Hepatocarcinogenesis in non-alcoholic fatty liver disease in Japan

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hepatocellular carcinoma (HCC), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH).

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Abstract
In Japan, there has been a gradual increase in cases of non-viral chronic liver diseases, including non-alcoholic fatty liver disease (NAFLD), occurring with hepatocellular carcinoma (HCC). First, a national survey investigating the etiology of HCC in Japan was performed. Among HCCs based on non-viral disease, alcoholic liver disease with HCC accounted for 7.2% of all HCCs, followed by chronic liver disease of unknown etiology with HCC (5.1%) and NAFLD with HCC (2.0%). The clinical characteristics of these three HCC groups were clearly different. In our second analysis, the HCC development rates among liver cirrhosis with NAFLD, alcoholic cirrhosis, and cirrhosis with hepatitis C virus (HCV) were compared. HCC development rates were 11.3%/5 years in NAFLD cirrhosis, 30.5%/5 years in HCV cirrhosis, and 12.5%/5 years in alcoholic cirrhosis, suggesting that the hepatocarcinogenesis in NAFLD and alcoholic liver disease were similar but were lower than that in HCV.

Using Cox hazards analysis, older age, higher serum γ-glutamyl transpeptidase level, and higher Child–Pugh score as risk factors of HCC were identified. Finally, clinical data of NAFLD-HCC with the data for HCC with HCV (HCV-HCC) were compared. The percentage of NAFLD-HCC patients with des-gamma-carboxy prothrombin-positive was higher than that with α-fetoprotein-positive. The 5-year survival and recurrence rates for NAFLD-HCC were almost similar to those for HCV-HCC. In Asian countries, the prevalence of NAFLD is increasing. Therefore, elucidating the pathogenesis and clinical features of HCC in patients with NAFLD is indeed an urgent problem.

Introduction
Primary liver cancer is the fifth most common cancer worldwide and the third most common cause of cancer mortality. Hepatocellular carcinoma (HCC) accounts for about 90% of primary liver cancers. With respect to the underlying liver disease, the latest nationwide report of the Liver Cancer Study Group of Japan showed that hepatitis C virus (HCV)-related liver disease is the most common underlying cause of HCC. HCV-related HCC accounts for 67% of all HCC cases, followed by 16% for hepatitis B virus (HBV)-related HCC. The incidence of HCV-related HCC has been gradually decreasing in recent years, while the incidence of HCC associated with non-viral chronic liver disease has gradually been increasing.

In Pacific and Asian countries, the prevalence of non-alcoholic fatty liver disease (NAFLD) in the general population is increasing dramatically and ranges from 5% to 40%. NAFLD consists of simple steatosis and non-alcoholic steatohepatitis (NASH), while NASH comprises a wide spectrum of conditions from NASH without fibrosis to cirrhosis. Obesity and diabetes mellitus have been established as significant risk factors for HCC by epidemiological observations and experimental studies, and there is increasing evidence that NASH is a risk factor for HCC. We reported that HCC was a critical factor in the prognosis of NAFLD cirrhosis. Therefore, there is an urgent need to elucidate pathogenesis, clinical features, and treatments for these diseases, especially NAFLD advanced stages and NAFLD-related HCC (NAFLD-HCC).

In this review, we describe the survey of HCC in Japan that my colleagues and I conducted, the rate at which HCC develops from NAFLD, the risk factors for HCC in NAFLD, the clinical features of NAFLD-HCC.

National survey of HCC
We performed a national survey investigating the etiology of HCC in the Japanese population in 2010. The nationwide survey included 14,530 HCC patients diagnosed during 2006–2009, of whom 14.1% were positive for hepatitis B surface (HBs) antigen, 66.3% were positive for HCV-RNA, and 3.7% were positive for both HBs antigen and HCV-RNA. Among those surveyed, 15.8% of patients were diagnosed as having non-HBV, non-HCV HCC.
Among HCCs based on non-viral disease, alcoholic liver disease with HCC (ALD-HCC) accounted for 7.2% of all HCCs, followed by chronic liver disease of unknown etiology with HCC (unknown HCC) (5.1%) and NAFLD with HCC (2.0%) (Fig. 1). The characteristics of these three groups were clearly different from one another (median age was 72 years for NAFLD-HCC, 68 years for ALD-HCC, and 73 years for unknown HCC, \( P < 0.01 \); female gender was 38%, 4%, and 37%, respectively, \( P < 0.01 \) (Table 1). Body mass index (BMI) and the prevalence of diabetes, hypertension, and dyslipidemia were significantly higher in patients with NAFLD-HCC than in those with ALD-HCC and unknown HCC. Serum levels of total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and \( \gamma \)-glutamyl transpeptidase (GTP) were significantly higher in the ALD-HCC group compared with the other groups, while the platelet count and serum albumin level were lowest in the ALD-HCC group. The hemoglobin A\(_{1C} \) and fasting blood glucose levels were highest in the NAFLD-HCC group. These data suggested that clinical characteristics of these three HCC groups were clearly different from one another.

Regarding the etiology of HCC in Western countries, NAFLD-HCC has been reported to account for 3.8–13% of all HCCs.\(^1\)\(^2\),\(^1\)\(^3\) In comparison with Western countries, the prevalence of NAFLD-HCC is lower in Japan. This is not only due to the low incidence of NAFLD-HCC but also to the high incidence of hepatitis virus-related HCC in Japan. However, the incidence of NAFLD-HCC in Japan is expected to increase in the future because of the rising prevalence of NAFLD associated with obesity and/or diabetes.

To determine whether modest alcohol intake could influence carcinogenesis in patients with unknown HCC, we divided the patients into a no alcohol subgroup (alcohol consumption < 20 g/day) and a modest alcohol intake subgroup (alcohol consumption of 20–70 g/day) (Table 2). Among the no alcohol subgroup, the prevalence of women was markedly higher (\( P < 0.001 \)) at 58% compared with the other two groups (62% and 60%, respectively) (Table 2). Among HCC patients with non-viral liver diseases, alcoholic liver disease with HCC (ALD-HCC) (7.2%) was the most common diagnosis, followed by unknown HCC (5.1%). Non-alcoholic fatty liver disease (NAFLD)-HCC (2.0%) was the third most common etiology. HCV; (HBV; , alcoholic; (NAFLD; (etiology unknown; *AIH, autoimmune hepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis. **Metabolic disease; Wilson disease, hemochromatosis, etc. Adapted from Tokushige et al.\(^1\)\(^1\)

**Table 1** Comparison among NAFLD-HCC, ALD-HCC, and unknown HCC

|                  | NAFLD-HCC (n = 292) | ALD-HCC (n = 991) | Unknown-HCC (n = 614) | \( P \) value |
|------------------|---------------------|-------------------|-----------------------|--------------|
| Age at diagnosis | 72 ± 8.4            | 68 ± 9.1          | 73 ± 10.1             | \(< 0.001\)  |
| Gender (female)  | 38%                 | 4%                | 37%                   | \(< 0.001\)  |
| BMI (kg/m\(^2\)) | 27.0 ± 4.0          | 23.8 ± 3.7        | 23.5 ± 4.1            | \(< 0.001\)  |
| Diabetes         | 70%                 | 49%               | 43%                   | \(< 0.001\)  |
| Hypertension     | 60%                 | 43%               | 46%                   | \(< 0.001\)  |
| Dyslipidemia     | 35%                 | 14%               | 15%                   | \(< 0.001\)  |
| Liver cirrhosis  | 62%                 | 78%               | 52%                   | \(< 0.001\)  |
| Albumin (g/dL)   | 3.8 ± 0.6           | 3.6 ± 0.6         | 3.6 ± 0.6             | \(< 0.001\)  |
| Total bilirubin  | 0.9 ± 1.3           | 1.1 ± 1.9         | 0.9 ± 1.7             | \(< 0.001\)  |
| AST (IU/L)       | 40 ± 36             | 80 ± 301          | 43 ± 71               | \(< 0.001\)  |
| ALT (IU/L)       | 35 ± 35             | 45 ± 176          | 30 ± 44               | 0.03         |
| \( \gamma \)-GTP (IU/L) | 91 ± 202    | 147 ± 271         | 88 ± 198              | \(< 0.001\)  |
| FBS (mg/dL)      | 119 ± 57            | 111 ± 63          | 107 ± 53              | \(< 0.001\)  |
| HbA\(_{1C} \) (%)| 6.3 ± 1.4           | 5.9 ± 1.6         | 5.7 ± 1.4             | \(< 0.001\)  |
| Platelet count (\( \times 10^4 \)/mm\(^3\)) | 14.1 ± 7.4 | 12.6 ± 8.0 | 15.2 ± 9.1 | \(< 0.001\)  |
| AFP (mg/mL)      | 12 ± 427 557        | 11 ± 368 512      | 13.0 ± 94 155         | 0.284        |

Adapted from Tokushige et al.\(^1\)\(^1\)

\( \gamma \)-GTP, gamma-glutamyl transpeptidase; AFP, \( \alpha \)-fetoprotein; ALD, alcoholic liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FBS, fasting blood sugar; HbA\(_{1C} \), hemoglobin A\(_{1C} \); HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease.
Adapted from Tokushige et al. 11

γ-GTP, gamma-glutamyl transpeptidase; AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FBS, fasting blood sugar; HbA1C, hemoglobin A1C; HCC, hepatocellular carcinoma.

versus only 8% in the modest alcohol subgroup. The mean age at diagnosis of HCC was higher in the no alcohol subgroup than in the modest alcohol intake subgroup (75.5 years vs 72 years, \( P < 0.001 \)). Between the two subgroups, the modest alcohol intake subgroup showed different clinical features in terms of unknown HCC and showed the same trends in regard to gender, BMI, lifestyle-related diseases, and γ-GTP levels as the ALD-HCC group.

These data suggested that a relatively low alcohol intake may lead to the development of non-viral HCC. The alcohol consumption criteria for diagnosis of alcoholic liver disease vary around the world,14,15 and the alcohol consumption criteria for alcoholic liver disease proposed by the Japanese Study Group of Alcoholic Liver Disease is more than 70 g/day. Our data suggest that social or modest intake of alcohol might have a more significant role in hepatic carcinogenesis than is presently thought. In the future, more detailed studies will need to be performed, including assessment of alcohol metabolism genotypes.

HCC rate in patients with NAFLD

Kawamura et al. reported that rate of HCC was 0.51%/12 years from all NAFLD, including simple steatosis.16 In NAFLD as a whole, the development of HCC is rare. However, liver fibrosis is the most important factor for development of HCC in any liver disease. To make clear the hepatocarcinogenic power in NAFLD, we compared the HCC development rates among liver cirrhosis (LC) with NAFLD (NAFLD-LC), alcoholic cirrhosis (ALD-LC), and cirrhosis infected with HCV (HCV-LC) in our hospital. HCC development rates were 11.3%/5 years in NAFLD-LC, 30.5%/5 years in HCV-LC, and 12.5%/5 years in ALD-LC (Fig. 2).10,17 Sanyal et al. and Ascha et al. reported that the HCC development rate in NAFLD cirrhosis was about 10–13% in 5 years and lower than that of HCV-LC in the USA.18,19 These data almost match the Japanese data. The rates of hepatocarcinogenesis in NAFLD and alcoholic liver disease were almost identical but were lower than that in HCV-LC. Adapted from Yatsuji et al. and Kodama et al.10,17

Table 2 The comparison between the no alcohol and modest alcohol subgroups of unknown HCC

|                     | No alcohol intake | Modest alcohol intake | P value |
|---------------------|-------------------|-----------------------|---------|
| Age at diagnosis    | 75.5 ± 10.2       | 72 ± 9.0              | < 0.001 |
| Gender (female)     | 58%               | 8%                    | < 0.001 |
| BMI (kg/m²)         | 23.8 ± 4.5        | 23.5 ± 3.4            | 0.396   |
| Diabetes            | 41%               | 46%                   | 0.214   |
| Hypertension        | 45%               | 49%                   | 0.424   |
| Dyslipidemia        | 15%               | 15%                   | 0.989   |
| Liver cirrhosis     | 57%               | 42%                   | 0.001   |
| Albumin (g/dL)      | 3.6 ± 0.7         | 3.8 ± 0.6             | 0.030   |
| Total bilirubin (mg/dL) | 0.9 ± 1.5     | 0.8 ± 1.2             | 0.266   |
| AST (IU/L)          | 44 ± 63           | 39 ± 73               | 0.081   |
| ALT (IU/L)          | 29 ± 45           | 29 ± 42               | 0.455   |
| γ-GTP (IU/L)        | 75 ± 184          | 103.5 ± 213           | 0.003   |
| FBS (mg/dL)         | 106 ± 51          | 110 ± 56              | 0.050   |
| HbA1C (%)           | 5.7 ± 1.3         | 5.7 ± 1.5             | 0.307   |
| Platelet count (×10^9/mm³) | 14.6 ± 9.0     | 16.8 ± 8.7            | 0.001   |
| AFP (ng/mL)         | 13.3 ± 77 396     | 10 ± 31 196           | 0.378   |

5-year HCC rates

- NAFLD-LC: 11.3%
- HCV-LC: 30.5%
- ALD-LC: 12.5%

Figure 2 Hepatocellular carcinoma (HCC) rate in non-alcoholic fatty liver disease (NAFLD) cirrhosis (NAFLD-LC), alcoholic liver disease-cirrhosis (ALD-LC), and hepatitis C virus (HCV)-liver cirrhosis (HCV-LC). The HCC rates in NAFLD-LC and ALD-LC were similar, and were lower than that in HCV-LC. Adapted from Yatsuji et al. and Kodama et al.10,17

Table 3 The comparison between NAFLD-HCC (n = 41) and NAFLD without HCC (n = 533) by multivariate logistic regression model

|                     | Odds ratio | 95% CI      | P value |
|---------------------|------------|-------------|---------|
| Age (older)         | 1.103      | 1.050–1.159 | < 0.001 |
| Gender (male)       | 4.680      | 1.803–12.146| 0.002   |
| Liver fibrosis      | 2.718      | 1.745–4.233 | < 0.001 |
| Activity            | 0.361      | 0.163–0.802 | 0.012   |
| ALT                 | 0.974      | 0.955–0.993 | 0.007   |
| γ-GTP               | 1.005      | 1.001–1.009 | 0.008   |

Risk factors of NAFLD-HCC

To clarify the risk factors of HCC in NAFLD, we compared clinical data between NAFLD-HCC and NAFLD without HCC with a multivariate logistic regression model. Both NAFLD patients with and without HCC were admitted to our hospital between 1990 and 2011. NAFLD was diagnosed by liver biopsy. Age, gender, BMI, diabetes, hypertension, dyslipidemia, blood examinations (total bilirubin, albumin, AST, ALT, alkaline phosphatase [ALP], γ-GTP, platelet, prothrombin time [PT]), and liver histology findings (fibrosis grade, activity grade, and steatosis grade) were analyzed as risk factors of HCC. In the results, older age, male gender, advanced liver fibrosis, lower activity of liver histology, lower ALT level, and higher γ-GTP level were detected as risk factors of HCC in the population with NAFLD-HCC (Table 3). However, this analysis did not include the factor of duration, and liver fibrosis is the most important factor for...
development of HCC. In the next analysis, we investigated the risk factors for HCC in 72 NAFLD-LC patients with a Cox hazards model. All NAFLD-LC patients were admitted to our hospital between 1990 and 2011. NAFLD-LC was diagnosed by liver biopsy. The patients with NAFLDH-LC were assessed with regard to the development of HCC, and their risk factors for HCC were analyzed. Age, gender, BMI, ascites, varices, encephalopathy, diabetes, hypertension, dyslipidemia, blood examinations (total bilirubin, albumin, AST, ALT, ALP, hypertension, γ-GTP, platelet, PT), and Child–Pugh score were analyzed as risk factors of HCC. Older age, higher serum γ-GTP level, and higher Child–Pugh score were identified as risk factors in NAFLD-LC (Table 4), and older age and Child–Pugh were confirmed by log-rank test.17 Kawamura et al. reported the risk factors for HCC in all NAFLD patients as being old age. AST > 40 IU/mL, advanced fibrosis, and diabetes mellitus.16 Ascha et al. reported that NASH patients with cirrhosis had a greatly increased risk of liver cancer, and even social alcohol consumption appeared to be the most significant factor associated with the risk of HCC.19 Considering all of these findings, we conclude that older age, male gender, advanced fibrosis, γ-GTP level, which was the marker of oxidative stress, diabetes mellitus, and mild alcohol intake might be important factors in the pathogenesis of HCC in NAFLD.

Clinical features of NAFLD-HCC

Finally, we compared the clinical data of NAFLD-HCC with the data for HCC caused by HCV infection (HCV-HCC) in our hospital. The percentage of NAFLD-HCC patients with des-gamma-carboxy prothrombin-positive results was higher than that of patients with α-fetoprotein-positive results.20 Yasui et al. also showed the same profile of tumor markers in NASH-HCC.21 In our hospital, the 5-year survival rate in the treated NAFLD-HCC group was 55.2%, and the cumulative HCC recurrence rate at 5 years was 69.8% as opposed to a 5-year survival rate of 50.6% and recurrence rate of 83.1% in the HCV-HCC group.22 The 5-year survival and recurrence rates for NAFLD-HCC were almost similar to those for HCV-HCC.

Zen et al. reported a case of HCC arising in a patient diagnosed with NASH at 62 years old. At 66 years old, her first hepatic tumor appeared. The pathological diagnosis of the first nodule was “pseudolymphoma.” When she was 72 years old, three hepatic tumors appeared and were diagnosed as moderately differentiated HCC. At age 73, two more tumors appeared and were diagnosed as well-differentiated HCC and a dysplastic nodule.23 These results suggested a multicentric occurrence of HCC in NASH, similar to HCC based on viral hepatitis.

We had measured anti-hepatitis B core (HBc) antibody to investigate the influence of HBV on the carcinogenesis of NAFLD-HCC. The difference between the NAFLD-HCC group and HCV-HCC group was not significant, and none of the NAFLD-HCC patients had high HBc antibody titers that would have led to the suspicion that they were HBV carriers. These findings therefore suggested that even if HBV did influence carcinogenesis in NAFLD, the influence would be minimal.

We reported that HCC was a critical factor in the prognosis of NAFLD.10 Regular screening for HCC is extremely important, especially in NAFLD patients with advanced fibrosis, and the strong possibility of recurrence also warrants close attention.

In conclusion, in Asian countries, the prevalence of NAFLD is increasing dramatically. Elucidating the pathogenesis, clinical features, and treatment of HCC in NAFLD is an urgent problem.

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