Incidence of and risk factors for hepatocellular carcinoma in primary biliary cirrhosis: National data from Japan

doi: 10.1002/hep.26176

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)], Takafumi Ichida[6)], Yasuni Nakanuma[1)]

Kenichi Harada and Junko Hirohara contributed equally to this work.

1) Department of Human Pathology, Kanazawa University Graduate School of Medicine, Kanazawa, Japan
2) Third Department of Internal Medicine, Kansai Medical University, Osaka, Japan
3) Department of Gastroenterology, Yamagata University Faculty of Medicine, Yamagata, Japan
4) University Information Center, Kansai Medical University, Osaka, Japan
5) Digestive and Lifestyle Diseases, Department of Human and Environmental Sciences, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan
6) Division of Gastroenterology and Hepatology, Juntendo University School of Medicine, Shizuoka Hospital, Izunokuni, Japan

Short title: HCC in PBC

Address correspondence to:

Kenichi Harada, MD

Department of Human Pathology
Kanazawa University Graduate School of Medicine
Kanazawa 920-8640, Japan
FAX : (0)76-234-4229 (Japan)
TEL : (0)76-265-2199 (Japan)
E-mail: kenichih@med.kanazawa-u.ac.jp

ABSTRACT

Primary biliary cirrhosis (PBC) primarily affects females and is rarely complicated by hepatocellular carcinoma (HCC). Although 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, HCC incidence was 2.4% (71/2946). 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. In 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.

INTRODUCTION

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% 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

A 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 Labour Sciences Research Grants in Japan. The subjects included 7376 patients registered in the 1st–14th surveys performed between 1980 and 2009.[9,14] Of the 7376 patients, the absence or presence of HCC was confirmed during follow-up in 2946 (70 males, 2576 females), who were then investigated in the current study. HBV carriers and HB antigen- and anti-HCV antibody-positive patients were excluded.

A 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 dehydrogenase 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. A p value of <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 2946. Of these, 2100 cases available for analysis of histological stage of PBC at diagnosis underwent liver biopsy. HCC incidence during follow up was 2.4% (71/ 2946). This incidence was 5.1% (19/370) in males and 2.0% (52/2576) 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 approximately 16.0% in both male and female PBC patients without HCC (Table 2), whereas that 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 and 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 (supplementary Table 1), whereas no significant independent factors were revealed for males (supplementary 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 (supplementary Table 3).

PBC patients with HCC in Japan

From the data of the national survey specially set up at the 47th Annual Meeting of the Liver Cancer Study Group of Japan, we collected and investigated those for 178 PBC patients with HCC from a total of 39 hospitals included in the study group. These cases included 100 fatalities in the past years as well as 78 patients followed up from each hospital or institute as of June, 2011. Among the followed-up patients, four 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 for 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.

Pathological examination for HCC and background liver tissue assessment by biopsy or hepatectomy was conducted for 66 and 82 patients, respectively. Clinicopathological data at the time of HCC diagnosis are shown in Table 3. There were more males with prior HBV infection and a history of alcohol consumption compared with females. There were no differences in the history of blood transfusion, diabetes mellitus, AMA levels, anti-nuclear antibody levels, body mass index, serum triglyceride levels, serum total cholesterol levels associated with nonalcoholic fatty liver disease (including nonalcoholic steatohepatitis), and use of UDCA (Table 3) between males and females. 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 = 24/104, 18.5%) remained higher than that of the total PBC male patients (male/female = 370/2576, 12.6%; $p < 0.05$; supplementary Table 4). Moreover, in females, 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 histological 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 (supplementary Table 5). There was also no significant difference in tumor number and differentiation between males and females (supplementary 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.[11,17,18] 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% vs 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,[19] others showed that HCC incidence was high in PBC patients.[5,7,20] HCC incidence among PBC patients is reportedly low at 0.76%–5.9% according to previous reports.[4-9] 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, HCC incidence was 2.4%. As for risk factors associated with HCC in PBC patients, several conflicting results have been reported.[4,5,11-13] In general, male gender, advanced stage, HCV infection, and a history of blood transfusion were reported to be associated with HCC in PBC patients.[5,7,20] In a proportional hazards analysis of patients with PBC in Japan, Shibuya et al.[5] 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 USA,[4,5,11-13] 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. 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, 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 via downregulation of 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.[21,22] 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.[18] 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 (non-cirrhosis *vs.* cirrhosis) suggested that past HBV infection and alcohol consumption were not directly associated with progression to cirrhosis in PBC patients with HCC.

In addition to male gender, the national survey by the Intractable Hepato-Biliary Diseases

Study Group demonstrated that old age, low serum albumin levels, low total cholesterol levels, advanced histological stage, and symptomatic status at the time of PBC diagnosis were statistically significant in PBC patients with HCC compared to those without HCC. However, multivariate analysis by gender revealed that histological stage at the time of diagnosis of PBC was an independent risk factor for HCC in females, but not in males. In addition to at the time of diagnosis of PBC, at that of HCC, histological stage is associated with HCC by national survey for PBC with HCC patients. However, there was no difference in any clinical or biological characteristics between PBC patients with HCC with or without cirrhosis at HCC diagnosis. In females, HCC incidence gradually increased according to histological stage, indicating that the terminal stage of PBC, which is a cirrhotic state, may be a risk factor for HCC development in females, whereas males are likely to develop HCC at any stage. The carcinogenesis of HCC in PBC patients should be further clarified. PBC is pathologically characterized by CNSDC, and the main inflammatory lesions associated with PBC are not hepatocytes but cholangiocytes, which may be one of the reasons why HCC incidence in PBC patients is relatively low compared with the incidence of sustained hepatitic diseases such as chronic viral hepatitis and autoimmune hepatitis. Male PBC patients with HCC are thought to be a good model because they lack estrogen-mediated prevention of HCC. Unlike that in hepatitic diseases, intrahepatic cholestasis is found from the early stage in PBC,[1,23] and some mitogenic factors in the bile of PBC patients presumably participate in the carcinogenesis of HCC from an early stage.[17,24] However, this hypothesis remains a matter of speculation, and further study is required to clarify the molecular mechanism involved in the carcinogenesis of HCC in PBC patients.

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.

ACKNOWLEDGEMENTS

The authors thank for cooperating questionary investigation of PBC with HCC projected at 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 Medicine), Dr. Taniai and Dr. Hashimoto (Tokyo Women's Medical University, Internal Medicine and Gastroenterology), Dr. Tateishi and Dr. Koike (The University of Tokyo, Gastroenterology), Dr. Takahashi, Dr. Abe, and Dr. Ohira (Fukushima Medical University, Internal Medicine), Dr. Korenaga, Dr. Tomiyama, and Dr. Hino (Kawasaki Medical School, Internal Medicine), Dr. Shida and Dr. Maguchi (Teine Keijinkai Hospital, Gastroenterology), Dr. Kita and Dr. Osaki (Osaka Red Cross Hospital, Gastroenterology), Dr. Makino and Dr. Fukuda (Ikeda Municipal Hospital, Gastroenterology), Dr. Yoshihara and Dr. Yoshimatsu (Oita University, Internal Medicine 1 Faculty of Medicine), Dr. Inoue and Dr. Enomoto (University of Yamanashi, First Department of Internal Medicine), Dr. Shinomura and Dr. Sasaki (Sapporo Medical University, Internal Medicine), Dr. Sakamoto (Hirosaki University, Graduate School of Medicine, Internal Medicine), Dr. Mukai (Ohta Nishinouchi Hospital, Gastroenterology), Dr. Sugimoto (Nagoya University, Surgery), Dr. Fujiyama, Dr. Nishimura and Dr. Fukumoto (Shiga University of Medical Science, Internal Medicine), Dr. Kon (Kansai Medical University, Surgery), Dr. Kaibori (Kansai Medical University, Hirakata Hospital, Surgery), Dr. Okazaki (Kansai Medical University, Internal Medicine), Dr. Seki (Kansai Medical University, Takii Hospital, Internal Medicine), Dr. Katayama (Osaka Medical Center for Cancer and Cardiovascular Diseases, Internal Medicine), Dr. Nakamura (Okayama University, Internal Medicine), Dr. Fujioka (Okayama Saiseikai General Hospital, Hepatology), Dr. Taura (Nagasaki University, Internal Medicine), Dr. Koga and Dr. Katafuchi (Kurume University, Internal Medicine), Dr. Sakaguchi (Hamamatsu University School of Medicine, Surgery), Dr. Inui (Fujita Health

University School of Medicine, Banbuntane Houtokukai Hospital, Internal Medicine), Dr. Yoshioka (Fujita Health University School of Medicine, Internal Medicine), Dr. Yoshikawa (Sanraku Hospital, Internal Medicine), Dr. Misawa (Sapporo City General Hospital, Surgery), Dr. Kakizoe (Kakizoe Hospital, Surgery), Dr. Tsuchiya, Dr. Kurosaki and Dr. Izumi (Musashino Red Cross Hospital, Gastroenterology), Dr. Ogawa (Nishinomiya Municipal Central Hospital, Internal Medicine), Dr. Kaiho (Kimitsu Chuo Hospital, Surgery), Dr. Horigome (Iida Municipal Hospital, Surgery), Dr. Shibata and Dr. Ariyoshi (University of Occupational and Environmental Health, Japan, Internal Medicine), Dr. Muro (National Hospital Organization Ohita Medical Center, Internal Medicine), Dr. Ikeda (Yokosuka Kyosai Hospital, Internal Medicine), Dr. Takahashi and Dr. Nakamura (Hokkaido Cancer Center, Internal Medicine), Dr. Matsunami (Matsunami General Hospital, Surgery), Dr. Doi (Otemae Hospital, Internal Medicine), Dr. Itamoto and Dr. Nakahara (Hiroshima Prefectural Hospital, Surgery), Dr. Kitamoto (Hiroshima Prefectural Hospital, Internal Medicine), Dr. Miyazaki and Dr. Yakushijin (Osaka University, Graduate School of Medicine, Internal Medicine), Dr. Watanabe (Yame General Hospital, Surgery), Dr. Kawada and Dr. Iwai (Osaka City University Graduate School of Medicine, Internal Medicine), Dr. Ishihara (Yokkaichi-Shoukaki Center, Internal Medicine), Dr. Inoue (Osaka General Medical Center, Internal Medicine), Dr. Hayashi and Dr. Ishizu (Kinki Central Hospital of the Mutual Aid Association of Public School Teachers, Internal Medicine), Dr. Aoki (Kinki Central Hospital of the Mutual Aid Association of Public School Teachers, Surgery), Dr. Usuda (Aizawa Hospital, Gastroenterology), Dr. Nakagome (Shirakawa Kosei General Hospital, Internal Medicine), Dr. Onji and Dr. Abe (Ehime University Graduate School of Medicine, Internal Medicine), Dr. Chayama and Dr. Aikata (Hiroshima University, Graduate School of Biomedical Sciences, Internal Medicine), Dr. Ishibashi (National Hospital Organization Nagasaki Medical Center, Clinical Research Center), Dr. Seki (Daito Central Hospital), Dr. Itsubo and Dr. Koike (The Jikei University School of Medicine, Internal Medicine), Dr. Hijikata

(The Jikei University School of Medicine, Daisan Hospital, Internal Medicine), Dr. Sakisaka and Dr. Takeyama (School of Medicine, Fukuoka University, Internal Medicine), Dr. Iijima and Dr. Tanaka (Hyogo College of Medicine, Internal Medicine), Dr. Yamamoto (Hyogo College of Medicine, Internal Medicine), Dr. Takamori (Teikyo University School of Medicine, Internal Medicine), Dr. Kikuchi (Teikyo University School of Medicine University Hospital, Mizonokuchi, Internal Medicine), Dr. Shinohara (Yokkaichi Municipal Hospital, Surgery), Dr. Nagao (Matsushita Memorial Hospital, Gastroenterology), Dr. Kirikoshi and Dr. Morimoto (Yokohama City University Medical Center, Internal Medicine), Dr. Okumoto and Dr. Saito (Yamagata University, Internal Medicine), Dr. Saito and Dr. Nikami (Nishisaitama-Chuo National Hospital, Gastroenterology), Dr. Makita (National Hospital Organization Nishigunma Hospital, Digestive Organ Internal Medicine and Surgery), Dr. Nakanishi, Dr. Todo and Dr. Chuma (Hokkaido University Hospital, Internal Medicine), Dr. Kawamura (Sapporo Hokuyu Hospital, Institute for artificial organs transplanation and gene therapy), Dr. Yamamoto and Dr. Katagiri (Chiba Cancer Center, Surgery), Dr. Watanabe and Dr. Shimizu (Gifu Prefectural General Medical Center, Internal Medicine), Dr. Nishimura (Toyota Kosei Hospital, Internal Medicine), Dr. Taoka (Suzuka General Hospital, Surgery), Dr. Okano (Suzuka General Hospital, Internal Medicine), Dr. Fujita (Tango Central Hospital, Surgery), Dr. Monden (NTT WEST Osaka Hospital, Surgery), Dr. Kaneko (NTT WEST Osaka Hospital, Internal Medicine), Dr. Nishihara and Dr. Iwasaki (Kochi Medical School, Internal Medicine), Dr. Yamaguchi (Kochi Medical School, Surgery), Dr. Saitsu and Dr. Takami (Kyusyu Medical Center, Surgery), Dr. Kajiwara (Steel Memorial Yawata Hospital, Gastroenterology), Dr. Deguchi and Dr. Suzuki (Faculty of Medicine, Kagawa University, Gastroenterology and Neurology), Dr. Ogura and Dr. Kuroda (Matsuzaka City Hospital, Surgery), Dr. Kamiike (National Hospital Organization Kure Medical Center, Surgery), Dr. Takano (National Hospital Organization Kure Medical Center, Internal Medicine), Dr. Takeuchi (National Hospital Organization Iwakuni Clinical Center, Surgery), Dr.

Miyashita (National Hospital Organization Iwakuni Clinical Center, Internal Medicine), Dr. Kobayashi (Tokyo Kyosai Hospital, Internal Medicine), Dr. Mizusawa (Yasugi Municipal Hospital, Surgery), Dr. Watanabe and Dr. Adachi (Yasugi Municipal Hospital, Internal Medicine), Dr. Tawada and Dr. Okabe (Graduate School of Medicine, Chiba University, Internal Medicine), Dr. Yoshida and Dr. Kamimura (Saiseikai Niigata Daini Hospital, Internal Medicine), Dr. Hayashi (Hayashi Hospital, Surgery), Dr. Yoshikawa (Kokuho Central Hospital, Surgery), Dr. Sato and Dr. Fukuda (Kokuho Central Hospital, Internal Medicine), Dr. Kumagai (Showa University School of Medicine, Toyosu Hospital, Surgery), Dr. Nomura (Showa University School of Medicine, Toyosu Hospital, Internal Medicine), Dr. Sasaki (Showa University School of Medicine, Internal Medicine), Dr. Inoue and Dr. Watanabe (Showa University Fujigaoka Hospital, Internal Medicine), Dr. Shimamura (Kurashiki Riverside Hospital, Internal Medicine), Dr. Mima (Tokushima Kensei Hospital, Surgery), Dr. Kadota (Tokushima Kensei Hospital, Internal Medicine), Dr. Yamazaki (Osaka Koseinenkin Hospital, Surgery), Dr. Naito (Osaka Koseinenkin Hospital, Internal Medicine), Dr. Sakamoto and Dr. Sakurai (Tokyo Medical and Dental University, Internal Medicine), Dr. Nishida and Dr. Sakamoto (Aiseikai Yamashina Hospital, Internal Medicine), Dr. Makuuchi, Dr. Takamoto, and Dr. Sano (Japan Red Cross Medical Center, Surgery), Dr. Kobayashi and Dr. Omori (Kameda Medical Center, Internal Medicine), Dr. Hirose (Japan Red Cross Otsu Hospital, Internal Medicine), Dr. Kawanami, Dr. Takenaka, Dr. Kondo, and Dr. Yamada (Japan Red Cross Otsu Hospital, Gastroenterology), Dr. Omura (Hokkaido P.W.F.A.C Sapporo-Kosei General Hospital, Gastroenterology), Dr. Ishizu (Hokkaido P.W.F.A.C Sapporo-Kosei General Hospital, Surgery), and Health and Labour Sciences Research Grants for Research on Measures for Intractable Diseases for a national survey of PBC patients. Finally, the authors would like to thank Enago (www.enago.jp) for the English language review.

REFERENCE

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: S449-454.
4. Jones DE, Metcalf JV, 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, Hashimoto N, et al. Hepatocellular carcinoma and survival in patients with primary biliary cirrhosis. Hepatology 2002; 35: 1172-1178.
6. Deutsch M, Papatheodoridis GV, Tzakou 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, Chiaramonte M, Milani S, Surrenti C, Naccarato R. Incidence of hepatic and extra-hepatic malignancies in primary biliary cirrhosis (PBC). Ital J Gastroenterol 1993; 25: 473-476.
8. Miyake Y, Iwasaki Y, Terada R, Okamaoto R, Ikeda H, Makino Y, Kobashi H, 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, Toda G. Long-term prognosis of primary biliary cirrhosis (PBC) in Japan and analysis of the factors of stage progression in asymptomatic PBC (a-PBC). Hepatol Res 2002; 22: 250-260.
10. Findor J, He XS, Sord J, Terg R, Gershwin ME. Primary biliary cirrhosis and hepatocellular carcinoma. Autoimmun Rev 2002; 1: 220-225.
11. Silveira MG, Suzuki A, Lindor KD. Surveillance for hepatocellular carcinoma in patients with primary biliary cirrhosis. Hepatology 2008; 48: 1149-1156.
12. Cavazza A, Caballeria L, Floreani A, Farinati F, Bruguera M, Caroli D, Pares A. Incidence, risk factors, and survival of hepatocellular carcinoma in primary biliary cirrhosis: comparative analysis from two centers. Hepatology 2009; 50: 1162-1168.
13. Suzuki A, Lymp J, Donlinger 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.
14. Sasaki H, Inoue K, Higuchi K, Yasuyama T, Koyata H, Kuroki T, Yamamoto S, et al. Primary biliary cirrhosis in Japan: national survey by the Subcommittee on Autoimmune hepatitis.

Gastroenterol Jpn 1985; 20: 476-485.

15. Prince MI, Chetwynd A, Craig WL, Metcalf JV, James OF. Asymptomatic primary biliary cirrhosis: clinical features, prognosis, and symptom progression in a large population based cohort. Gut 2004; 53: 865-870.
16. Scheuer P, Lefkowitch JH, editors. Liver biopsy interpretation. 7th ed. Philadelphia: Elsevier Saunders Ltd; 2007.
17. Kadokawa Y, Omagari K, Ohba K, Kitamura S, Ohara H, Takeshima F, Mizuta Y, et al. Hepatocellular carcinoma in a male patient with early stage (stage I) primary biliary cirrhosis. Intern Med 2005; 44: 207-211.
18. Watanabe T, Soga K, Hirono H, Hasegawa K, Shibasaki K, Kawai H, Aoyagi Y. Features of hepatocellular carcinoma in cases with autoimmune hepatitis and primary biliary cirrhosis. World J Gastroenterol 2009; 15: 231-239.
19. Turissini SB, Kaplan MM. Hepatocellular carcinoma in primary biliary cirrhosis. Am J Gastroenterol 1997; 92: 676-678.
20. Caballeria 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.
21. Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, Karin M. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science 2007; 317: 121-124.
22. Yeh SH, Chen PJ. Gender disparity of hepatocellular carcinoma: the roles of sex hormones. Oncology 2010; 78 Suppl 1: 172-179.
23. Harada K, Ozaki S, Gershwin ME, Nakanuma Y. Enhanced apoptosis relates to bile duct loss in primary biliary cirrhosis. Hepatology 1997; 26: 1399-1405.
24. 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.

FIGURE LEGENDS

Fig. 1 Kaplan–Meier curve for survival in patients with primary biliary cirrhosis with (+) or without (−) hepatocellular carcinoma. There is a statistically significant difference between the curves ($p < 0.05$).

Fig. 2 Cumulative appearance rates of hepatocellular carcinomsa in patients with primary biliary cirrhosis by gender. There is a statistically significant difference between males and females.

Fig. 3 Histological stage at the diagnosis of primary biliary cirrhosis (PBC) in patients with or without hepatocellular carcinoma (HCC) by gender. The proportion of patients with histological stages 3 and 4 at the time of PBC diagnosis is approximately 16.0% for both male and female PBC patients without HCC. However, the proportion of patients with histological stages 3 and 4 is 14.2% in male and 57.1% in female PBC patients with HCC. Moreover, there is a significant difference in the proportion of female PBC patients with HCC and that without. The parentheses identify the number of patients examined.

Fig. 4 Average age at the time of diagnosis of primary biliary cirrhosis (PBC) and hepatocellular carcinoma (HCC), and the duration between the diagnosis of PBC and that of HCC. The duration between the diagnosis of PBC and that of HCC is shorter in males than in females ($p < 0.05$). The parentheses identify the number of patients examined.

Fig. 5 Histologic stage by gender at the time of hepatocellular carcinoma (HCC) diagnosis in patients with primary biliary cirrhosis. In females, HCC incidence gradually increases

according to histological stage, with a statistically significant difference ($p < 0.05$). The parentheses indicate the number of patients examined.

Table 1

Clinical and biological characteristics of PBC patients with or without HCC at PBC diagnosis

24	HCC (+)	HCC (−)	p
Number	71	2875	
Sex (M:F)	19:52	351:2524	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

150x112mm (300 x 300 DPI)

Table 2

Factors associated with increased risk of HCC in PBC patients (multivariate analysis)

	regression coefficient	standard deviation	χ^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

150x112mm (300 x 300 DPI)

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%

(*$p < 0.05$)

150x112mm (300 x 300 DPI)

Supplementary Table 1

Factors associated with increased risk of HCC in female PBC patients (multivariate analysis)

	regression coefficient	standard deviation	χ^2	odds ratio	P value
Age	−0.0130	0.0174	0.56	0.9870	0.4531
T-Bilirubin	0.0817	0.1171	0.49	1.0851	0.4852
Albumin	−0.1771	0.3366	0.28	0.8376	0.5987
T-cholesterol	0.0038	0.0033	1.32	1.0038	0.2512
Histological stage (I/II/III/IV)	−1.0255	0.1964	27.25	0.3586	<0.0001
Use of UDCA (%)	−0.1607	0.3151	0.26	1.3791	0.6100
Clinical stage (asymptomatic:symptomatic)	0.4252	0.1913	4.94	0.4271	0.0263

150x112mm (300 x 300 DPI)

Supplementary Table 2

Factors associated with increased risk of HCC in male PBC patients (multivariate analysis)

	regression coefficient	standard deviation	$\chi 2$	odds ratio	P value
Age	−0.0542	0.0319	2.89	0.9472	0.0893
T-Bilirubin	−0.1018	0.1790	0.32	0.9032	0.5697
Albumin	0.5884	0.5591	1.11	1.8011	0.2926
T-cholesterol	0.0001	0.0020	0.00	1.0001	0.9511
Histological stage (I/II/III/IV)	0.2484	0.4096	0.37	1.2819	0.5443
Use of UDCA (%)	−0.5367	0.4254	1.59	2.9258	0.2071
Clinical stage (asymptomatic:symptomatic)	−0.3590	0.4635	0.60	2.0506	0.4385

150x112mm (300 x 300 DPI)

Supplementary Table 3

Clinical and biological characteristics of PBC patients with or without cirrhosis at PBC diagnosis

	Non-cirrhosis	Cirrhosis	
Gender (M:F)	13:28	1:7	NS
Age (yrs)	59.8 ± 9.7	57.3±10.5	NS
T-Bilirubin	1.15±0.52	1.42±1.13	NS
Albumin	3.89±0.56	3.77±0.79	NS
T-cholesterol	201.0±59.4	229.4±77.9	NS
Duration of the diagnosis from PBC to HCC (years)	9.5±5.8	7.9±4.2	NS
Use of UDCA（No：Yes）	5:36	0:8	NS
Clinical stage (asymptomatic:symptomatic)	25:16	4:4	NS

Noncirrhosis, Scheuer's stage 1-3; Cirrhosis, Scheuer's stage 4; NS, not significant

150x112mm (300 x 300 DPI)

Supplementary Table 4

Clinical and biological characteristics of male and female PBC patients with HCC at HCC diagnosis

(excluding cases with past HBV infection or history of alcohol intake)

	Male (n = 24)	Female (n = 104)	Total (n = 128)
Blood transfusion	8%	7%	7%
Diabetes mellitus	18%	22%	22%
AMA levels	89%	93%	92%
ANA levels	41%	59%	56%
BMI(≥25%)	20%	33%	31%
Triglyceride (≥150)	11%	9%	10%
Total cholesterol (>220)	9%	11%	11%
associated with NAFLD	0%	6%	4%
Use of UDCA	83%	90%	89%

150x112mm (300 x 300 DPI)

Supplementary Table 5

Clinical and biological characteristics of PBC patients with or without cirrhosis at HCC diagnosis

	Non-cirrhosis (n = 43)	Cirrhosis (n = 39)	
Blood transfusion	9 %	10 %	NS
Past HBV infection	26 %	23 %	NS
Alcohol intake	9 %	10 %	NS
Diabetes mellitus	23 %	28 %	NS
AMA levels	93 %	97 %	NS
ANA levels	48 %	50 %	NS
BMI(>25%)	19 %	39 %	NS
Triglyceride (≥150)	13 %	10 %	NS
Total cholesterol (>220)	15 %	12 %	NS
associated with NAFLD	0 %	3 %	NS
Use of UDCA	81 %	82%	NS

Non-cirrhosis, Scheuer's stage 1-3; Cirrhosis, Scheuer's stage 4; NS, not significant

150x112mm (300 x 300 DPI)

Supplementary Table 6

Characteristics of HCC in male and female PBC patients with HCC

	Male	Female	Total
Number of HCC	(n = 49)	(n = 128)	(n = 178)
Solitary	65 %	60 %	62 %
Multiple	35 %	38 %	37 %
Unknown	0 %	2 %	2 %
Differentiation of HCC	(n = 25)	(n = 41)	(n = 66)
Well differentiated	44 %	37 %	39 %
Moderately differentiated	48 %	56 %	53 %
Poorly differentiated	8 %	7 %	8 %

150x112mm (300 x 300 DPI)

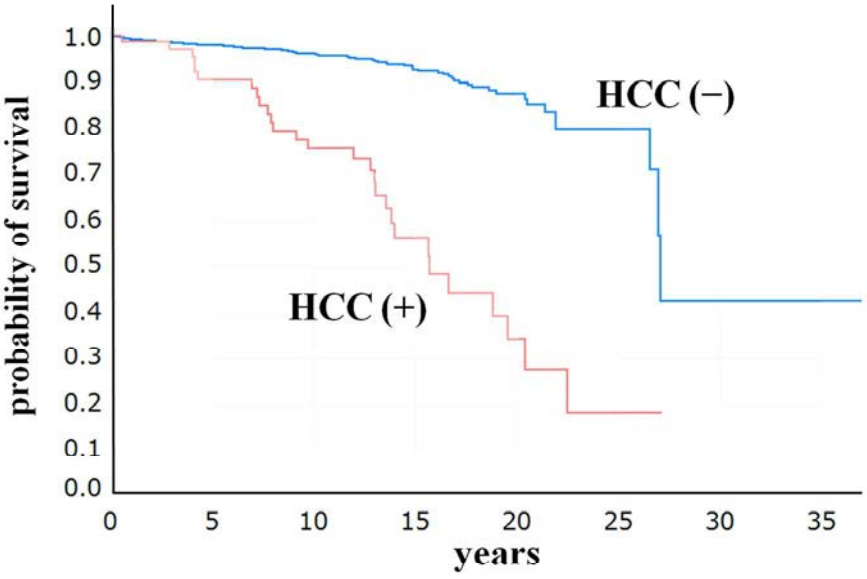

Fig. 1

150x112mm (300 x 300 DPI)

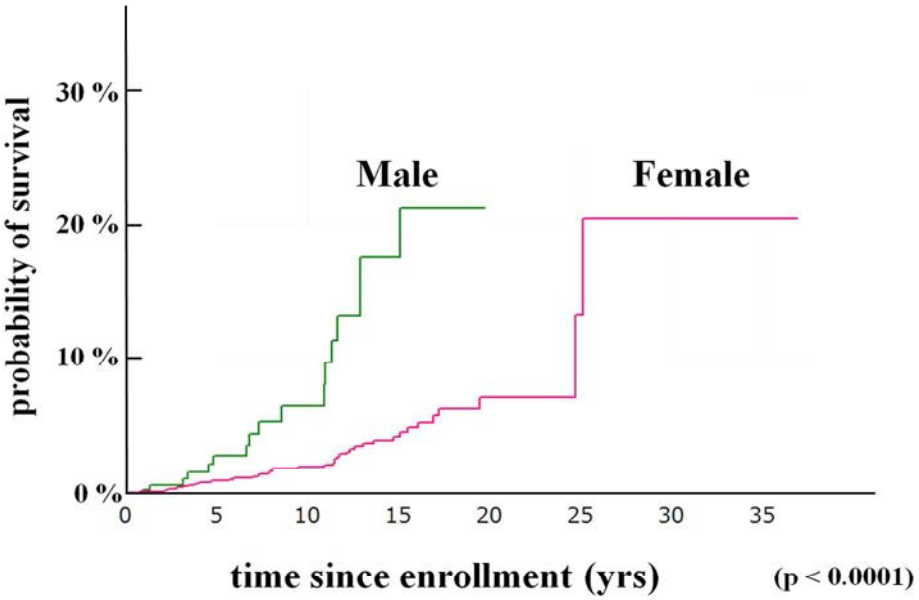

Fig. 2

150x112mm (300 x 300 DPI)

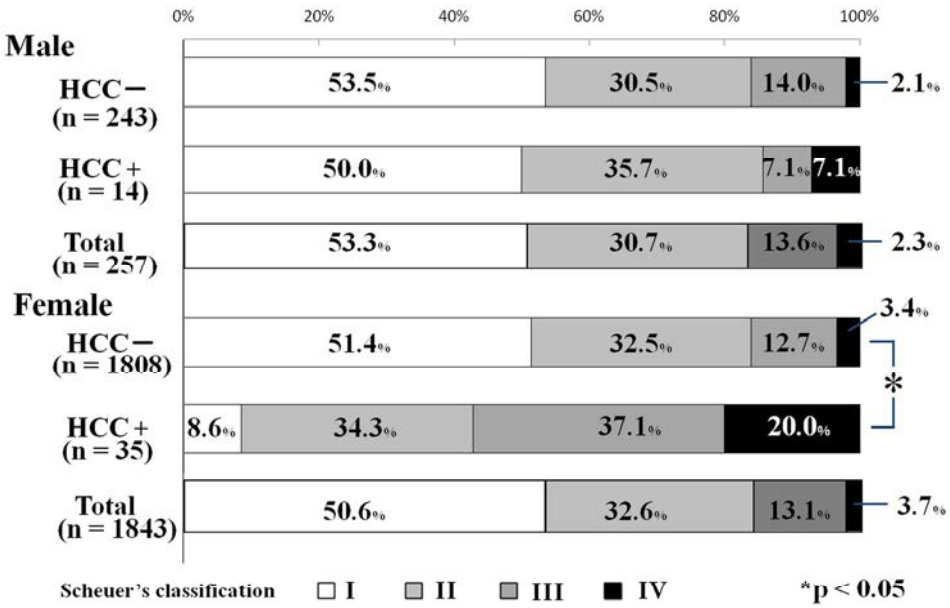

Fig. 3

150x112mm (300 x 300 DPI)

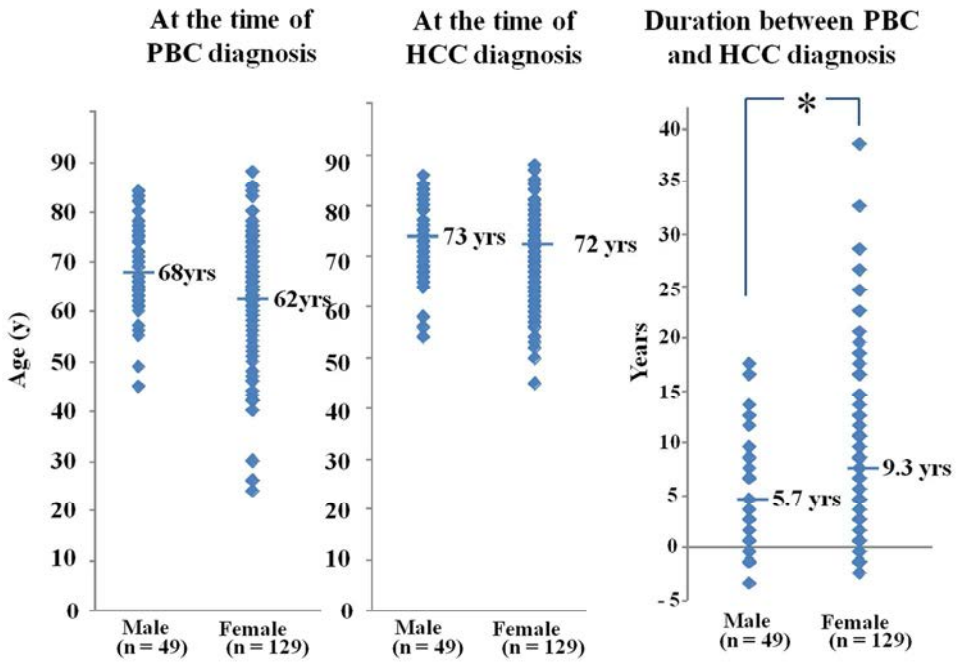

Fig. 4

(*p < 0.05)

150x112mm (300 x 300 DPI)

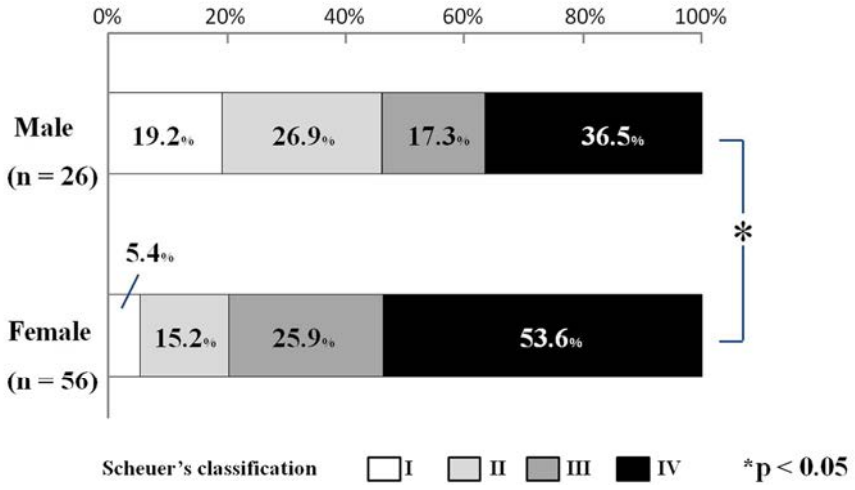

Fig. 5

150x112mm (300 x 300 DPI)