hepatic tumors including HCC developed in 5 of 11 (45%) transgenic mice fed the excess-iron diet at 12 months after the initiation of feeding, but did not in control mice or transgenic mice fed the control diet (50). These results indicate the importance of oxidative stress and subsequent mitochondrial injury synergistically induced by iron loading and HCV proteins in the development of HCC. Thus, there seems to be a close relationship between the development of HCC and oxidative DNA damage synergistically induced by hepatic iron accumulation and HCV proteins. However, further investigations are needed to clarify the detailed mechanisms by which hepatic iron accumulation results in the development of HCC in chronic hepatitis C.

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Figure legends

Figure 1. Schematic diagram depicting the mechanisms underlying the hepatic iron accumulation in transgenic mice expressing the HCV polyprotein. HCV protein-induced ROS increase hepatic expression of C/EBP homology protein (CHOP) and subsequently reduce DNA binding activity of C/EBP/α, which leads to reduction of hepcidin transcription. Decreased hepcidin expression increases ferroportin (FPN) expression in the enterocytes and reticuloendothelial macrophages, resulting in increased duodenal iron transport and macrophage iron release, which lead to hepatic iron accumulation.

Figure 2. Schematic diagram depicting the assumed mechanisms underlying the hepatic iron accumulation in patients with chronic hepatitis C. Hepcidin transcription in chronic hepatitis C may be potentially regulated by the opposing effects of HCV related ROS-induced hepcidin suppression and iron load-induced hepcidin stimulation. Inflammation may also have the opposing effects of stimulation and suppression of hepcidin transcription through the IL-6/STAT pathway and ROS pathway, respectively. Consequent relative suppression of hepcidin expression is potentially one of the mechanisms underlying the hepatic iron accumulation in patients with chronic hepatitis C.

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Figure 1

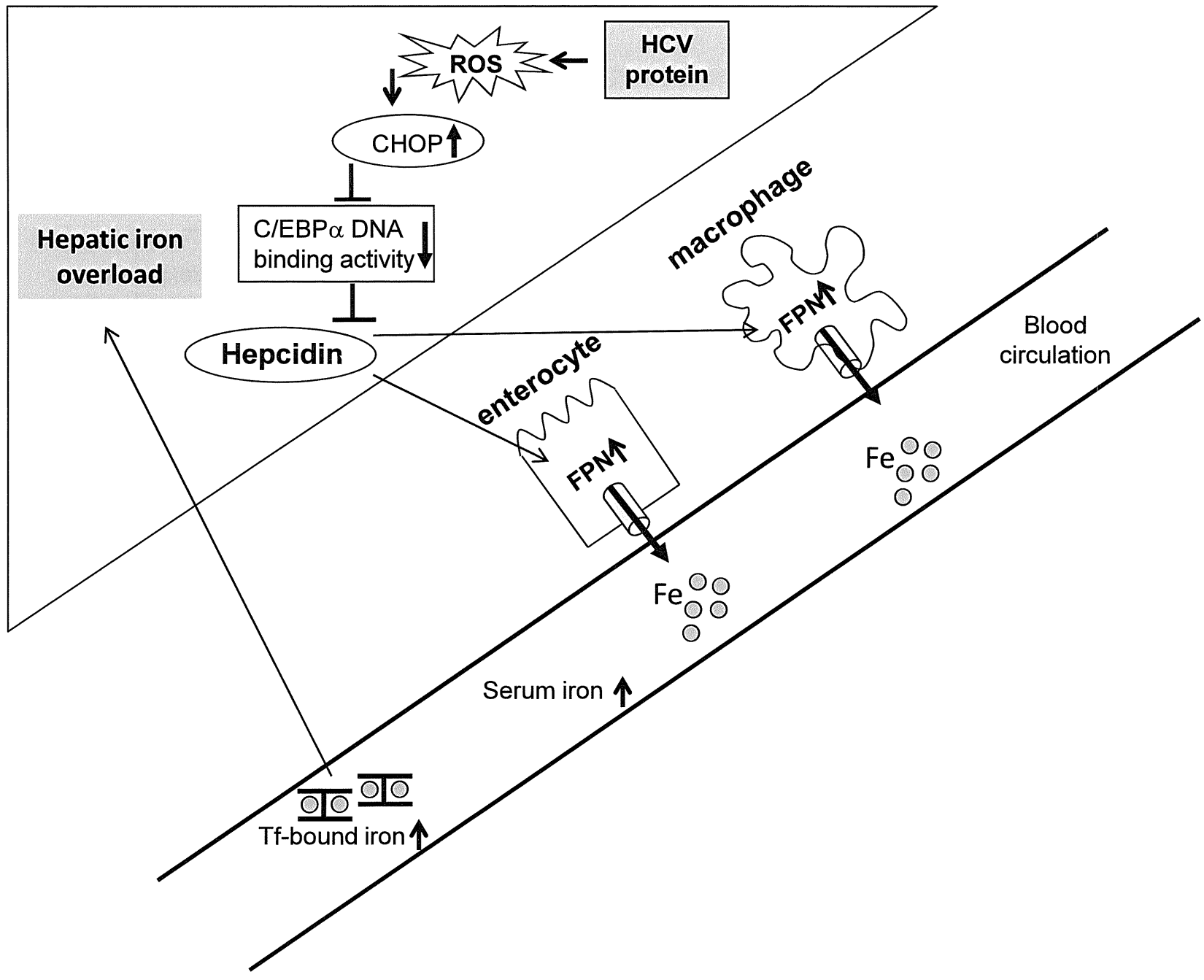
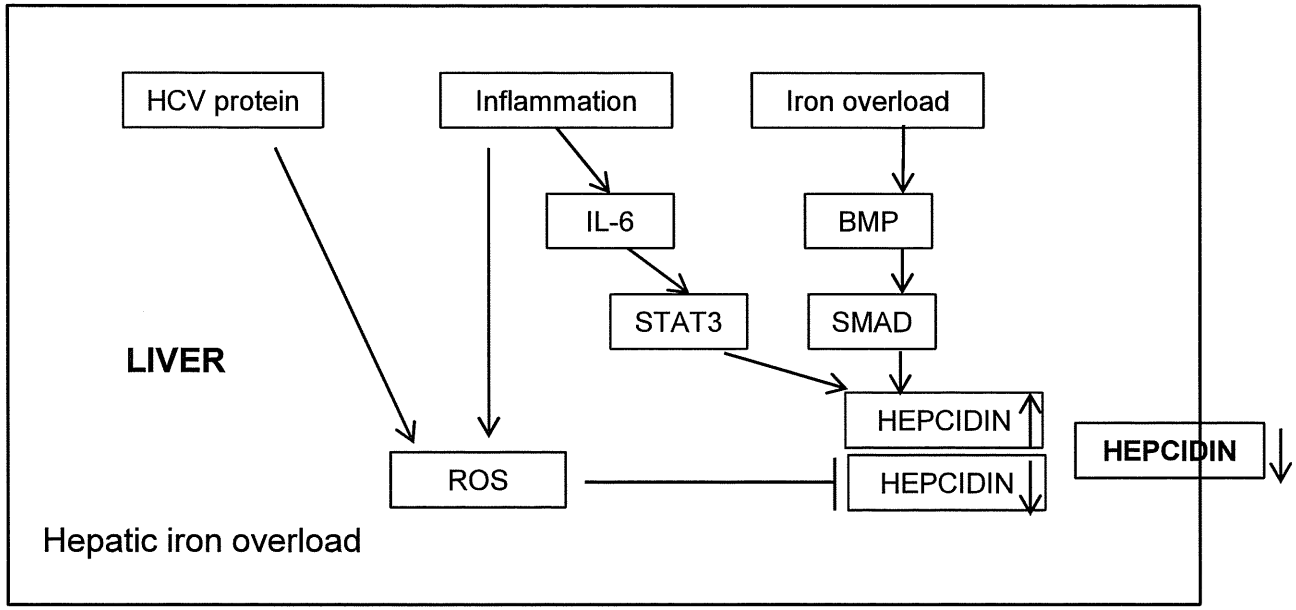

ROS
HCV protein
CHOP
C/EBPα DNA binding activity
Hepatic iron overload
Hepcidin
macrophage
FPN
enterocyte
FPN
Blood circulation
Fe
Fe
Serum iron
Tf-bound iron

Figure 2


HCV protein
Inflammation
Iron overload
IL-6
BMP
STAT3
SMAD
LIVER
HEPCIDIN
ROS
HEPCIDIN
HEPCIDIN
Hepatic iron overload

Risk factors for survival and development of hepatocellular carcinoma in primary biliary cirrhosis

A short running title: Risk factors for survival and HCC in PBC

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ABSTRACT

Objective: Earlier diagnosis of hepatocellular carcinoma (HCC) may be critical in management of patients with primary biliary cirrhosis (PBC), since the prognosis of PBC has improved. The aim of this study was to investigate whether HCC development affects the prognosis of PBC and to identify the risk factors for HCC in Japanese patients with PBC. **Methods:** We compared the survival between patients with HCC and those without and analyzed the risk factors for HCC development in 210 patients with PBC who were followed-up for a median period of 8.5 years. **Results:** HCC developed during follow-up in 11 patients (5.2%) and was diagnosed simultaneously at the time of diagnosis of PBC in 5 patients (2.4%) who were excluded from analysis. Kaplan-Meier analysis showed a significant difference in the overall survival between patients who did and did not develop HCC (P<0.001). Multivariate analysis revealed age (OR: 1.08, 95% confidence interval [CI]: 1.03-1.13, P=0.001), albumin level (OR: 0.24, 95% CI: 0.10-0.56, P=0.001), total bilirubin level (OR: 1.60, 95% CI: 1.09-2.36, P=0.017) and HCC development (OR: 2.97, 95% CI: 1.24-7.15, P=0.015) to be significant prognostic factors, and identified only advanced histological stage (Scheuer's classification III or IV, OR: 6.27, 95% CI: 1.80-21.83, P=0.004) as a risk factor associated with HCC. **Conclusions:** HCC development significantly affected survival of patients with PBC, and advanced histological stage was only risk factor associated with HCC development. These results highlighted an important role of liver fibrosis in hepatocarcinogenesis in patients with PBC.

Key words: diabetes mellitus; liver fibrosis; prognosis; survival; ursodeoxycholic acid;

INTRODCTION

Primary biliary cirrhosis (PBC) is a progressive cholestatic liver disease characterized by the presence of highly specific antimitochondrial antibodies, portal inflammation and lymphocyte-dominated destruction of intralobular bile ducts, which eventually leads to cirrhosis (1). The incidence of PBC has increased over recent decades, possibly attributable to augmented testing of liver biochemistry rather than a rise in disease incidence. The routine use of biochemical screening also has made it possible to diagnose PBC at an earlier stage (2). In addition to the diagnosis at an earlier stage, high prevalence of treatment with ursodeoxycholic acid (UDCA) potentially had made it possible for patients with PBC to live longer. However, the natural history of PBC is still debated and depends on several variables and on the symptoms of liver disease (3). In general the risk at cancer development increases as humans live longer, and the development of hepatocellular carcinoma (HCC) is no exception.

Patients with PBC have been considered at low risk for the development of HCC (4), while several reports revealed that PBC was associated with an increased risk of HCC (5-8). Additionally, whether the development of HCC affects the overall survival of patients with PBC is still controversial (5, 7, 9, 10). This matter and the identification of the risk factors for HCC development are of importance to improve the prognosis of patients with PBC. There are, however, few studies reporting the risk factors for HCC among patients with PBC (5-7, 9, 11). Age at diagnosis (7, 11), male sex (5, 7, 10, 11), a history of blood transfusion (7, 11), cigarette smoking (6), portal hypertension (11), more advanced histologic stages (9, 10), and superinfection with hepatitis C virus

(HCV) (6) have been reported to be risk factors for HCC in patients with PBC. Such risk factors are still a matter of debate due to recent changes of the nutritional environment and/or lifestyle. For instance, previous studies did not include obesity or diabetes mellitus as clinical parameters for assessing risk factors related to the development of HCC in patients with PBC, even though these parameters have been recognized as risk factors for the development of HCC (12, 13).

The aim of this study was to determine whether HCC development affects the prognosis of PBC and to identify the risk factors for HCC development in Japanese patients with PBC based on recent clinical evidences.

PATIENTS AND METHODS

This study was carried out in three series of patients from three different liver disease centers who were seen with a diagnosis of PBC between 1984 May and 2010 May. The total of 245 patients consisted of 116 from Kawasaki Medical School affiliated hospital, 72 from Yamaguchi Grand Medical Center and 57 from Kawasaki Hospital. The diagnosis of PBC was established if the patients fulfilled at least one of the following criteria defined by the Intractable Hepato-Biliary Disease Study Group of Japan: (i) laboratory abnormalities consistent with chronic cholestatic liver disease and the presence of chronic nonsuppurative destructive cholangitis, (ii) positive antimitochondrial antibodies and compatible liver histology with PBC, (iii) a medical history and laboratory abnormalities consistent with chronic cholestatic liver disease and positive antimitochondrial antibodies. Histological stage was classified according to the Scheuer's classification (14). The following 35 patients were excluded from analysis due to confounding risk factors for HCC or incomplete clinical parameters: hepatitis B surface antigen (HBsAg) positivity in 2, anti-HCV antibody (anti-HCV) positivity in 5, history of excessive alcohol consumption (>40 g/day) in 4, lack of follow-up period more than 0.3 years in 12, and lack of pathological diagnosis in 12, (Figure 1).

The diagnosis of diabetes mellitus was based on the history of anti-diabetic medications such as oral hypoglycemic agents or insulin since the diagnostic criteria of diabetes mellitus proposed by the Japan Diabetes Society were not applied to all the patients because of the lack of several biochemical parameters. The diagnosis of portal hypertension was based on the complication of esophageal or gastric varices, ascites or

splenomegaly. Clinical characteristics at diagnosis of PBC are shown in Table 1. Patients were regularly assessed for biochemical tests every 1-4 months and followed for a median period of 8.5 (range, 0.3-25.8) years. To determine the development of HCC, an abdominal ultrasonography was performed for all the patients without HCC at intervals of 4 to 12 months. HCC was diagnosed by abdominal ultrasonography and confirmed by computed tomography (CT), magnetic resonance imaging, hepatic arteriography, and/or fine-needle aspiration liver biopsy.

Overall survival was defined as the period from the moment of PBC diagnosis until death, liver transplantation or the last medical examination, and compared between patients who did and did not develop HCC. Laboratory parameters at the time of PBC diagnosis were compared to those at the time of HCC diagnosis in patients who developed HCC during the follow-up period. The study protocol conformed to the 1975 declaration of Helsinki Declaration, and was approved by the ethics committees of the institutions.

Statistical analysis

Baseline continuous variables were expressed as mean ± SD. Comparison between groups were performed using the Mann-Whitney test for continuous variables, and the χ^2 test with Yates correction or the Fisher-exact test for categorical variables. Cumulative survival was calculated using the Kaplan-Meier method and the differences among the groups were analyzed with the log-rank test. Univariate and multivariate analyses of predictors of survival were assessed using the Cox proportional hazards