ORIGINAL ARTICLES—LIVER, PANCREAS, AND BILIARY TRACT

Characteristics of Patients With Nonalcoholic Steatohepatitis Who Develop Hepatocellular Carcinoma

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This article has an accompanying continuing medical education activity on page e50. Learning Objectives—At the end of this activity, the learner should identify the clinical features of patients with nonalcoholic steatohepatitis who develop hepatocellular carcinoma and the role of hepatic fibrosis in the development of hepatocellular carcinoma.

See related article, Villanueva A et al, on page 1501 in *Gastroenterology*.

BACKGROUND & AIMS: Nonalcoholic steatohepatitis (NASH) can progress to hepatocellular carcinoma (HCC). We aimed to characterize the clinical features of NASH patients with HCC. **METHODS:** In a cross-sectional multicenter study in Japan, we examined 87 patients (median age, 72 years; 62% male) with histologically proven NASH who developed HCC. The clinical data were collected at the time HCC was diagnosed. **RESULTS:** Obesity (body mass index ≥25 kg/m^2), diabetes, dyslipidemia, and hypertension were present in 54 (62%), 51 (59%), 24 (28%), and 47 (55%) patients, respectively. In nontumor liver tissues, the degree of fibrosis was stage 1 in 10 patients (11%), stage 2 in 15 (17%), stage 3 in 18 (21%), and stage 4 (ie, liver cirrhosis) in 44 (51%). The prevalence of cirrhosis was significantly lower among male patients (21 of 54, 39%) compared with female patients (23 of 33, 70%) ($P = .008$). **CONCLUSIONS: Most patients with NASH who develop HCC are men; the patients have high rates of obesity, diabetes, and hypertension. Male patients appear to develop HCC at a less advanced stage of liver fibrosis than female patients.**

Keywords: Liver Cancer; Incidence; Sex; Retrospective Study.

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third leading cause of cancer mortality.[1] HCC mostly occurs within an established background of chronic liver disease and cirrhosis. Although the risk factors for HCC, including infection with hepatitis B and C viruses as well as alcohol consumption, are well-defined, 5%–30% of patients with HCC lack a readily identifiable risk factor for their cancer. It has been suggested that a more severe form of nonalcoholic fatty liver disease (NAFLD), namely nonalcoholic steatohepatitis (NASH), might account for a substantial portion of cryptogenic cirrhosis and HCC cases.[2]

NAFLD is one of the most common causes of chronic liver disease in the world.[3,4] NAFLD is associated with obesity, diabetes, dyslipidemia, and insulin resistance and is recognized as a hepatic manifestation of metabolic syndrome. The spectrum of NAFLD ranges from a relatively benign accumulation of lipid (simple steatosis) to progressive NASH associated with fibrosis, necrosis, and inflammation. Despite its common occurrence and potentially serious nature, relatively little is known about the natural history or prognostic significance of NAFLD. Although prospective studies on the natural history of NAFLD and NASH with a larger cohort are awaited, these

Abbreviations used in this paper: AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CT, computed tomography; DCP, des-γ-carboxy prothrombin; γ-GTP, γ-glutamyl transpeptidase; HCC, hepatocellular carcinoma; HDL, high-density lipoprotein; MRI, magnetic resonance imaging; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

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studies might be limited by the long and asymptomatic clinical course of these diseases, by their high prevalence in the general population, and by the lack of serologic markers for NASH. The evidence suggesting that NASH can progress to HCC comes from (1) case reports and case series,[5–8] (2) retrospective studies,[9–12] and (3) prospective studies.[13–17] These studies generally examined limited numbers of cases and follow-ups; therefore, the incidence of HCC and risk factors for HCC in NASH patients remain unclear.

The Japan NASH Study Group (representative, Takeshi Okanoue)[18] was established in 2008 by the Ministry of Health, Labour and Welfare of Japan to address unmet research needs in the area of liver diseases. As a part of this mandate, the study group conducted a cross-sectional multicenter study to characterize the clinical features of histologically proven NASH patients who developed HCC.

Methods

Patients

We retrospectively identified and reviewed 87 Japanese patients with NASH, who developed HCC between 1993 and 2010, at 15 hepatology centers that belong to the Japan NASH Study Group[18] and their affiliated hospitals in Japan. The diagnosis of NASH was based on (1) the histologic features of steatohepatitis, (2) negligible alcohol consumption, and (3) exclusion of liver diseases of other etiology. To determine alcohol consumption as accurately as possible, we reviewed medical records in our institutions, and when patients had been transferred from other institutions, we also reviewed a summary of medical records from those institutions. According to the medical records, alcohol consumption was assessed on the basis of a detailed history that was obtained by physicians and by interviewing family members. Exclusion criteria included consumption of more than 20 g of alcohol per day, positivity for hepatitis B virus surface antigen, positivity for anti–hepatitis C virus antibody, the presence of other types of liver diseases (eg, primary biliary cirrhosis, autoimmune hepatitis, Wilson's disease, or hemochromatosis), previous treatment with drugs known to produce hepatic steatosis, and a history of gastrointestinal bypass surgery. The sections of nontumor liver tissues were reanalyzed by experienced hepatopathologists (T.O., E.H.) who were blinded to the laboratory parameters and clinical data. We excluded patients whose histologic diagnosis of NASH was not confirmed by central review and patients with insufficient or inconclusive information concerning alcohol consumption, body mass index (BMI), and laboratory data including fasting glucose and lipid.

Of the 87 patients, 14 patients had been previously diagnosed as NAFLD or NASH and had been followed at our institutions; 73 patients had been transferred from other institutions to our institutions for investigation and treatment of HCC. Most patients had been identified as having HCC during screening, which included ultrasound and/or computed tomography (CT) of the liver and alpha-fetoprotein (AFP) testing.

The diagnosis of HCC was based on liver histology and, in the absence of histology, on typical features of HCC as assessed by dynamic CT or magnetic resonance imaging (MRI) (ie, hypervascular with washout in the portal/venous phase).[19] Of the 87 patients, 49 patients were diagnosed as HCC after hepatic resection, 21 patients were diagnosed after ultrasound-guided tumor biopsy, and 17 patients were diagnosed by dynamic CT or MRI.

The Ethics Committees of each participating center approved this study. Informed consent was obtained from each patient in accordance with the Declaration of Helsinki.

Clinical Assessment and Laboratory Tests

The clinical and laboratory data were collected at the time HCC was diagnosed. BMI was calculated by using the following formula: weight in kilograms/(height in meters)^2. Obesity was defined as BMI $\geq$25 kg/m^2 according to the criteria of the Japan Society for the Study of Obesity.[20] Diabetes was defined as fasting plasma glucose concentration of $\geq$126 mg/dL or 2-hour plasma glucose concentration of $\geq$200 mg/dL during an oral glucose (75 g) tolerance test or by the use of insulin or oral hypoglycemic agents to control blood glucose.[21] Hypertension was defined as systolic blood pressure $\geq$130 mm Hg or diastolic blood pressure $\geq$85 mm Hg or by the use of antihypertensive agents.[22] Dyslipidemia was defined as serum concentrations of triglycerides $\geq$150 mg/dL or high-density lipoprotein (HDL) cholesterol $<$40 mg/dL and $<$50 mg/dL for men and women, respectively, or by the use of specific medication.[22]

Venous blood samples were taken in the morning after 12-hour overnight fast. The laboratory evaluations included blood cell count and measurement of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), fasting plasma glucose, HbA1c, total cholesterol, HDL cholesterol, triglyceride, ferritin, hyaluronic acid, AFP, and des-γ-carboxy prothrombin (DCP). These parameters were measured by using standard clinical chemistry techniques.

Histopathologic Examination

Nontumor liver tissues were obtained from all 87 patients to diagnose the background liver tissue at the time HCC was diagnosed. In 49 patients who underwent hepatic resection for HCC, we examined nontumor liver tissues that were surgically resected. In 21 patients who underwent ultrasound-guided tumor biopsy, nontumor liver tissues far from HCC tumors were biopsied separately. In 17 patients who were diagnosed as HCC by dynamic CT or MRI and did not undergo either hepatic resection or tumor biopsy, only nontumor liver tissues far from HCC tumors were obtained by ultrasound-guided biopsy.

The specimens were fixed in formalin, embedded in paraffin, and stained with hematoxylin-eosin, with Masson trichrome, and by silver impregnation. NASH was defined as steatosis with lobular inflammation, hepatocellular ballooning, and Mallory's hyaline (Mallory's body) or fibrosis.[23–25] The necroinflammatory grade and the degree of fibrosis were evaluated and scored according to the criteria proposed by Brunt et al.[26]

Statistical Analysis

Results are presented as numbers with percentages in parentheses for qualitative data or as the medians and ranges (25th–75th percentiles) for quantitative data. Comparisons were made by using a χ^2 test for qualitative factors or a Mann–