by Cox proportional hazard model.

Variable	HR	95% CI	P-value
Univariate analysis			
Age	1.14	0.64-2.04	0.649
BMI	0.96	0.43-2.15	0.920
Alcohol drinking	1.27	0.70-2.31	0.428
Child-Pugh class[a]	3.79	1.96-7.14	<0.0001
Glucose intolerance	1.95	1.03-3.62	0.039
AFP	1.15	0.65-2.05	0.628
Tumor stage[b]	2.05	1.11-3.80	0.023
Maximal tumor size	0.94	0.40-2.21	0.880
Multivariate analysis			
Child-Pugh class[a]	4.26	1.95-9.34	0.0003
Glucose intolerance	2.30	1.05-5.03	0.036

BMI, body mass index; AFP, alpha-fetoprotein. [a]Child-Pugh scoring system (23). [b]TNM classification (24).

the normal glucose tolerance group. On the other hand, the cumulative survival rate in the glucose intolerance group was significantly poorer than that in the normal glucose tolerance groups (Fig. 2B) (P=0.036). The 1-, 3-, and 5-year cumulative survival rates were 100, 50, and 36.4%, respectively, in the glucose intolerance group and 98, 84.3, and 56.5%, respectively, in the normal glucose tolerance group.

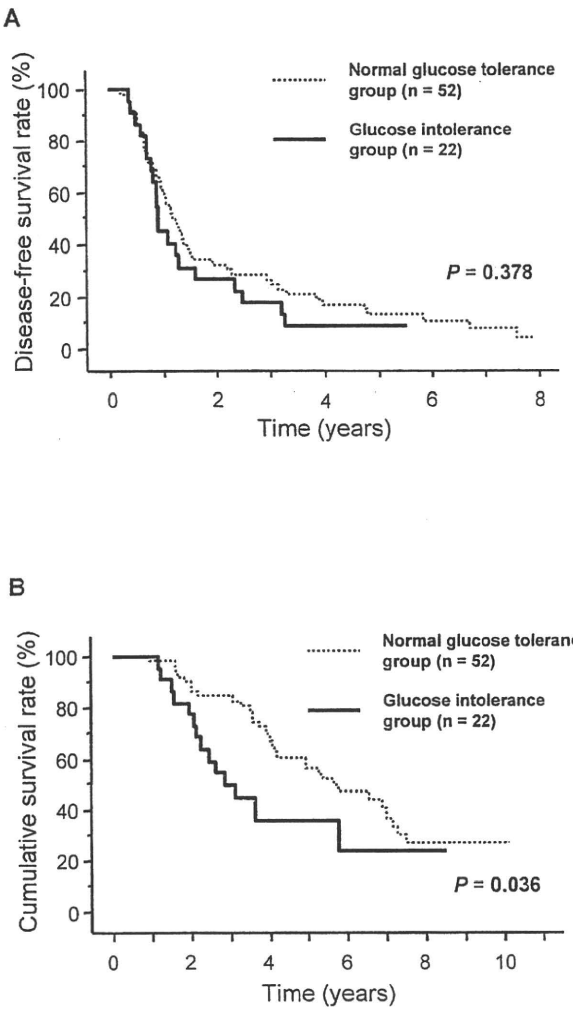

Figure 2. Comparison of disease-free survival rate (A) and cumulative survival rate (B) for male HCC patients between the normal glucose tolerance and the glucose intolerance groups.

Univariate and multivariate analysis in male patients. The results of univariate analysis of male patients are shown in Table IV. Child-Pugh class (HR: 3.79, 95% CI: 1.96-7.14, P<0.0001), glucose intolerance (HR: 1.95, 95% CI: 1.03-3.62, P=0.039) and tumor stage (HR: 2.05, 95% CI: 1.11-3.80, P=0.023) were identified as being statistically independent prognostic factors. In the multivariate analysis, Child-Pugh class (HR: 4.26, 95% CI: 1.95-9.34, P=0.0003) and glucose intolerance (HR: 2.30, 95% CI: 1.05-5.03, P=0.036) were identified as independent prognostic factors.

Protein expression levels of SHIP2 in the tumor and nontumor livers from HCC patients. Protein expression levels of SHIP2 in tumor and nontumor livers from patients with HCC were examined by immunoblotting and immunostaining. Immunoblotting revealed that SHIP2 expression was decreased in HCC tissues compared to that in nontumor tissues (Fig. 3A). Quantitation of immunoblotting intensity confirmed that SHIP2 expression was significantly down-regulated in HCC tissues compared to that in nontumor tissues (48.5±17.2 vs. 151.2±35.3 arbitrary units, P<0.05) (Fig. 3B). Immunostaining demonstrated cytoplasmic SHIP2 expression in nontumor hepatocytes, but not in peripherally located well-differentiated

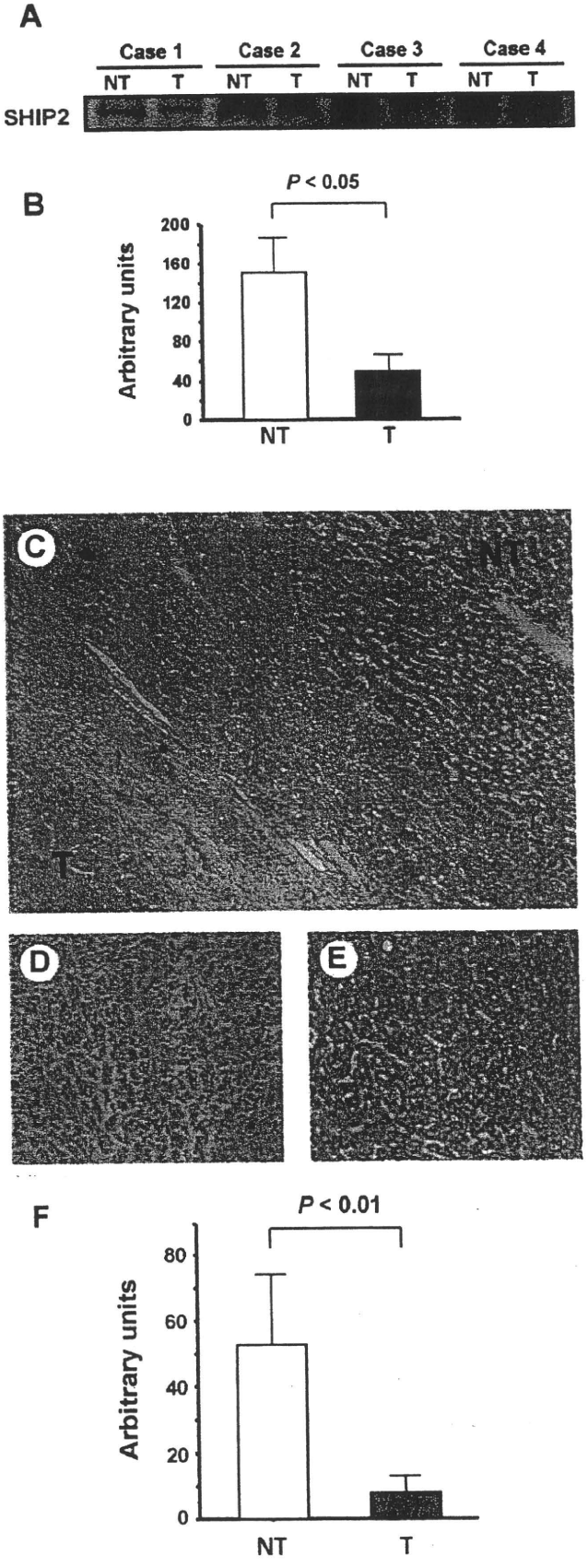

Figure 3. Expression levels of SHIP2 in tumor and nontumor livers from patients with HCC. (A) Immunoblotting for SHIP2. Proteins from tumor and nontumor extracts were immunoblotted with anti-SHIP2 antibodies. (B) Quantitation of immunoblotting intensity for SHIP2 in tumor and nontumor tissues. (C) Immunostaining for SHIP2. SHIP2 staining of liver sections from border areas between HCC tissue (T) and nontumor tissue (NT), (D) tumor tissue, and (E) nontumor tissue. (F) Quantitation of immunostaining intensity for SHIP2 in tumor and nontumor sections.

HCC cells (Fig. 3C-E). Quantitation of immunostaining intensity confirmed that SHIP2 expression was significantly decreased in HCC cells compared to that of nontumor hepatocytes (13.5±3.1 vs. 192.2±15.9 arbitrary units, $P<0.01$) (Fig. 3F).

Discussion

In Japanese HCC patients, survival is poorer in patients with diabetes mellitus compared to those without diabetes mellitus (29). On the other hand, two other cohort studies had reported that diabetes mellitus does not have a significant influence on the perioperative outcome or prognosis after hepatic resection for HCC (30,31). Thus, the impact of glucose intolerance for HCC patients remains controversial. A major contrast between their studies is that the majority of Japanese patients had HCV infection, whereas the patients of other studies had predominantly HBV infection. In a large population based cohort study, the relative risk for the development of HCC among those with HBV infection was 9.6, and the relative risk among those with HCV infection was 2.7. This result suggests that HBV itself, compared to HCV, may have greater potential for being carcinogenic (32). Furthermore, we previously reported that more severe insulin resistance was present in patients with HCV infection than in patients with HBV infection. HCV core protein has been reported as a responsible factor to the development of glucose intolerance (9). Similarly, HCV core protein level was associated with glucose intolerance. Therefore, glucose intolerance may have a significant influence on survival of HCC patients with HCV infection.

In this study, we investigated the impact of glucose intolerance in HCC patients with HCV infection. We observed no change in either disease-free survival or long-term survival. Barbara *et al* reported that Child-Pugh class was independently correlated with survival in the natural history of small untreated HCC among patients with cirrhosis (33). In agreement with the previous study, Child-Pugh class was an independent prognostic factor in the multivariate analysis of this study. Thus, our data imply that the ability to change the clinical course of the disease is strongly influenced by liver function.

Although the effect of glucose intolerance as a survival predictor was not significant, the significance of glucose intolerance in HCC patients was disclosed by stratification of gender. In male HCC patients, glucose intolerance was associated with significant decrease in long-term survival rate, although no difference was observed in disease-free survival between patients with and without glucose intolerance. It is presently uncertain why a gender difference was observed in the negative relationship between glucose intolerance and long-term survival of HCC patients with HCV. However, we showed that fasting insulin level was significantly higher in males than in females. It is possible that increased insulin resistance in male patients is associated with long-term survival of HCC patients.

In order to examine risk factors for long-term survival of male HCC patients, we performed univariate and multivariate analyses. In both analyses, the Child-Pugh class and glucose intolerance was identified as statistically independent prognostic factors. It remains unclear how glucose intolerance is involved in survival. It could be explained as insulin resistance contributing to fibrotic progression in chronic hepatitis with HCV infection (34). Furthermore, diabetes mellitus is an independent prognostic factor associated with the occurrence of hepatic decompensation in HCC patients (35). Thus, our findings suggest that the presence of glucose intolerance in HCC patients causes a more severely impaired liver function, which results in poor long-term survival. An alternative mechanism is that glucose intolerance could be a significant factor contributing to the growth rate of HCC. Saito *et al* reported the effect of hyperinsulinaemia on growth of human HCC (36). In addition, clinical studies have reported that high cell proliferation activity is an important survival predictor for HCC patients (37). Collectively, increased insulin levels may stimulate the growth of HCC, and survival may be reduced in male HCC patients.

Suppression of insulin signaling is also related to growth of HCC. Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a suppressor of insulin signaling and it is frequently mutated or deleted in a variety of human cancers (38). Decreased PTEN expression levels are involved in the pathogenesis of HCC, and this decrease was correlated with tumor progression and poorer prognosis (39). SHIP2 is also known to be a negative regulator of insulin signaling (16,17). In chronic myeloid leukemia cells, overexpression of SHIP2 results in decreased insulin sensitivity, which strongly reduces cell proliferation (40). Thus, although the important role of SHIP2 as a tumor suppressor has been clarified in insulin signaling, the changes in SHIP2 on HCC has not been investigated. In this study, immunoblotting and immunostaining showed significant decreases in SHIP2 expression level in HCC tissues compared to nontumor tissue. These results suggest that down-regulated SHIP2 expression leads to increased sensitivity to insulin, which is linked to cell proliferation in HCC.

In conclusion, in this study we showed that glucose intolerance is an independent factor of poor prognosis in male HCC patients with HCV infection and expression of SHIP2 is significantly down-regulated in human HCC.

Acknowledgements

We thank Syunichi Hattori (Ortho-Clinical Diagnostics, K.K.), Hidetoshi Itoya (Ortho-Clinical Diagnostics, K.K.) and Miwa Sakai for technical assistance. This study was supported in part by a Grant-in-Aid for Scientific Research (C) (No. 16590648 to M.S.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1. Bosch FX, Ribes J, Diaz M and Cleries R: Primary liver cancer: worldwide incidence and trends. Gastroenterology 127: S5-S16, 2004.
2. Kiyosawa K, Umemura T, Ichijo T, *et al*: Hepatocellular carcinoma: recent trends in Japan. Gastroenterology 127: S17-S26, 2004.
3. Megyesi C, Samols E and Marks V: Glucose tolerance and diabetes in chronic liver disease. Lancet 2: 1051-1056, 1967.
4. Marchesini G, Pacini G, Bianchi G, Patrono D and Cobelli C: Glucose disposal, beta-cell secretion, and hepatic insulin extraction in cirrhosis: a minimal model assessment. Gastroenterology 99: 1715-1722, 1990.

5. Petrides AS, Vogt C, Schulze-Berge D, Matthews D and Strohmeyer G: Pathogenesis of glucose intolerance and diabetes mellitus in cirrhosis. Hepatology 19: 616-627, 1994.
6. Mason AL, Lau JY, Hoang N, *et al*: Association of diabetes mellitus and chronic hepatitis C virus infection. Hepatology 29: 328-333, 1999.
7. Kawaguchi T, Yoshida T, Harada M, *et al*: Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. Am J Pathol 165: 1499-1508, 2004.
8. Kawaguchi T, Nagao Y, Tanaka K, *et al*: Causal relationship between hepatitis C virus core and the development of type 2 diabetes mellitus in a hepatitis C virus hyperendemic area: A pilot study. Int J Mol Med 16: 109-114, 2005.
9. Shintani Y, Fujie H, Miyoshi H, *et al*: Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. Gastroenterology 126: 840-848, 2004.
10. Adami HO, Chow WH, Nyren O, *et al*: Excess risk of primary liver cancer in patients with diabetes mellitus. J Natl Cancer Inst 88: 1472-1477, 1996.
11. El-Serag HB, Tran T and Everhart JE: Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. Gastroenterology 126: 460-468, 2004.
12. Gruppuso PA, Boylan JM, Bienieki TC and Curran TR Jr: Evidence for a direct hepatotrophic role for insulin in the fetal rat: implications for the impaired hepatic growth seen in fetal growth retardation. Endocrinology 134: 769-775, 1994.
13. Virkamaki A, Ueki K and Kahn CR: Protein-protein interaction in insulin signaling and the molecular mechanisms of insulin resistance. J Clin Invest 103: 931-943, 1999.
14. Cantley LC: The phosphoinositide 3-kinase pathway. Science 296: 1655-1657, 2002.
15. Boulton TG, Nye SH, Robbins DJ, *et al*: ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. Cell 65: 663-675, 1991.
16. Pesesse X, Deleu S, De Smedt F, Drayer L and Erneux C: Identification of a second SH2-domain-containing protein closely related to the phosphatidylinositol polyphosphate 5-phosphatase SHIP. Biochem Biophys Res Commun 239: 697-700, 1997.
17. Clement S, Krause U, Desmedt F, *et al*: The lipid phosphatase SHIP2 controls insulin sensitivity. Nature 409: 92-97, 2001.
18. Milburn CC, Deak M, Kelly SM, Price NC, Alessi DR and Van Aalten DM: Binding of phosphatidylinositol 3,4,5-trisphosphate to the pleckstrin homology domain of protein kinase B induces a conformational change. Biochem J 375: 531-538, 2003.
19. Taylor V, Wong M, Brandts C, *et al*: 5' phospholipid phosphatase SHIP-2 causes protein kinase B inactivation and cell cycle arrest in glioblastoma cells. Mol Cell Biol 20: 6860-6871, 2000.
20. Ishihara H, Sasaoka T, Hori H, *et al*: Molecular cloning of rat SH2-containing inositol phosphatase 2 (SHIP2) and its role in the regulation of insulin signaling. Biochem Biophys Res Commun 260: 265-272, 1999.
21. Monzillo LU and Hamdy O: Evaluation of insulin sensitivity in clinical practice and in research settings. Nutr Rev 61: 397-412, 2003.
22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF and Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412-419, 1985.
23. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC and Williams R: Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 60: 646-649, 1973.
24. Sobin LH and Witteking CH: TNM Classification of malignant tumors. 5th edition, John Wiley & Sons, Inc., New York, 1997.
25. Donato F, Tagger A, Gelatti U, *et al*: Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. Am J Epidemiol 155: 323-331, 2002.
26. Aoyagi K, Ohue C, Iida K, *et al*: Development of a simple and highly sensitive enzyme immunoassay for hepatitis C virus core antigen. J Clin Microbiol 37: 1802-1808, 1999.
27. Liver Cancer Study Group of Japan: Primary liver cancer in Japan. Clinicopathologic features and results of surgical treatment. Ann Surg 211: 277-287, 1990.
28. Kawaguchi T, Sakisaka S, Sata M, Mori M and Tanikawa K: Different lobular distributions of altered hepatocyte tight junctions in rat models of intrahepatic and extrahepatic cholestasis. Hepatology 29: 205-216, 1999.
29. Ikeda Y, Shimada M, Hasegawa H, *et al*: Prognosis of hepatocellular carcinoma with diabetes mellitus after hepatic resection. Hepatology 27: 1567-1571, 1998.
30. Poon RT, Fan ST and Wong J: Does diabetes mellitus influence the perioperative outcome or long term prognosis after resection of hepatocellular carcinoma? Am J Gastroenterol 97: 1480-1488, 2002.
31. Huo TI, Wu JC, Lui WY, *et al*: Differential mechanism and prognostic impact of diabetes mellitus on patients with hepatocellular carcinoma undergoing surgical and nonsurgical treatment. Am J Gastroenterol 99: 1479-1487, 2004.
32. Yang HI, Lu SN, Liaw YF, *et al*: Hepatitis B e antigen and the risk of hepatocellular carcinoma. N Engl J Med 347: 168-174, 2002.
33. Barbara L, Benzi G, Gaiani S, *et al*: Natural history of small untreated hepatocellular carcinoma in cirrhosis: a multivariate analysis of prognostic factors of tumor growth rate and patient survival. Hepatology 16: 132-137, 1992.
34. Hui JM, Sud A, Farrell GC, *et al*: Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression [corrected]. Gastroenterology 125: 1695-1704, 2003.
35. Huo TI, Lui WY, Huang YH, *et al*: Diabetes mellitus is a risk factor for hepatic decompensation in patients with hepatocellular carcinoma undergoing resection: a longitudinal study. Am J Gastroenterol 98: 2293-2298, 2003.
36. Saito K, Inoue S, Saito T, *et al*: Augmentation effect of postprandial hyperinsulinaemia on growth of human hepatocellular carcinoma. Gut 51: 100-104, 2002.
37. Donato MF, Arosio E, Del Ninno E, *et al*: High rates of hepatocellular carcinoma in cirrhotic patients with high liver cell proliferative activity. Hepatology 34: 523-528, 2001.
38. Li J, Yen C, Liaw D, *et al*: PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275: 1943-1947, 1997.
39. Hu TH, Huang CC, Lin PR, *et al*: Expression and prognostic role of tumor suppressor gene PTEN/MMAC1/TEP1 in hepatocellular carcinoma. Cancer 97: 1929-1940, 2003.
40. Giuriato S, Blero D, Robaye B, Bruyns C, Payrastre B and Erneux C: SHIP2 overexpression strongly reduces the proliferation rate of K562 erythroleukemia cell line. Biochem Biophys Res Commun 296: 106-110, 2002.

厚生労働科学研究費補助金
肝炎等克服緊急対策研究事業

肝炎状況・長期予後の疫学に関する研究

平成１９年度～２１年度　研究成果－２

研究代表者　　吉澤　浩司　　田中　純子

平成２２（2010）年　３月

Liver International ISSN 1478-3223

CLINICAL STUDIES

Serum C-reactive protein levels predict survival in hepatocellular carcinoma

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Keywords
C-reactive protein – hepatocellular carcinoma – prognosis

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Received 13 April 2007
accepted 26 June 2007

DOI:10.1111/j.1478-3231.2007.01550.x

Abstract

Background/Aims: C-reactive protein (CRP) was recently identified as a prognostic factor for patients with hepatocellular carcinoma (HCC) after surgical resection. We investigated the relationship between the serum levels of high sensitivity CRP (H-CRP) and the prognosis of HCC patients. *Method:* We conducted a cohort study of 90 HCC patients enrolled from 1997 to 1998. All patients were treated and followed for a mean period of 3.2 years. Clinical variables were compared between patients positive for H-CRP (serum H-CRP levels ≥ 3.0 mg/L, $n = 47$) and those negative for H-CRP (serum H-CRP levels < 3.0 mg/L, $n = 43$). We also determined the relationship between serum H-CRP and prognosis in HCC patients. *Results:* The survival rate of patients of the H-CRP-positive group was lower than that of H-CRP-negative patients. Tumour stage (stages 3 or 4), total bilirubin ≥ 1.2 mg/dL, albumin (Alb) < 3.5 g/dL, des-γ-carboxy prothrombin ≥ 40 mAU/mL, positive H-CRP and initial treatment (transcatheter arterial chemoembolization, hepatic arterial infusion chemotherapy or best supportive care) were identified as significant poor prognostic factors by univariate analysis, while positive H-CRP [hazard ratio (HR), 1.58; $P = 0.048$], Alb < 3.5 g/dL (HR, 2.10; $P = 0.004$), tumour stage (stages 3 or 4; HR, 3.05; $P = 0.001$) and initial treatment (HR, 1.88; $P = 0.029$) were considered to be significant determinants of poor prognosis by multivariate Cox proportional hazards analysis. *Conclusions:* The prognosis of H-CRP-positive patients was poorer compared with H-CRP-negative patients. This study confirmed that H-CRP, like CRP, is a marker of poor prognosis in HCC patients.

Hepatocellular carcinoma (HCC) is one of the most common cancers in Japan, Southeast Asia, northern Europe and the USA (1, 2). Recent advances in treatment including liver transplantation, surgical resection, percutaneous ethanol injection therapy and transcatheter arterial chemoembolization (TACE) have improved the prognosis for patients with HCC (3, 4).

C-reactive protein (CRP) is an acute-phase reactant synthesized by hepatocytes as part of the inflammatory response and regulated by proinflammatory cytokines. Interleukin (IL)-6 is another inflammatory cytokine that is central in the induction of CRP in human primary cultured hepatocytes and in human hepatoma cell lines. Cancer cells show autocrine production of IL-6 in multiple myeloma, as well as in prostate, renal, colorectal and oesophageal cancer *in vivo* (5–8). Serum CRP level is a prognostic marker in various malignancies, such as oesophageal cancer, colorectal cancer, gastric cancer, multiple myeloma, malignant fibrous histiocytoma and HCC treated with surgical resection (9–13). Recently, the value of the serum level of high-sensitivity CRP (H-CRP) has been investigated. Some authors have identified H-CRP as a predictor of future cardiovascular events, atherosclerosis and colorectal cancer (14–16).

In the present study, we analysed the relationship between serum H-CRP concentration and prognosis in patients with HCC based on the reported prognostic value of standard CRP levels.

Materials and methods

Patients and diagnosis

Between January 1997 and November 1998, 184 patients with untreated HCC were admitted to the Division of Gastroenterology, Department of Medicine, at Kurume University School of Medicine for HCC therapy. This was a cohort study comprising a collection of serial serum samples. The diagnosis of HCC was confirmed by needle biopsy or based on the findings of hypervascular liver masses with increased serum α-foetoprotein (AFP) levels exceeding 400 ng/mL. Ultrasonography (US), computed tomography (CT), magnetic resonance imaging and digital subtraction angiography were used for imaging techniques. In most of the patients, a percutaneous fine-needle aspiration liver biopsy was also performed under US guidance to confirm the diagnosis. Clinical staging was determined based on the TNN Classification of Malignant Tumors/ International Union Against Cancer (UICC) classification system (17). The degree of tumour differentiation was determined histologically according to a modified Edmondson and Steiner classification (18). On the basis of nuclear overcrowding, increased cytoplasmic basophilia and microacinar formation, tumours were defined as well differentiated, moderately differentiated (grade III) or poorly differentiated (grade IV).

Treatment and follow-up of patients

The following treatment criteria were used in this study: (i) Percutaneous ethanol injection therapy (PEIT) was performed to allow US detection of less than four HCC lesions measuring less than 3 cm each in size; (ii) operation was performed for solitary lesions measuring > 3 cm or solitary lesions measuring < 3 cm that were undetectable by US; (iii) TACE was performed for multiple nodular HCC lesions where they numbered more than four or were larger than 3 cm in size; (iv) hepatic arterial infusion chemotherapy (HAIC) was performed for multiple nodular lesions with major portal tumour thrombus (19); (v) Patients with Child–Pugh grade C or distant metastases were treated with best supportive care (BSC).

After the initial treatment, the condition of each patient was carefully followed. Serum AFP and des-γ-carboxy prothrombin (DCP) concentrations were measured once every month. US and CT were performed every 3 months until 6 months post-treatment and every 6 months thereafter until 30 months post-treatment. The recurrence of HCC was confirmed by tumour enlargement or the appearance of new lesions in the imaging studies. When recurrence was suspected, an angiography or a percutaneous fine-needle aspiration liver biopsy was performed under US guidance. Subsequent treatments for recurrent HCC were selected according to tumour number and liver function. Therapy for a recurrent tumour included the following: (i) PEIT was usually selected for recurrent HCC where tumours were < 2 cm and the number of nodules was less than three; (ii) TACE was selected for single nodules more than 3 cm or for multiple nodules with unequivocal tumour stains; (iii) HAIC was selected for multiple nodules with major portal tumour thrombus; (iv) Patients with Child–Pugh grade C or distant metastases were treated with BSC.

This study was closed in December 2005, or at the time of a patient's death. The possible causes of death were defined as: (i) liver-related diseases: patients who died of tumour progression, liver failure or bleeding form oesophageal–gastric varices or (ii) others: patients who died of other diseases.

If a patient had not been monitored in our hospital or in a related private Hepatology Clinic for more than 1 year, the patient was considered to have been lost to the follow-up procedure.

High sensitivity C-reactive protein determinations

Serial serum samples have been collected over many years at our hospital and maintained at $-20\,^{\circ}C$. Serum H-CRP levels were measured with an Assay Max Human C-Reactive Protein ELISA kit (Assay Pro, Winfield, MO, USA), using the protocol provided by the manufacturer (20). The H-CRP values in preserved serum and fresh serum were compared to determine the influence of preservation at $-20\,^{\circ}C$ using 10 samples. Because the standard deviation was $< 10\%$ (data not shown), we concluded that serum preservation at $-20\,^{\circ}C$ did not affect the measured values.

Statistical analysis

Survival rates were determined by the Kaplan–Meier method, and differences in the survival rates between the two groups were compared using the log rank test. Analysis of multiple covariates of prognostic factors for the patient's background was performed with the Cox proportional hazards model. The χ^2-test and Kruskal–Wallis rank test were used for comparisons of discrete variables. Statistical significance was defined as a P value of < 0.05. All statistical analyses were performed using the SPSS and SAS systems. The 14 factors examined were age at the diagnosis of HCC, sex, hepatitis B surface antigen (HBsAg), total bilirubin (TB), albumin (Alb), serum aspartate aminotransferase, serum alanine aminotransferase, platelet count, prothrombin time,

positive H-CRP, positive AFP, positive DCP and tumour stage. The cut-off value for the AFP and DCP levels was set at 200 ng/mL and 40 mAU/mL respectively. The initial treatment was classified into two groups of hepatectomy or PEIT, and TACE or HAIC or BSC.

Results

Patient characteristics

Ninety patients were enrolled in this study, and prognostic factors for HCC were prospectively analysed by follow-up for a mean period of 3.2 years (median follow-up period, 2.5 years; range, 0.03–8.6 years). The patient characteristics are listed in Table 1. Patient age ranged from 14 to 83 years (median: 65 years) and there were 68 males and 22 females. Of these patients, 15 (16.7%) were positive for HBsAg, but negative for antibodies to hepatitis C virus (anti-HCV), while 66 (73.3%) patients were positive for anti-HCV, but negative for HbsAg; nine (10.0%) patients were negative for both HBsAg and anti-HCV. Of all patients, 65 (72.2%) were DCP positive at the time of diagnosis, and 29 (32.2%) patients were AFP positive.

Percutaneous fine-needle aspiration liver biopsy performed under US guidance was used to confirm the diagnosis of HCC in 35 of 90 patients. In 35 (38.8%) patients, HCC was diagnosed pathologically. The remaining 55 (61.2%) patients showed clinical features of HCC in the imaging study. Thirty patients underwent hepatectomy or PEIT, 50 patients were treated with transarterial chemoembolization or HAIC and 10 patients received BSC.

Table 1. Characteristics of patients ($n = 90$)

Variables	(%)
Age (years, median)	65
Sex (M/F)	68/22
Positive for HBsAg and negative for anti-HCV (%)	15 (16.7)
Positive for anti-HCV and negative for HBsAg (%)	66 (73.3)
Negative for both HBsAg and anti-HCV (%)	9 (10)
Serum albumin (g/dL)	3.4
Total bilirubin (mg/dL)	1.2
Serum aspartate aminotransferase (U/L)	63.5
Serum alanine aminotransferase (U/L)	50.5
Prothrombin time (%)	78.5
Platelet count ($\times 10^4$)	10.2
α-foetoprotein (ng/mL)	55.4
Des-γ-carboxy prothrombin (mAU/mL)	113.5
Highly sensitive C-reactive protein (mg/L)	2.65
Child–Pugh grade (A/B+C)	60/30
Tumour stage (1/2/3A+B+C/4)	37/36/14/3
Histology (well/moderate or poor)	10/25
Initial treatment (hepatectomy or PEI/TAE or HAI/BSC)	30/50/10
Average interval period (years)	3.2

BSC, best supportive care; HAI, hepatic arterial injection; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; PEI, percutaneous ethanol injection; TAE, transarterial embolization; Tumour stage: TNM classification of malignant tumour 6th edition.

Long-term outcomes

Follow-up data were obtained on 85 (94.4%) patients while five patients (5.6%) were lost during the follow-up period. In total, 74 (82.2%) patients died from hepatic disease (72 died from tumour progression and hepatic failure, and two patients died from gastrointestinal bleeding), and two patients died from other causes (one died in a traffic accident, and the other from a different kind of cancer).

Characteristics of the two groups classified according to high sensitivity C-reactive protein

The patients with HCC were divided into two groups: 47 patients were H-CRP positive at the time of diagnosis of HCC, whereas 43 patients were H-CRP negative. As shown in Table 2, the patients in these groups were similar with regard to age, aetiology of cirrhosis, degree of liver function, AFP levels and DCP levels. Only the H-CRP levels were significantly different between the two groups.

Survival rates

Three-year survival rates for the 90 patients averaged 41.1% and the 5-year survival rate was 24.4% (Fig. 1). The overall survival of patients in the H-CRP-positive group (Fig. 2) was significantly lower than for patients classified as H-CRP negative, using the log rank test ($P < 0.05$).

Univariate and multivariate analyses

The independent predictors of survival are summarized in Table 3. According to univariate analysis, H-CRP positive, tumour stage, positive DCP, TB $\geq$ 1.2 mg/dL and Alb $<$ 3.5 g/dL significantly correlated with survival. Multivariate Cox regression analyses were performed on the five variables in the model. Three factors were found to be independently associated with survival: Alb $<$ 3.5 g/dL, positive H-CRP, tumour stages 3 or 4 and initial treatment (TACE or HAIC or BSC). The risk of death in patients with Alb $<$ 3.5 g/dL at entry was 2.01-fold higher compared with patients with Alb $\geq$ 3.5 g/dL. The risk of death in patients in the H-CRP-positive group was 1.82-fold higher than for H-CRP-negative patients. The risk of

Table 2. Univariate and multivariate analyses of survival in patients with hepatocellular carcinoma

Variables		Number of patients	Univariate analyses Hazard ratio (95% CI)	*P* value	Multivariate analyses Hazard ratio (95% CI)	*P* value
Age	≥65	47	0.98 (0.59–1.63)	0.96		
Sex	Male	47	0.79 (0.52–1.21)	0.29		
Hepatitis B virus	HBsAg positive	15	1.72 (0.97–3.03)	0.075		
Albumin	< 3.5 g/dL	45	1.75 (1.13–2.70)	0.011	2.01 (1.20–3.37)	0.008
Total bilirubin	≥1.2 mg/dL	47	1.52 (1.00–2.33)	0.049	1.11 (0.65–1.88)	0.69
Serum aspartate aminotransferase	≥64 U/L	45	1.49 (0.98–2.28)	0.062		
Serum alanine aminotransferase	≥51 U/L	45	0.90 (0.59–1.38)	0.65		
Prothrombin time	< 80%	46	1.10 (0.72–1.68)	0.65		
Platelet count	< 10 ($\times 10^4$)	44	1.07 (0.70–1.63)	0.73		
Highly sensitive C-reactive protein	≥3 mg/L	47	1.76 (1.15–2.69)	0.01	1.82 (1.13–2.93)	0.012
α-fetoprotein	≥200 ng/mL	29	1.32 (0.85–2.07)	0.21		
Des-γ-carboxy prothrombin	≥40 mAU/mL	65	1.96 (1.22–3.16)	0.004	1.49 (0.89–2.51)	0.12
Stage	Stages 3 or 4	17	2.69 (1.54–4.69)	0.001	2.26 (1.16–4.39)	0.016
Initial treatment	TAE or HAI or BSC	60	2.88 (1.69–4.93)	0.001	1.88 (1.065–3.32)	0.029

BSC, best supportive care; CI, confidence intervals; HAI, hepatic arterial injection; TAE, transarterial embolization.

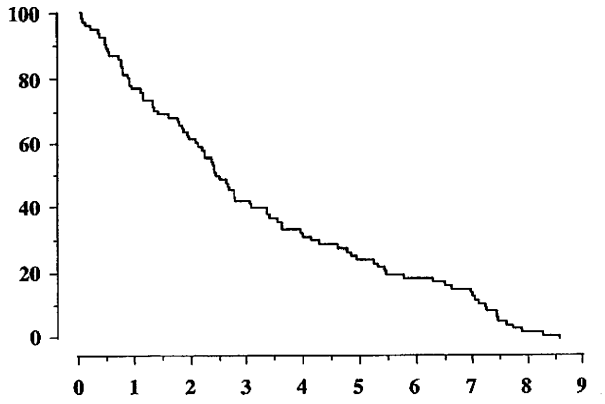

Fig. 1. Overall survival rate of 90 patients who underwent initial treatment for hepatocellular carcinoma.

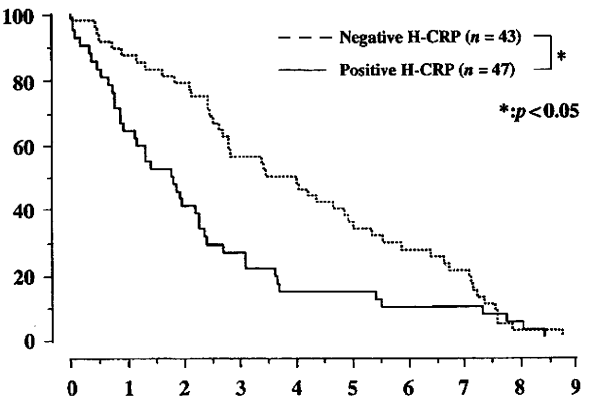

Fig. 2. Survival rates according to the Kaplan–Meier method for the hepatocellular carcinoma patient groups defined by H-CRP. The survival rate for the H-CRP-positive group (solid line; $n = 47$) was significantly lower than that for the H-CRP-negative group (dotted line; $n = 43$). H-CRP, high sensitivity C-reactive protein.

death in patients with stages 3 or 4 tumours was 2.26-fold higher than for those with stages 1 or 2 HCC. The risk of death in patients treated by TACE or HAIC or BSC was 1.88-fold higher than in those treated by hepatectomy or PEIT.

Discussion

This study shows that H-CRP is a marker of poor prognosis and tumour progression. Following the identification of CRP as a prognostic marker for various malignant tumours, predictive scoring systems that included CRP were reported for HCC, oesophageal cancer and multiple myeloma (10, 11, 13). We identified CRP in some HCC samples and surrounding tissues obtained by surgical resection (data not shown), and Nozoe *et al.* (8) used immunohistochemistry to demonstrate CRP expression in tumour cells of patients with squamous cell carcinoma of the oesophagus. They found that elevated serum CRP levels might be at least in part owing to the production of CRP by the tumour itself, in addition to CRP synthesis by hepatocytes in response to the tumour growth. Considering the poor prognosis associated with elevated CRP in various cancer patients, serum CRP is probably correlated with malignant potential and tumour growth.

Infection with hepatitis B virus or HCV is an important risk factor for HCC, and various chronic

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Table 3. Comparison of characteristics of patients positive for highly sensitive C-reactive protein (H-CRP) and negative for H-CRP

Variables	Positive H-CRP ($n=47$)	Negative H-CRP ($n=43$)
Age (years)	63	65
Sex (M/F)	38/9	30/13
Positive for HBsAg and Negative for anti-HCV (%)	10 (21.2)	5 (11.6)
Positive for anti-HCV and Negative for HBsAg (%)	31 (66.0)	35 (72.6)
Negative for both HBsAg and anti-HCV (%)	6 (12.8)	3 (8.1)
Serum albumin (g/dL)	3.3	3.5
Total bilirubin (mg/dL)	1.4	1.1
Serum aspartate aminotransferase (U/L)	67	59
Serum alanine aminotransferase (U/L)	49	55
Prothrombin time (%)	76	82
Platelet count ($\times 10^4$)	11	9.9
α-foetoprotein (ng/mL)	99.1	25.6
Des-γ-carboxy prothrombin (mAU/mL)	174	64
Highly sensitive C-reactive protein (mg/L)*	6.5	0.9
Child–Pugh grade (A/B+C)*	25/22	35/8
Tumour stage (1/2/3A+B+C/4)*	14/22/8/3	23/14/6/0
Maximum tumour size (cm)	4.6	3.5
Vascular invations (portal vein/hepatic vein/bile duct)	5/1/1	5/0/0
Histology (well/moderate or poor)	5/12	5/13
Initial treatment (hepatectomy or PEI/TAE or HAI/BSC)	15/23/9	15/27/1

Data expressed as median values.

*$P < 0.05$.

BSC, best supportive care; HAI, hepatic arterial injection; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; PEI, percutaneous ethanol injection; TAE, transarterial embolization.

inflammations have been linked to both the occurrence and the progression of malignancies including HCC, oesophageal cancer, lung cancer and cervical cancer. Nonspecific inflammatory responses include the release of proinflammatory cytokines and growth factors, some of which may promote tumour growth (21). There are also some epidemiological data that link inflammation with cancer; tumour necrosis factor and IL-1 are proinflammatory cytokines that have been associated with poor prognosis and disease severity in patients with non-Hodgkin's lymphoma (22) and gastric cancer (23). In addition, some authors have reported a relationship between prognosis and various cytokines including IL-6, IL-8, IL-10 and VEGF in HCC (24–26). These results, however, remain controversial.

Little is known about the association between the role of CRP in inflammation and its role in the progression, invasion and metastasis of cancer cells. If CRP is proven to be important in the signalling for tumour growth or metastases as this study suggests, then inhibition of CRP production may potentially be used to treat HCC patients. The success of this therapeutic modality was hinted at recently by Yoshida *et al.* (27), who showed that pigment epithelium-derived factor can block CRP production in Hep3B cells.

Lin *et al.* (28) reported significantly high serum CRP levels in patients with diffuse-type hepatoma, and Hashimoto *et al.* (13) reported CRP as a prognostic indicator and developed a scoring system including CRP for HCC patients treated by surgical resection. These authors associated serum CRP levels with related portal invasion, extrahepatic metastases and infiltration of hepatoma cell into the systemic circulation. Our study shows significantly high serum CRP levels in patients with extra hepatic metastases, and child B or C. However, average of maximum tumour size, vascular invasions, histological grades and initial treatment were not significant between the positive H-CRP group and the negative H-CRP group (Table 3). Some authors explain the reasons for malignant potential of the tumour with high CRP levels as follows: i, resistance to chemotherapy; ii, anti-apotopic activity and tumorigenic potency; iii, T cell impairment; iv, increased levels of serum angiogenic factors and v, the metastatic potential of the tumour (29). In addition, multivariate analyses identified H-CRP as a poor prognostic factor, but did not flag clinical tumour markers AFP and DCP as significant prognostic factors. Our results therefore suggest that H-CRP could also be a useful prognostic marker in other kinds of cancers; many authors reported that the prognosis of patients with various malignancies is related to CRP.