Incidence of and Risk Factors for Hepatocellular Carcinoma in Primary Biliary Cirrhosis: National Data from Japan

Kenichi Harada,1* Junko Hirohara,2* Yoshiyuki Ueno,3 Toshiaki Nakano,4 Yuko Kakuda,1 Hirohito Tsubouchi,5 Takaumi Ichida,6 and Yasuni Nakanuma1

Primary biliary cirrhosis (PBC) primarily affects females and is rarely complicated by hepatocellular carcinoma (HCC). Although the HCC incidence in PBC patients is low, several characteristics and risk factors associated with its development have been reported. In this study, national data concerning the current status of carcinogenesis in PBC patients in Japan are reviewed. Using data from two national questionnaire surveys, we investigated the clinicopathological findings associated with HCC in PBC patients. According to the data of all reviewed PBC patients, the HCC incidence was 2.4% (71/2946). The HCC incidence by gender was 5.1% (19/370) in males and 2.0% (52/2576) in females, and the proportion of males was 26.7%. Prognosis was significantly poorer in the PBC patients with HCC than in those without. Multivariate analysis of risk factors associated with HCC by gender revealed histological stage at the time of PBC diagnosis as an independent risk factor associated with the development of HCC in females, but not in males. Furthermore, data from another national survey of 178 PBC patients with HCC (male/female 49/129; proportion of males 27.5%) revealed that the duration between the diagnosis of PBC and that of HCC was significantly shorter in males than in females. In addition, histological stage at the time of HCC diagnosis was an independent risk factor for HCC in females, whereas no risk factors were identified in males. Conclusion: these data indicate that males are at risk of developing HCC at any histological stage of PBC. Therefore, male PBC patients in particular should be carefully screened for HCC from the early stages of PBC. (HEPATOLOGY 2013;57:1942-1949)

Primary biliary cirrhosis (PBC) primarily affects middle-aged females. Histologically, the interlobular bile ducts are primarily damaged and show characteristic findings such as chronic nonsuppurative destructive cholangitis (CNSDC) followed by progressive bile duct loss.1,2 A terminal feature of PBC is irreversible biliary cirrhosis, and liver transplantation is the sole treatment for hepatic failure.3 Although hepatic failure defines the prognosis in most PBC patients, hepatocellular carcinoma (HCC) is also reported to occur in 0.76%-5.9% of PBC patients.4-9 Recently, however, the incidence of PBC complicated by HCC has been gradually increasing with improvements in PBC treatment and survival. In general, HCC is typically encountered in the terminal stage, when irreversible biliary cirrhosis sets in. Moreover, the hepatitis virus is a major risk factor for HCC development in patients with chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infections. In PBC patients, however, no carcinogenic factors directly associated with HCC have been identified. The proposed risk factors for HCC arising from PBC-affected livers include the hepatitis virus, cirrhosis, older age, diabetic mellitus, and male gender.4,5,10-13 However, epidemiologic studies are limited and provide conflicting results, perhaps because of the low prevalence of the disease and geographical and environmental differences.
In the present study we evaluated data from two nationwide surveys performed in Japan. Our aim was to clarify the current status of carcinogenesis in PBC patients, identify the associated clinicopathological risk factors, and understand how the pathogenesis of PBC is directly associated with HCC.

Materials and Methods
Setting and Patient Selection

Survey of PBC in Japan (National Survey by the Intractable Hepato-Biliary Diseases Study Group). National surveys of PBC patients in Japan have been performed 14 times biennially or triennially by the Intractable Hepato-Biliary Diseases Study Group for Research on Measures for Intractable Disease, which is supported by Health Labor Sciences Research Grants in Japan. The subjects included 7,376 patients registered in the 1st-14th surveys performed between 1980 and 2009.9,14 Of the 7,376 patients, the absence or presence of HCC was confirmed during follow-up in 2,946 (70 males, 2,576 females), who were then investigated in the current study. HBV carriers and HB antigen- and anti-HCV antibody-positive patients were excluded.

Survey of PBC Patients with HCC in Japan (National Survey by the Liver Cancer Study Group of Japan). This project was set up at the 47th Annual Meeting of the Liver Cancer Study Group of Japan (President, Professor Ichida), and it was executed in 2011. Questionnaires were sent to 340 hospitals or institutions included in the Liver Cancer Study Group of Japan. Eighty-six of the 340 hospitals responded, and data from 178 PBC patients with HCC from 39 hospitals or institutions were eventually included. HBV carriers and HB antigen- and anti-HCV antibody-positive patients were excluded. The cooperating institutions are listed in the Appendix.

PBC Diagnosis

PBC was diagnosed according to criteria established by the Intractable Hepato-Biliary Diseases Study Group of Japan. Patients whose condition met one of the following criteria were diagnosed as having PBC: (1) histologically confirmed CNSDC with laboratory findings positive for PBC; (2) positivity for antimitochondrial (AMA) and/or anti-pyruvate dehydrogenase (PDH) antibodies, absence of histological findings of CNSDC, and presence of histological findings compatible with PBC; and (3) no histological examination, but positivity for AMA and/or anti-PDH antibodies and clinical findings and course indicative of PBC. PBC symptoms were defined as pruritus, overt jaundice, esophageal varices, ascites, and hepatic encephalopathy.15 Histological findings were classified according to Scheuer’s system.16

Statistical Analysis

The Mann-Whitney U and chi-square tests test were used as nonparametric and independence tests, respectively. Logistic regression analysis was used for the multivariate analysis of prognostic factors. Survival rate was obtained by the Kaplan-Meier method. \( P < 0.05 \) was considered statistically significant.

Results

HCC Incidence in the Japanese PBC Population.

The current status of and risk factors for HCC in PBC patients in Japan were analyzed on the basis of data from the national survey conducted by the Intractable Hepato-Biliary Diseases Study Group. The total number of PBC patients was 2,946. Of these, 2,100 cases available for analysis of histological stage of PBC at diagnosis underwent liver biopsy. HCC incidence during follow-up was 2.4% (71/2,946). This incidence was 5.1% (19/370) in males and 2.0% (52/2,576) in females, and the proportion of males was 26.7%. The mean ± standard deviation and median values for the observation period were 80.1 ± 70.8 (range, 1-443) and 58 months, respectively. The mean value for males was 65.1 ± 57.2 (range, 1-237; median, 45) months, while that for females was 82.2 ± 72.2 (range, 1-443; median, 60) months.

A comparative analysis of PBC patients with and without HCC revealed male gender, old age, low serum albumin levels, low serum total cholesterol levels, advanced histological stage, and symptomatic status at the time of PBC diagnosis as significant risk factors for HCC (Table 1). There was no difference in total bilirubin levels and the presence or absence of
ursodeoxycholic acid (UDCA) treatment between the two groups (Table 1). Prognosis was significantly poorer in the PBC patients with HCC than in those without (Fig. 1). The cumulative incidence of carcinogenesis was 6.5% in males and 2.0% in females during the 10 years after PBC diagnosis; the difference between males and females was statistically significant ($P < 0.0001$) (Fig. 2). In particular, analyses of HCC incidence in patients aged 10-80 years revealed that male PBC patients in their 40s and 50s had an increased risk of HCC compared with female PBC patients in the same age groups (data not shown). In multivariate analysis for risk factors of HCC, gender and histological stage were selected as significant factors ($P < 0.00001$) (Table 2). There was no difference in the proportion of males and females who underwent histological staging at PBC diagnosis. The incidence of histological stages 3 and 4 was $\sim16.0$% in both male and female PBC patients without HCC (Fig. 3), whereas it was 14.2% and 57.1% in male and female PBC patients with HCC, respectively.

Advanced histological stage was a risk factor for HCC in females ($P < 0.0001$; Fig. 3; Table 2). Multivariate analysis for risk factors of HCC by gender revealed that histological stage at the time of PBC diagnosis was an independent risk factor for HCC in females (Supplemental Table 1), whereas no significant independent factors were revealed for males (Supplemental Table 2). Moreover, although we assessed PBC patients with HCC according to histological stage, we found no difference in any clinical or biological characteristics between patients with and without cirrhosis at PBC diagnosis (Supplemental Table 3).

**Table 1. Clinical and biological characteristics of PBC patients with or without HCC at PBC diagnosis**

|                          | HCC(+) | HCC(−) | P     |
|--------------------------|--------|--------|-------|
| Number                   | 71     | 2875   |       |
| Sex (M:F)                | 19:52  | 351:252| 0.0003|
| Age (Mean ± SD)          | 60.5 ± 10.4 | 56.4 ± 11.2 | 0.0023|
| T-Bilirubin (Mean ± SD)  | 1.37 ± 1.63 | 0.99 ± 1.52 | 0.1061|
| Albumin (Mean ± SD)      | 3.81 ± 0.58 | 4.05 ± 0.51 | 0.0002|
| T-cholesterol (Mean ± SD)| 201.3 ± 60.5 | 217.4 ± 86.7 | 0.0397|
| Histological stage (I/II/III/IV) | 10/17/14/8 | 1060/662/263/66 | <0.0001|
| Use of UDCA (%)          | 89.7   | 91.8   | 0.5291|
| Clinical stage (asymptomatic: symptomatic) | 38:33 | 2775/100 | <0.0001|

**Fig. 1. Kaplan-Meier curve for survival in patients with PBC with (+) or without (−) HCC. There is a statistically significant difference between the curves ($P < 0.05$).**

**Table 2. Factors associated with increased risk of HCC in PBC patients (multivariate analysis)**

|                          | regression coefficient | standard deviation | $\chi^2$ | odds ratio | P value |
|--------------------------|------------------------|--------------------|----------|------------|---------|
| Sex (M:F)                | −0.5646                | 0.1737             | 10.56    | 3.0932     | 0.0012  |
| Age                      | −0.0242                | 0.0149             | 2.63     | 0.9760     | 0.1050  |
| T-Bilirubin              | 0.0302                 | 0.0880             | 0.12     | 1.0307     | 0.7313  |
| Albumin                  | 0.0274                 | 0.3087             | 0.01     | 1.0277     | 0.9292  |
| T-cholesterol            | 0.0021                 | 0.0026             | 0.65     | 1.0021     | 0.4210  |
| Histological stage (I/II/III/IV) | −0.7294 | 0.1661 | 19.27 | 0.4821 | <0.0001 |
| Use of UDCA (%)          | −0.2823                | 0.2473             | 1.3      | 1.7590     | 0.2537  |
| Clinical stage (asymptomatic: symptomatic) | 0.2990 | 0.1674 | 3.19 | 0.5498 | 0.0741  |

**Fig. 2. Cumulative appearance rates of HCC in patients with PBC by gender. There is a statistically significant difference between males and females.**
underwent liver transplantation, which was performed at the time of HCC discovery in three and 3 years after HCC discovery in one. There were 49 male and 129 female PBC patients with HCC, and the proportion of males was 27.5%, which was similar to that from the previously described national survey of PBC. Although the average age at the time of PBC diagnosis was slightly higher for males (68 years) than for females (62 years), that at the time of HCC diagnosis was similar between males (73 years) and females (72 years; Fig. 4). Moreover, the duration between the diagnosis of PBC and that of HCC was shorter in males than in females. HCC was diagnosed simultaneously with or prior to the diagnosis of PBC in 32.7% (16/49) males and 14.7% (19/129) females (Fig. 4).

Pathological examination for HCC and background liver tissue assessment by biopsy or heptectomy was conducted for 66 and 82 patients, respectively. Clinicopathological data at the time of HCC diagnosis are shown in Table 3. There were no differences in the history of blood transfusion, diabetes mellitus, AMA levels, anti-nuclear antibody levels, body mass index (BMI), or triglyceride levels, but there were higher rates of prior HBV infection and alcohol intake in males. Total cholesterol levels were slightly higher in males, but there were no significant differences in the rates of use of ursodeoxycholic acid (UDCA).

Table 3. Clinical and biological characteristics of male and female PBC patients at HCC diagnosis

|                | Male (n = 49) | Female (n = 129) | Total (n = 178) |
|----------------|---------------|------------------|-----------------|
| Blood transfusion | 9%            | 8%               | 9%              |
| past HBV infection* | 33%          | 18%              | 22%             |
| Alcohol intake*    | 27%           | 2%               | 9%              |
| Diabetes mellitus  | 24%           | 23%              | 24%             |
| AMA levels         | 86%           | 82%              | 83%             |
| ANA levels         | 41%           | 49%              | 47%             |
| BMI (>25%)         | 25%           | 31%              | 29%             |
| Triglyceride (>150) | 8%            | 9%               | 9%              |
| Total cholesterol (>220) | 15%        | 9%               | 11%             |
| associated with NAFLD | 0%         | 4%               | 3%              |
| Use of UDCA        | 84%           | 84%              | 84%             |

(*p < 0.05)
index, serum triglyceride levels, serum total cholesterol levels associated with nonalcoholic fatty liver disease (including nonalcoholic steatohepatitis), and use of UDCA between males and females (Table 3). However, an analysis excluding patients with past HBV infection and a history of alcohol consumption revealed that there was no difference in other clinical findings, although the proportion of males (male/female = 370/2,576, 12.6%; \( P < 0.05 \); Supplemental Table 4). Moreover, in females the HCC incidence gradually increased with histologic stage, while the incidence in males showed no trend or statistical significance. There was a significant difference in the distribution of histologic stage between males and females (Fig. 5). An analysis of PBC patients with HCC according to histological stage revealed no clinical findings (including past HBV infection and alcohol consumption) that were significantly different between patients with and without cirrhosis at HCC diagnosis (Supplemental Table 5). There was also no significant difference in tumor number and differentiation between males and females (Supplemental Table 6).

Discussion

Recently, we encountered PBC patients with HCC during routine pathological assessments, and the number of these patients appears to have increased according to reports from other institutes. In most patients, HCC is detected during follow-up for PBC, whereas some patients are simultaneously diagnosed with PBC and HCC or diagnosed with HCC prior to PBC. Although prognosis has improved with advances in treatment for PBC, the precise reason for the increased number of PBC patients with HCC in recent decades remains unknown. Therefore, we analyzed data from Japanese PBC patients and those with PBC and HCC who were independently surveyed by two different study groups. One set of data was from a national survey of PBC patients performed 14 times between 1980 and 2009, while the other was from PBC patients with HCC who were evaluated as a special project of the Annual Meeting of the Liver Cancer Study Group of Japan in 2011. Both surveys collected data through questionnaires administered to foundation hospitals or specialized hospitals for hepatology in Japan. Therefore, although the investigative hospitals/institutions and objectives did not match, it is speculated that most PBC patients with HCC overlapped. Moreover, the proportion of males among PBC patients with HCC almost coincided in these two independent studies (26.7% versus 27.5%), validating the use of these studies together as representative of the situation in Japan.

Although some studies have reported that PBC patients do not have an increased risk of developing HCC, others showed that the HCC incidence was high in PBC patients. The HCC incidence among PBC patients is reportedly low, at 0.76%-5.9%, according to previous reports. In this study, we investigated the incidence of and risk factors for HCC in Japanese PBC patients. According to data from the nationwide survey by the Intractable Hepato-Biliary Diseases Study Group, the HCC incidence was 2.4%. As for risk factors associated with HCC in PBC patients, several conflicting results have been reported. In general, male gender, advanced stage, HCV infection, and a history of blood transfusion were reported to be associated with HCC in PBC patients. In a proportional hazards analysis of patients with PBC in Japan, Shibuya et al. reported three factors to be independently associated with HCC development: age at the time of diagnosis, male gender, and history of blood transfusion. While autoimmune liver disease, including PBC, is more common in females than in males, HCC incidence in PBC patients was higher in males than in females. In agreement with previous reports from Japan, Europe, and the US, gender was identified as a risk factor associated with HCC in the nationwide survey of PBC patients conducted by the Intractable Hepato-Biliary Diseases Study Group. HCC incidence was 5.1% in males and 2.0% in females (proportion of males, 26.7%), indicating that male PBC patients had a 2.1-fold higher risk of HCC compared with female PBC patients.
patients. The proportion of males among the PBC patients with HCC was consistent with that in the nationwide survey by the Liver Cancer Study Group of Japan (27.5%). Moreover, the cumulative HCC incidence was 6.5% in males and 2.0% in females during the 10 years after PBC diagnosis, and male PBC patients had a 3.3-fold higher risk of HCC compared with females. In general, during the carcinogenesis of HCC, estrogen can protect hepatocytes from malignant transformation by way of downregulation of interleukin (IL)-6 release from Kupffer cells, indicating that estrogen-mediated inhibition of IL-6 production by Kupffer cells potentially decreased the risk of HCC in females. Therefore, although PBC primarily affects females, HCC may be more common in male PBC patients because of a lack of estrogen-mediated prevention. The national survey by the Liver Cancer Study Group of Japan revealed that the duration between the diagnosis of PBC and that of HCC was shorter in males than in females and that the diagnosis of HCC was performed simultaneously at or prior to the diagnosis of PBC in 32.7% males and 14.7% females. Several reasons may be responsible for the delayed diagnosis of PBC and carcinogenesis in the early stage in males, but the details remain unspecified. Moreover, the rate of past HBV infection and alcohol consumption was significantly higher in males than in females, indicating that these factors also possibly affect the increased HCC incidence in male PBC patients. Watanabe et al. reported that past HBV infection is an important factor in the association of HCC with PBC. In a patient with HBV infection, HBV-DNA possibly integrates into the human genome, but the frequency of this integration in prior HBV-infected PBC patients with HCC remains unknown. Moreover, because the distribution of past HBV infection by gender in the whole PBC population could not be obtained, the extent to which previous infection with HBV is directly associated with HCC carcinogenesis in male PBC patients remains debatable. However, analysis excluding cases with past HBV infection and a history of alcohol consumption revealed that the proportion of males with HCC in PBC patients with HCC remained high compared with that of all PBC male patients. In addition, analysis according to histological stage (noncirrhosis versus cirrhosis) suggested that past HBV infection and alcohol consumption were not directly associated with progression to cirrhosis in PBC patients with HCC.

In conclusion, we investigated the risk factors for HCC using data from two nationwide surveys of PBC patients in Japan. Because male PBC patients are at risk of developing HCC at any histologic stage, they should be carefully screened for HCC from an early stage of PBC, irrespective of histological stage.

Acknowledgment: The authors thank Enago (www.enago.jp) for the English language review.

Appendix

The authors thank the cooperating questionnaire investigation of PBC with HCC project at the 47th Annual Meeting of the Liver Cancer Study Group of Japan (President, Professor Ichida); Dr. Komori and Dr. Ishibashi (National Hospital Organization Nagasaki Medical Center, Gastroenterology), Dr. Ueda and Dr. Kaneko (Kanazawa University Graduate School of Medicine, Internal
References

1. Nakanuma Y, Ohta G. Histometric and serial section observations of the intrahepatic bile ducts in primary biliary cirrhosis. Gastroenterology 1979;76:1326-1332.
2. Kaplan MM, Gershwin ME. Primary biliary cirrhosis. N Engl J Med 2005;353:1261-1273.
3. Yamagiwa S, Ichida T. Recurrence of primary biliary cirrhosis and primary sclerosing cholangitis after liver transplantation in Japan. Hepatol Res 2007;37(Suppl 3):S449-S454.
4. Jones DE, Mertz JF, Collier JD, Bassendine MF, James OF. Hepatocellular carcinoma in primary biliary cirrhosis and its impact on outcomes. HEPATOLOGY 1997;26:1138-1142.
5. Shibuya A, Tanaka K, Miyakawa H, Shibata M, Takatori M, Sekiyama K, et al. Hepatocellular carcinoma and survival in patients with primary biliary cirrhosis. HEPATOLOGY 2002;35:1172-1178.
6. Deutsch M, Papaevangelou GV, Trakou A, Hadziyannis SJ. Risk of hepatocellular carcinoma and extrahepatic malignancies in primary biliary cirrhosis. Eur J Gastroenterol Hepatol 2008;20:5-9.
7. Floreani A, Biagini MR, Chiaravon M, Milani S, Surrenti C, Naccarato R. Incidence of hepatic and extra-hepatic malignancies in primary biliary cirrhosis. Ital J Gastroenterol 1993;25:473-476.
8. Miyake Y, Iwasaki Y, Terada R, Okamaoto R, Ikeda H, Makino Y, et al. Persistent elevation of serum alanine aminotransferase levels leads to poor survival and hepatocellular carcinoma development in type 1 autoimmune hepatitis. Aliment Pharmacol Ther 2006;24:1197-1205.
9. Nakano T, Inoue K, Hirohara J, Arita S, Higuchi K, Omata M, et al. Enhanced apoptosis relates to bile duct loss in primary biliary cirrhosis. HEPATOLOGY 2008;48:1149-1156.
10. Cavaza A, Caballero L, Floreani A, Farinati F, Bruguera M, Caroli D, et al. Incidence, risk factors, and survival of hepatocellular carcinoma in primary biliary cirrhosis: comparative analysis from two centers. HEPATOLOGY 2009;50:1162-1168.
11. Suzuki A, Lymp J, Donilinger J, Mendes F, Angulo P, Lindor K. Clinical predictors for hepatocellular carcinoma in patients with primary biliary cirrhosis. Clin Gastroenterol Hepatol 2007;5:259-264.
12. Sasaki H, Inoue K, Higuchi K, Yasuyama T, Koyata H, Kuroki T, et al. Primary biliary cirrhosis in Japan: national survey by the Subcommittee on Autoimmune hepatitis. Gastroenterol Jpn 1985;20:1476-1485.
13. Prince MI, Chetwynd A, Craig WL, Metcalf JF, James OF. Asymptomatic primary biliary cirrhosis: clinical features, prognosis, and symptom progression in a large population based cohort. Gut 2004;53:865-870.
14. Scheuer P, Lefkowitzt JH (eds.). Liver biopsy interpretation, 7th ed. Philadelphia: Elsevier Saunders; 2007.
15. Tsurissini SB, Kaplan MM. Hepatocellular carcinoma in primary biliary cirrhosis. Am J Gastroenterol 1997;92:676-678.
16. Caballero L, Pares A, Castells A, Gines A, Bru C, Rodes J. Hepatocellular carcinoma in primary biliary cirrhosis: similar incidence to that in hepatitis C virus-related cirrhosis. Am J Gastroenterol 2001;96:1160-1163.
17. Naoultoue WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, et al. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science 2007;317:121-124.
18. Yeh SH, Chen PI. Gender disparity of hepatocellular carcinoma: the roles of sex hormones. Oncology 2010;78(Suppl 1):172-179.
19. Harada K, Ozaki S, Gershwin ME, Nakanuma Y. Enhanced apoptosis relates to bile duct loss in primary biliary cirrhosis. HEPATOLOGY 1997;26:1399-1405.
20. Nijhawan PK, Therneau TM, Dickson ER, Boynton J, Lindor KD. Incidence of cancer in primary biliary cirrhosis: the Mayo experience. HEPATOLOGY 1999;29:1396-1398.