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HEPATOBILIARY

# Premalignant lesions of cholangiocarcinoma: characteristics on ultrasonography and MRI

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## Abstract

**Background and objective** Cholangiocarcinoma (CCA) is an aggressive malignancy with high prevalence rate in Asia. The CCA premalignant lesions, including Biliary intraepithelial neoplasia (Bil-IN) and Intraductal papillary neoplasm of biliary tract (IPNB), share a common carcinogenesis; however, on imaging, patterns of presentation are different. Patterns and imaging characteristics on ultrasonography (US) and Magnetic resonance imaging (MRI) of both Bil-IN and IPNB are reported herein.

**Methods** In this retrospective study of imaging findings in premalignant CCA, pathology-proven cases of Bil-IN and IPNB at Chulabhorn Hospital were analyzed. Demographics, locations of lesions, imaging characteristics of both Bil-IN and IPNB were assessed, compared, and described.

**Results** Twenty-one premalignant lesions, 13 Bil-INs and 8 IPNBs, from 18 patients were included. Both Bil-IN and IPNB lesions were found more commonly at the right than left intrahepatic ducts (66.7% vs. 33.3%), and had more peripheral than central locations (85.7% vs. 14.3%). On US, Bil-IN commonly presented as focal bile duct dilatation (76.9%), whereas IPNB was more variable with hyperechoic nodules (37.5%), focal bile duct dilatation (37.5%), and diffuse bile duct dilatation with intraductal nodules (25%). On MRI, focal bile duct dilatation and nonfunctioning bile excretion are the most sensitive findings with sensitivities in the range of 84.6% to 100%. The presence of intraductal nodules and connection to the biliary system are findings that were significantly different between IPNB and Bil-IN, 62.5% versus 7.7% ($p=0.014$) and 75% versus 15.4% ($p=0.018$), respectively.

**Conclusions** Premalignant lesions of CCA, including Bil-IN and IPNB, have different imaging presentations. Knowledge of imaging presentations may improve early detection and increase confidence in diagnosis.

**Keywords** Premalignant lesion · Cholangiocarcinoma · Liver imaging · Malignant (non-HCC) · Biliary intraepithelial neoplasia · Bil-IN · Intraductal papillary neoplasm of biliary tract, IPNB · *Opisthorchis viverrini*

## Abbreviations

| | |
|---|---|
| Bil-IN | Biliary intraepithelial neoplasm |
| CCA | Cholangiocarcinoma |
| CS | *Clonorchis sinensis* |
| CT | Computed tomography |
| GRE | Gradient recoiled echo |
| IPNB | Intraductal papillary neoplasm of biliary tract |
| MRCP | Magnetic resonance cholangiopancreatography |
| MRI | Magnetic resonance imaging |
| OV | *Opisthorchis viverini* |
| US | Ultrasonography |

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## Introduction

Cholangiocarcinoma (CCA) is the second most common primary hepatic malignancy [1], responsible for 10–20% of all primary hepatic cancers worldwide [2]. In Asia, CCA is

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far more common than in the other parts of the world with the greatest prevalence in Southeast Asia [3]. A large burden of disease has been reported in Thailand, particularly in Northeastern and Northern regions [3, 4] with some reports of rates 100 times higher than those in Western countries [4]. The major risk factor in Asia is liver fluke infestation [5, 6] including liver fluke *Opisthorchis viverrini* (OV) and *Clonorchis sinensis* (CS). In contrast, the major risk factors for CCA in Western countries are primary sclerosing cholangitis and liver cirrhosis [1, 7].

CCA is an aggressive malignancy, often presenting in an advanced unresectable stage with a grave prognosis [8]. Overall, 1-year and 5-year survival rates are only 50% and 5%, respectively [8, 9]. Surveillance for CCA in endemic areas may improve survival [10]. In addition, detection of premalignant lesions and of early-stage resectable CCA resulted in improved clinical outcomes [10].

The current WHO classification of CCA classifies premalignant lesions of CCA as biliary intraepithelial neoplasia (Bil-IN) and intraductal papillary neoplasm of the bile duct (IPNB), which have differing histopathology and pathogenesis [11]. Bil-IN is characterized by abnormal cell division and presents as flat intraepithelial lesions with high nucleus/cytoplasm ratios Fig. 1 [12, 13]. IPNB is characterized by exophytic proliferation of the biliary

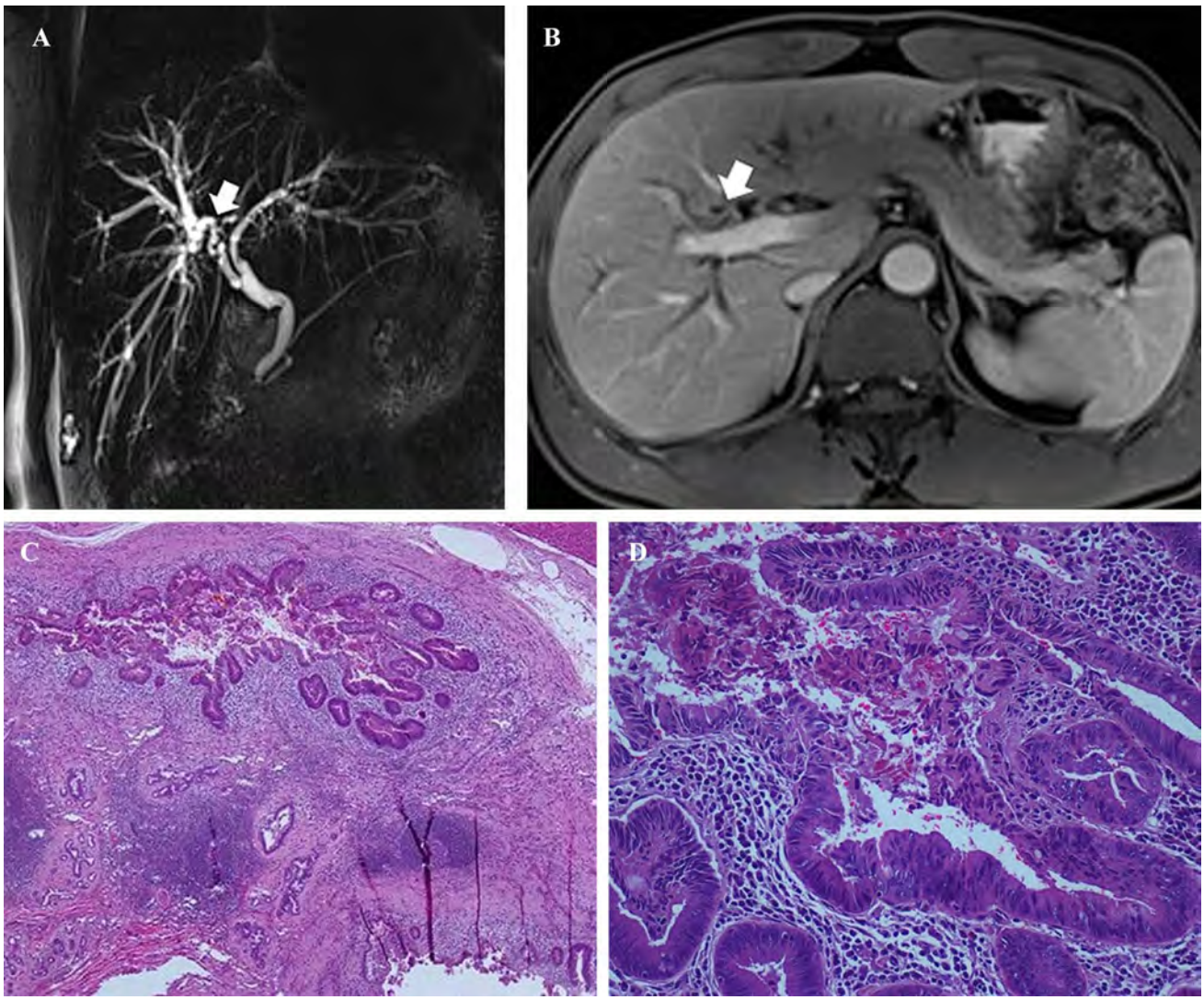


**Fig. 1** A 52-years old male patient who presented with focal duct dilatation with pathology-proven high grade Bil-IN grade III. **a** MRCP shows focal irregular bile duct narrowing at proximal right IHD associated with mild peripheral right intrahepatic bile duct dilatation. **b** Axial T1-weighted image post contrast shows focal bile duct thickening at proximal right intrahepatic bile duct corresponding to area of irregular narrowing bile duct segment on MRCP. **c** Microscopic examination of lesion after hepatic resection on low power field shows circumferential thickening of the bile duct wall. **d** On high power field the lesions show flat, pseudopapillary architecture with pseudostratification and dysplastic nuclear change, consistent with high grade Bil-IN

epithelium and presents as a fibrovascular stalk within the bile duct lumen Fig. 2 [13].

Ultrasonography (US) is an imaging modality used to screen for liver malignancy. The majority of cases are diagnosed and confirmed by more advanced imaging modalities, usually Computed tomography (CT) scan or Magnetic resonance imaging (MRI). Therefore, a better understanding of presentations of the premalignant changes of CCA on US and MRI may improve lesion detection and imaging diagnosis, and lead to more prompt diagnosis and treatment. However, now-a-days there are limited published data about the early findings of premalignant CCA lesions. Our study aimed to study imaging presentations and patterns of premalignant lesions of CCA by US and MRI.

## Materials and methods

### Study design and population

This was a retrospective study of patients who were diagnosed as having CCA within an operable stage, and who received hepatic resection from October 2011 to January 2017.

The study patients were from a CCA surveillance program in Nan Province, Thailand which involved the screening with abdominal US every 6 months of 4225 adults, age 30–60 years who were indigenous to the CCA endemic area [10]. A cohort flow chart illustrating the inclusion and exclusion criteria of participants in this study is shown as Fig. 3.

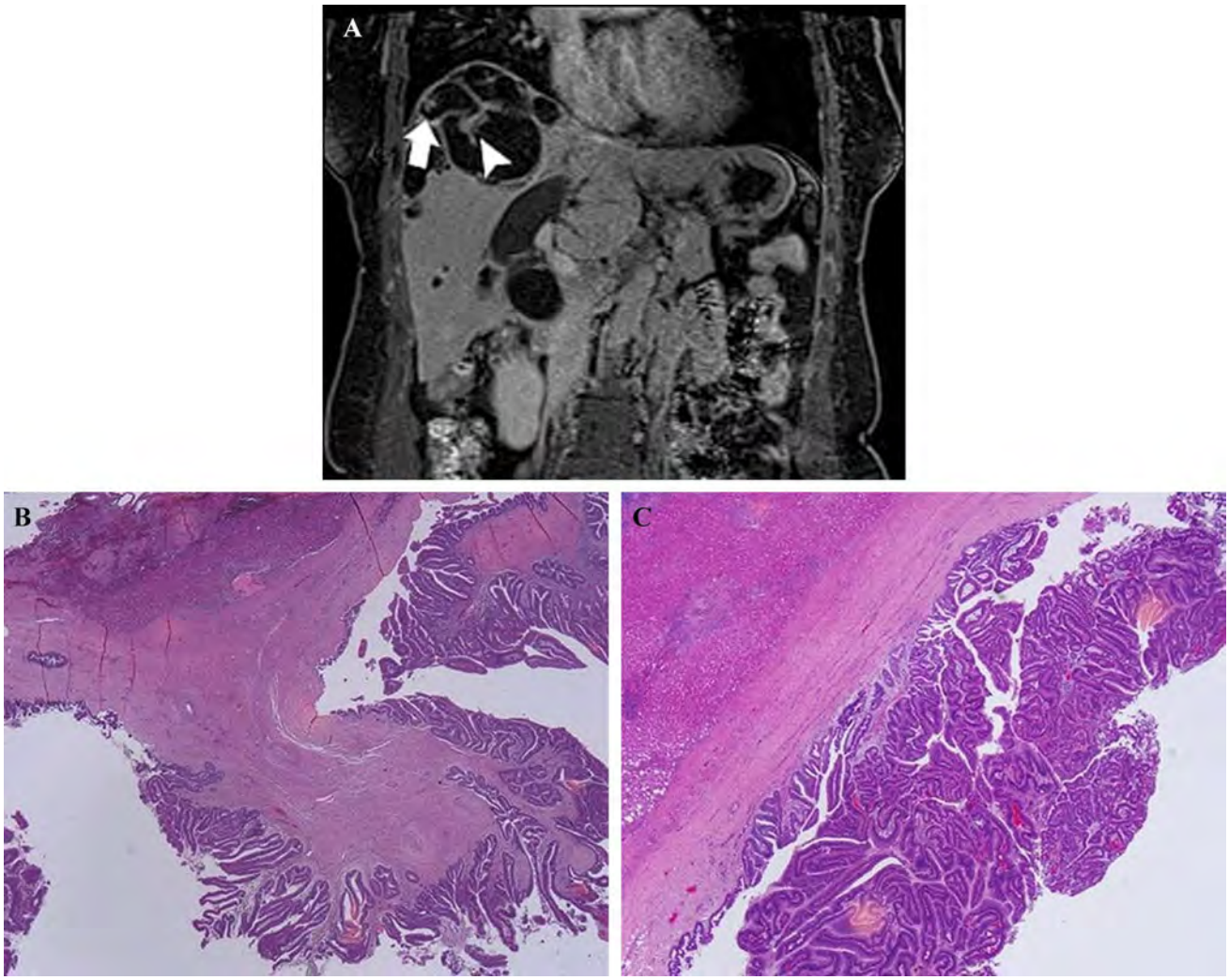


**Fig. 2** A 54-year-old female patient who presented with markedly dilated biliary system with pathology-proven IPNB. **a** Coronal T1-weighted image shows severe diffuse dilatation of biliary system with demonstrable small pedunculated polyp (arrow) and a sessile polyp (arrow head). **b** Corresponding to the pedunculated polyp, histopathology shows papillary projection of epithelial atypia with fibrovascular stroma which presents as a stalk, consistent with IPNB. **c** Corresponding to the sessilepolyp, histopathology shows fond-like papillary projection of dysplastic epithelium with fibrovascular stroma, consistent with IPNB

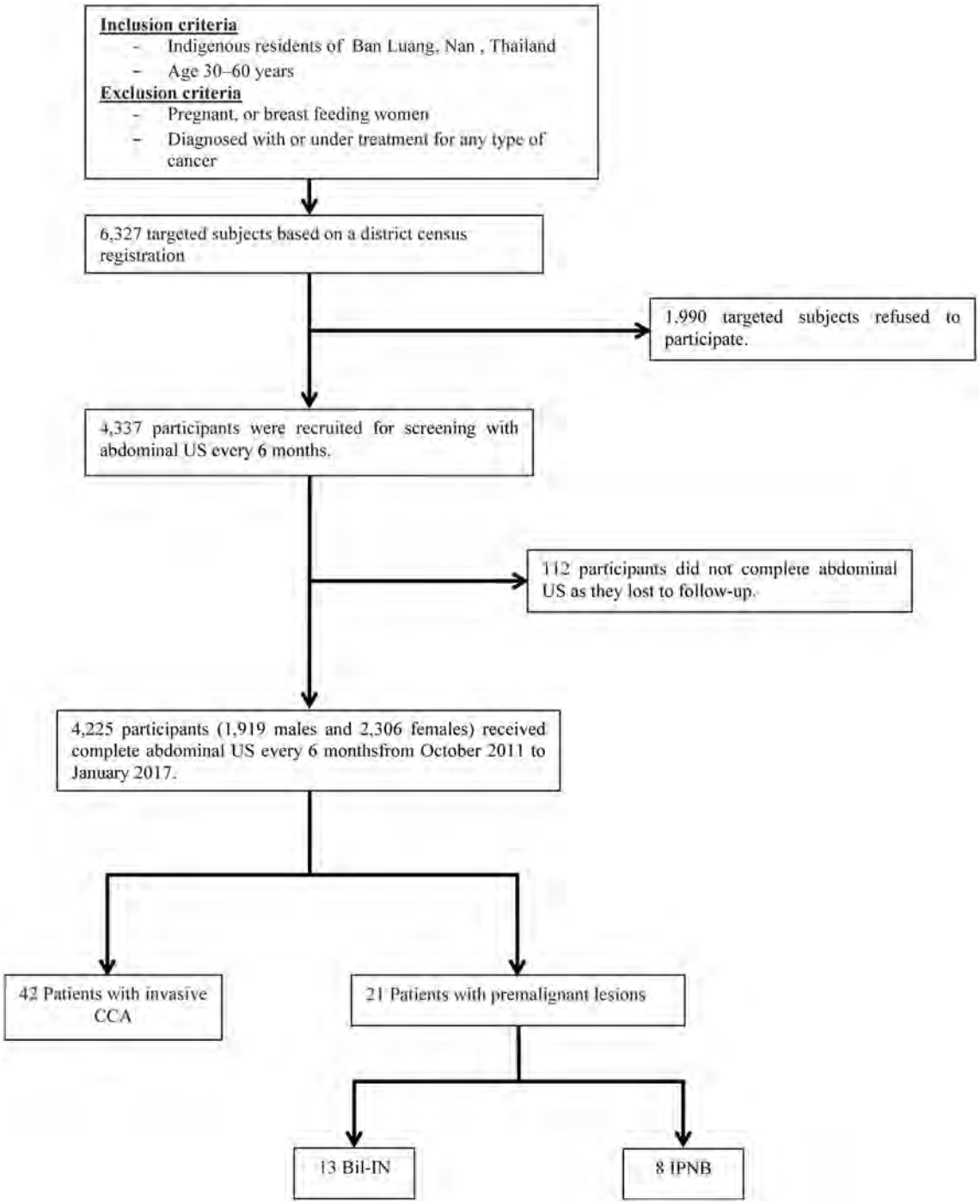


**Fig. 3** Cohort flow chart illustrating the inclusion and exclusion criteria of participants in the study

The study was approved by the Ethics Committee for Human Research, Chulabhorn Research Institute (Certificate No. 040/2560), and the need for individual consent was waived.

All cases with proven invasive CCA were excluded, and only premalignant lesions of CCA including Bil-IN and IPNB were included and analyzed.

All patients with US findings suspicious of CCA were referred for further MRI with or without magnetic resonance cholangiopancreatography (MRCP) for diagnosis. Further investigation and treatment of patients diagnosed as having CCA was directed by the institutional multidisciplinary tumor board. Diagnosis of premalignant bile duct lesions including Bil-IN and IPNB was based

on pathological criteria from the WHO classification of 2010 [11, 14].

All patients in this cohort had received either hepatic wedge resection or hepatic lobectomy of the suspicious lesion(s). Radiologic and pathologic correlations by radiologist (S.S.) and pathologist (T.S.) were reconfirmed as to the location of the lesion(s) in the specimen and on MRI. In cases of hepatic lobectomy or of a specimen having more than one lesion on pathology, the radiologic-pathology correlation was reviewed and it was confirmed that the same lesion was assessed by radiologist (S.S.) and pathologist (T.S.).

## Imaging techniques and definition of imaging findings

All patients in the CCA surveillance cohort received upper abdominal US using a 2–5 MHz curvilinear transducer under ultrasound system (Aplio 300; Toshiba Medical System Corporation, Tokyo, Japan). All US procedures were performed by diagnostic radiologists.

MRI was performed using 3-T Tim Trio (Siemens Healthcare, Erlangen, Germany) with a 16 Channel phase-array coil. The MRI and MRCP protocols were composed of in-phase and out-of-phase T1-weighted GRE (gradient recoiled echo), dynamic fat-suppressed pre- and post-contrast T1-weighted 3-dimensional GRE sequences with 30-, 60-, 90-s, 3-, 5-, 10-, and 90-120-min delays for Gadobenate Dimeglumine (Gd-BOPTA) hepatobiliary phases. T2-weighted sequences included multishot and single-shot spin echo techniques (SSFSE) with additional thin slice thicknesses of 2 mm, for suspected small bile duct lesions using respiratory gated fat-suppressed fast spin echo T2 (TR/TE 4000/80).

Diffusion-weighted imaging (DWI) with b values of 50, 400, and 800 s/mm$^2$ with apparent diffusion coefficient (ADC) mapping were included. For MRCP, an additional 3-dimensional T2 thick slap SSFE with radial rotation was performed. Gd-BOPTA administration (0.5 mmol/mL solution; Multihance, Bracco, Milan, Italy) at a dose of 15 mL followed by a 20 mL 0.9% normal saline flush; both were delivered at rate of 2 mL/s using power injector.

To characterize findings of the lesions on US and MRI, the following details were considered and defined as follows.

*Focal bile duct dilatation*: abnormal enlargement of a bile duct with a demonstrable point of narrowing or obstruction (Fig. 4).

*Hypoechoic/isoechoic/hyperechoicnodules:* nodules which have echogenicity less than, comparable to, or more than adjacent liver parenchyma, respectively.

Diffuse bile duct dilatation with an intraductal nodule: generalized dilatation of the biliary system with a detectable nodule originating from the bile duct wall.

*Irregular bile duct:* unsmooth or uneven surface of a bile duct.

*Non-functioning bile duct excretion:* no excretion of hepatocyte-specific contrast agent into a branch of the bile ducts (Fig. 5).

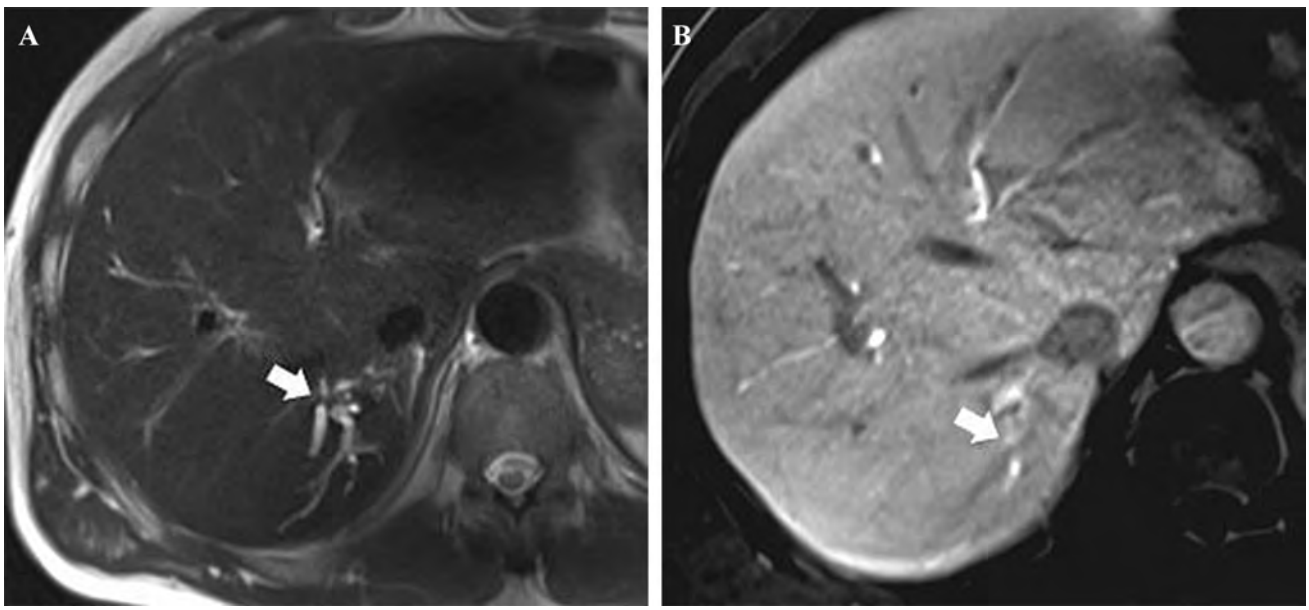


**Fig. 4** A 56-year-old female patient who presented with focal duct dilatation with pathology-proven Bil-IN grade II. **a** Axial T2-weighted MRI shows corresponding focal bile duct dilatation of hepatic segment 7 (arrow). **b** Axial T1WI hepatobiliary phase shows contrast excretion into biliary system with partial excretion of contrast at the narrowing bile segment (arrow)

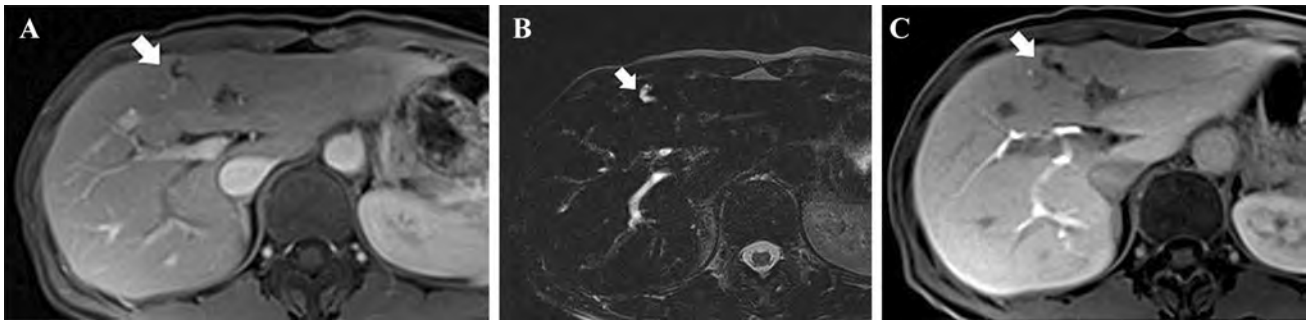


**Fig. 5** A 50-year-old male who presented with focal duct dilatation and pathology-proven Bil-IN. **a** Axial T1-weighted image shows focal bile duct dilatation at hepatic segment 4B (arrow). **b** Corresponding T2-weighted image reveals focal bile duct dilatation at hepatic segment 4B with focal bile duct dilatation (arrow). **c** Corresponding T1-weighted image post-gadolinium BOPTA at hepatobiliary phase corresponding to the same lesion. The affected peripheral duct showsno biliary contrast excretion (arrow)

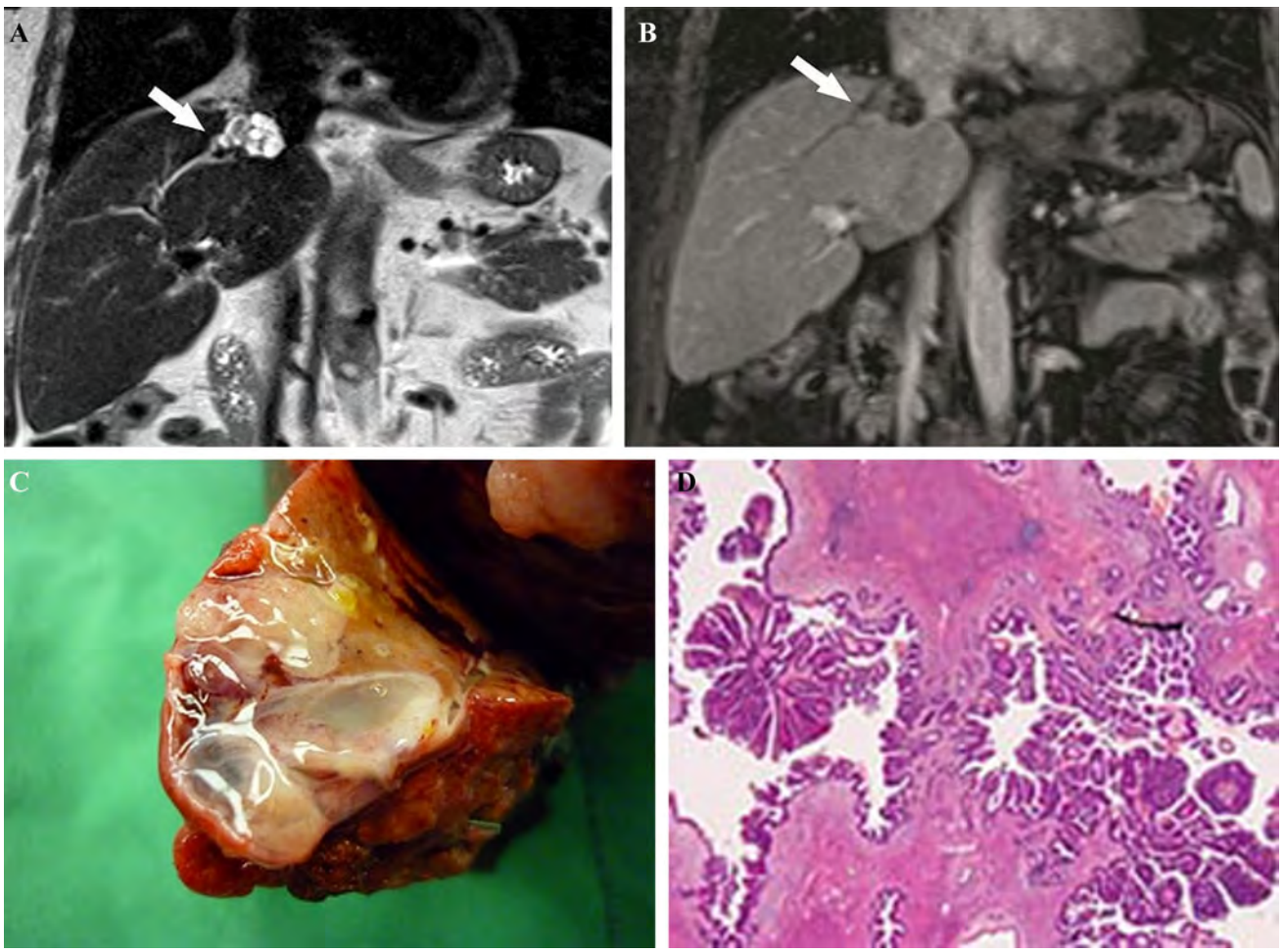


**Fig. 6** A 50-year-old male with pathologically proven IPNB. **a** Coronal T2-weighted image shows cystic bile duct dilatation with an internal solid nodule and demonstrates connection of the lesion to the biliary system (arrow). **b** Coronal T1-weighted image at venous phase, post-contrast study. This corresponding lesion shows mild enhancement of the intracystic nodule. **c** Corresponding gross pathology reveals the mucinous content inside the cystic bile duct, both dilated and solid parts. **d** Corresponding histopathology reveals polypoid lesion with papillary projection of epithelial atypia with fibrovascular stroma, consistent with IPNB

*Intraductal nodule:* nodule within bile duct lumen which originates from the bile duct wall (Fig. 6).

*Cystic dilatation of bile duct:* bulbous dilatation of the bile duct (Figs. 6, 7).

*Connection of a lesion to the biliary system:* demonstrable connection of the focal dilatation of bile duct or cystic lesion to the adjacent biliary system (Fig. 6a, b).

Two board-certified abdominal radiologists who have 10 and 15 years of experience in the field independently reviewed all US and MRI studies. The decision of presence or absence of US and MRI characteristic findings were based on the consensus of both readers.

## Statistical analysis

Demographic data of the patients, including age, gender, and family history of liver cancer, baseline tumor markers, liver function tests, and location of the lesion(s) were compared between the IPNB and Bil-IN groups using cluster analysis (with logistic regression for comparing demographic data).

Imaging findings of IPNB and Bil-IN on US and MRI studies were compared and analyzed using Chi-square and Fischer's exact tests. Interobserver agreements of MRI findings were assessed with the Cohen k statistic. The *k* values were interpreted as suggested by Landis and Koch [15]: 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, substantial agreement; 0.81–1.00, excellent agreement. Analyses were conducted using SPSS version 20 (IBM, NY, USA) with a 2-sided $p$ value $< 0.05$ defined as the level of significance.

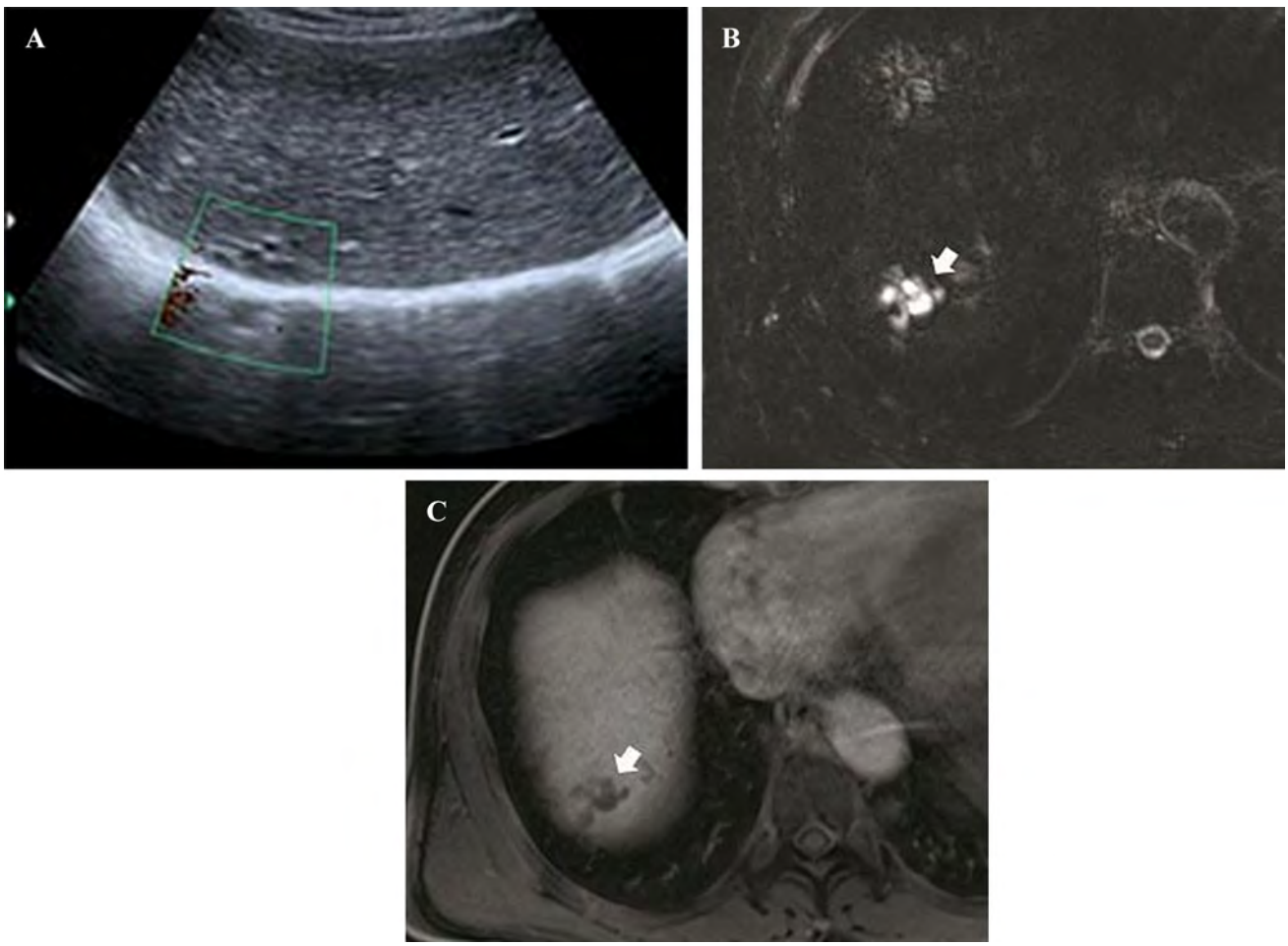


**Fig. 7** A 50-year-old male with cystic bile duct dilatation from Bil-IN. **a** Subcostal sonography shows a cluster of septate cysts without demonstrable solid components. No vascularity is detected using Doppler mode (Green Box). **b** Axial T2-weighted image of the corresponding lesion appears as a tortuous cystic dilatation of the peripheral bile duct of segment 7 (arrow). **c** Corresponding Axial T1WI hepatobiliary phase of the lesion shows no biliary contrast excretion into this lesion (arrow)

## Results

There were 63 patients who were diagnosed with CCA in resectable stage, and who underwent hepatic resection. Of these, invasive CCA were proven in 45 patients, whereas premalignant CCA were found in 18 patients.

Of all 18 patients, 15 had single premalignant lesion including 9 Bil-INs and 6 IPNBs, while 3 patients had synchronous lesions including one patient with 2 Bil-IN lesions, and 2 patients with both Bil-IN and IPNB. The total of premalignant lesions were accounted for 13 Bil-INs and 8 IPNBs.

The average ages of patients at initial screening and at the time of detection of the lesions were 50.9 ± 5.3 and 55.3 ± 6.7 years, respectively.

Both family history of CCA and history of OV infestation were found in 6 (33.3%) of all patients. Baseline tumor markers and liver function tests including cancer antigen 19-9 (CA 19-9), carcino embryonic antigen (CEA), alkaline phosphatase, albumin, aspatate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin (TB) are of normal range and shown in (Table 1).

Of all premalignant lesions, 14 (66.7%) and 7 (33.3%) lesions are found in the right and left hepatic lobes, respectively. Seventeen lesions (81%—11 Bil-IN and 6 IPNB) were found in peripheral bile duct locations, whereas only 4 (19%) lesions were observed in central or perihilar regions. None of the lesions were detected in extrahepatic bile ducts. Locations of each premalignant lesion are shown in Fig. 8.

The sonographic presentation of Bil-IN appeared as focal bile duct dilatation in 10/13 (76.9%) and as a hyperechoic nodule in 3/13 (23.1%). In contrast, IPNB presented as a hyperechoic nodule in 3/8 (37.5%), focal bile duct dilatation in 3/8 (37.5%), and diffuse bile duct dilatation with an intraductal nodule in 2/8 (25%).

Among the MRI findings, focal bile duct dilatation and nonfunctioning bile excretion of hepatocyte-specific agent were the most sensitive findings for detection of the premalignant lesions including Bil-IN and IPNB with sensitivities ranging from 84.6 to 100% (Table 2). The presence of intraductal nodules and connection of the lesion to the biliary system were found to be significantly different between IPNB and Bil-IN lesions [62.5% vs. 7.7% ($p = 0.014$) and 75% vs. 15.4% ($p = 0.018$), respectively]. Other findings, including focal duct dilatation with mass/nodule, irregular bile duct, and cystic dilatation of the bile duct, showed no significant differences between Bil-IN and IPNB lesions.

**Table 1** Demographic data of patients with premalignant lesions

| | Bil-IN (13 lesions) | IPNB (8 lesions) | All patients (18 patients) | *p* value[a] |
|---|---|---|---|---|
| Age at screening (mean ± SD) | 51.3 ± 5.5 | 51.1 ± 5.2 | 50.9 ± 5.3 | 0.962 |
| Age at diagnosis (mean ± SD) | 56.3 ± 7.5 | 56.4 ± 7.5 | 55.3 ± 6.7 | 0.875 |
| Sex | | | | 0.964 |
| Male | 5 (41.7%) | 3 (62.5%) | 8 (44.4%) | |
| Female | 7 (58.3%) | 5 (62.5%) | 10 (55.6%) | |
| Family history of CCA | | | | 0.777 |
| Yes | 4 (33.3%) | 2 (25.0%) | 6 (33.3%) | |
| No | 8 (66.7%) | 6 (75.0%) | 12 (66.7%) | |
| History of OV infection | | | | 0.586 |
| Yes | 4 (33.3%) | 2 (25.0%) | 6 (33.3%) | |
| No | 7 (58.3%) | 5 (62.5%) | 11 (61.1%) | |
| Unknown | 1 (8.3%) | 1 (12.5%) | 1 (5.6%) | |
| Baseline laboratory values (mean ± SD) | | | | |
| CA19-9 (U/mL) | 8.9 ± 7.4 | 7.2 ± 7.0 | 7.9 ± 7.3 | 0.590 |
| CEA (ng/mL) | 3.0 ± 2.3 | 3.3 ± 2.1 | 3.3 ± 2.3 | 0.624 |
| ALP (IU/L) | 78.6 ± 15.7 | 107.3 ± 71.7 | 91.4 ± 49.7 | 0.259 |
| Serum albumin (g/dL) | 4.3 ± 0.2 | 4.2 ± 0.4 | 4.3 ± 0.3 | 0.348 |
| AST (IU/L) | 24.4 ± 4.8 | 39.8 ± 27.5 | 31.4 ± 19.6 | 0.077 |
| ALT (IU/L) | 18.5 ± 8.2 | 28.1 ± 23.7 | 23.7 ± 16.7 | 0.122 |
| TB (mg/dL) | 0.8 ± 0.4 | 0.7 ± 0.3 | 0.8 ± 0.4 | 0.546 |

13 Bil-IN and 8 IPNB lesions from 18 patients

There are 2 patients with both Bil-IN and IPNB, and 1 patient with 2 Bil-IN lesions

[a]Logistic regression analysis with clustered patient

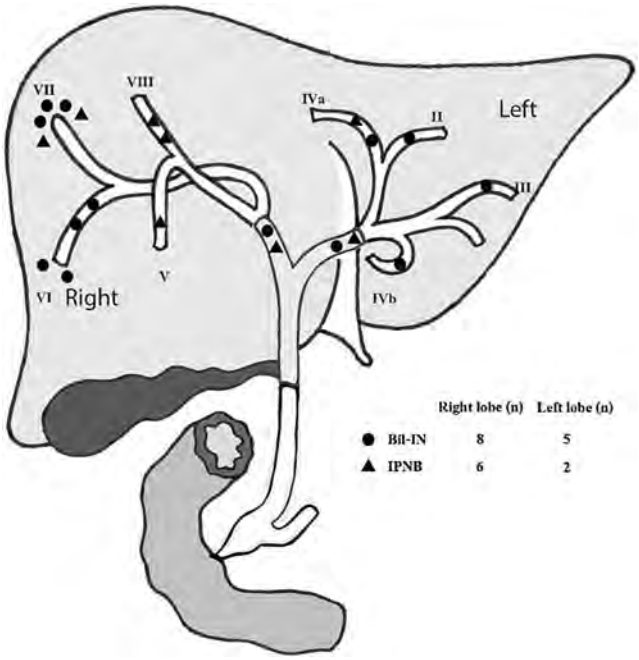


**Fig. 8** Locations of premalignant lesions of cholangiocarcinoma detected in the biliary system. Areas of biliary tract shown in white inside and outside liver parenchyma indicate peripheral intrahepatic and extrahepatic bile ducts. The areas of biliary tract shown in gray are perihilar and hilar bile ducts. Biliary intraepithelial neoplasias (Bil-IN) are shown as black circles and intraductal papillary neoplasms of biliary tract (IPNB) are shown as black triangles. Locations of Bil-IN and IPNB on liver parenchyma outside the biliary tract indicate ultradistal location of peripheral bile ducts

The degrees of interobserver agreement of MRI findings were substantial for focal bile duct dilatation, intraductal nodule, cystic dilatation of bile duct, and nonfunctioning bile duct excretion with *k* values of 0.64, 0.69, 0.73, and 0.77, respectively. Connection of the lesion to normal bile duct was in fair agreement with *k* value of 0.29.

## Discussion

The current WHO classification of premalignant CCA lesions, published in 2010 by Nakanuma et al. [14], introduces the histopathologic terms Bil-IN and IPNB. These two premalignant changes characterized by abnormal cholangiocyte proliferation are considered a transitional stage of the malignancy. Once these two lesions have developed an invasive component beyond the basement membrane, invasive CCA is diagnosed [16].

Bil-IN is caused by an abnormal cell division and presents as flat intraepithelial lesions with high nucleus/cytoplasm ratios. There are three grades, from Bil-IN I to III. Bil-IN III is the most invasive, the lesions having stratified epithelium and pseudostratification [14, 17]. The growth pattern of Bil-IN progresses from micropapillary projections to eventually develop the periductal growth pattern of CCA, similar to classic tubular adenocarcinoma [14, 18].

The key histology of IPNB is a fibrovascular core which is lined by papillomatous epithelial cells, consisting of any of four subtypes of epithelial-lining cells, gastric, intestinal, pancreatic, and oncocytic [19]. Mucin-producing IPNB has been reported in about 33% of cases [5] and commonly occurs as the pancreatic subtype. The mucin, a proteinaceous viscous material, causes mucinous bile duct obstruction and cystic bile duct dilatation associated with intraductal nodules [18, 20]. In Asia, gastric and intestinal subtypes are more common than pancreato-biliary and oncocytic subtypes, with the mortality and recurrence rates in lesions with the pancreato-biliary subtype being higher than with the others [12]. Unlike Bil-IN, IPNB have polypoid intraductal growth; therefore, the lesions tend to invade basement membranes more slowly and are associated with both colloid (mucinous) carcinoma and tubular adenocarcinoma of which the former has a better prognosis [14, 17].

Liver flukes, including OV and CS, are considered Group 1 carcinogens according to the International Agency for Research on Cancer [21]. It is estimated that over 10 million people in Southeast Asian countries are infected with OV [22], and that about 6 million of them are in Thailand [23]. Thus, OV infestation remains a major health problem in Thailand and neighboring countries [10]. In our cohort of patients, about 19% of 3663 patients had OV infestation in their stool; however, 68.8% of all CCA patients in this cohort were proven to have OV infestation on stool exam and PCR [10].

Interestingly, the majority of premalignant lesions in this study (both Bil-IN and IPNB) arose from the right intrahepatic duct, specifically from a peripheral branch of the inferoposterior and superoposterior segments of the right hepatic lobe (segments 6 and 7). This finding is in contrast with the CS infestation which describes the location of CS to be commonly found in the left hepatic lobe than in the right hepatic lobe [24].

CS is relatively larger, 1.5 to 4 mm wide and 8 to 15 mm long, as compared to OV and has a preferential location of infestation in medium size bile ducts. This is especially true of the left proximal intrahepatic duct which is larger and more straight than the right hepatic duct [24]. CS also commonly causes cholangitis and parasitic stone formation in the proximal left hepatic duct, referred to as 'Oriental-cholangio-hepatitis' [24]. In contrast, OV is about 1.5 mm wide and 7 mm long, smaller in size by half compared to CS [25]. With this size, the parasite could reside in any part of the biliary system. However, our study showed that the premalignant lesions tended to be in peripheral branches of the right intrahepatic duct, especially in the posterior right hepatic segments (segment 6 and 7) (Fig. 8). We postulate

**Table 2** Imaging findings of Bil-IN and IPNB on US and MRI

| | Bil-IN (13) (%) | IPNB (8) (%) | Total (21) (%) | *p*-value* |
|---|---|---|---|---|
| US patterns | | | | 0.079 |
| 1 Focal bile duct dilatation | 10 (76.9) | 3 (37.5) | 13 (61.9) | |
| 2.1 Hypoechoic nodule | 0 (0) | 0 (0) | 0 (0) | |
| 2.2 Isoechoic nodule | 0 (0) | 0 (0) | 0 (0) | |
| 2.3 Hyperechoic nodule | 3 (23.1) | 3 (37.5) | 6 (28.6) | |
| 3 Diffuse bile duct dilatation with intraductal nodule | 0 (0) | 2 (25) | 2 (9.5) | |
| MRI findings | | | | |
| Focal duct dilatation | | | | 1.000 |
| Presence | 12 (92.3) | 7 (87.5) | 19 (90.5) | |
| Absence | 1 (7.7) | 1 (12.5) | 2 (9.5) | |
| Focal bile dilatation with mass/nodule | | | | 0.253 |
| Presence | 1 (7.7) | 3 (37.5) | 4 (19) | |
| Absence | 12 (92.3) | 5 (62.5) | 17 (81) | |
| Irregular bile duct | | | | 1.000 |
| Presence | 8 (61.5) | 5 (62.5) | 13 (61.9) | |
| Absence | 5 (38.5) | 3 (37.5) | 8 (38.1) | |
| Non function bile duct excretion | | | | 0.505 |
| Presence | 11 (84.6) | 8 (100) | 19 (90.5) | |
| Absence | 2 (15.4) | 0 (0) | 2 (9.5) | |
| Cystic dilatation of bile duct | | | | 0.646 |
| Presence | 4 (30.8) | 4 (50) | 8 (38.1) | |
| Absence | 9 (69.2) | 4 (50) | 13 (61.9) | |
| Connection to biliary system | | | | 0.018 |
| Presence | 2 (15.4) | 6 (75) | 8 (38.1) | |
| Absence | 11 (84.6) | 2 (25) | 13 (61.9) | |
| Intraductal nodule | | | | 0.014 |
| Presence | 1 (7.7) | 5 (62.5) | 6 (28.6) | |
| Absence | 12 (92.3) | 3 (37.5) | 15 (71.4) | |

*Exact probability test

that this phenomenon may occur because the location of this right hepatic duct contains a downturn and is the most dependent part of the biliary system when in supine and right lateral decubitus positions. The parasite may do more migrating during the host's sleeping time, when there is little or no movement. However, more study of parasite behavior is needed to prove our hypothesis.

In our study, IPNB accounted for about 40% of premalignant lesions, more prevalent than in previously reported studies [12, 13]. A few reports show that a higher incidence of IPNB appears to be found in Asian countries where liver fluke infestation is highly prevalent [12, 26, 27]. Whereas in Western countries, IPNB appears to make up only 10–20% of all precancerous lesions, though a few studies have been reported with higher percentages [12, 13].

Liver fluke infestations, due to both eggs and mature flukes, induce Bil-IN. It is thought that the malignant changes are in response to metabolites of parasite origin, including oxysterols and N-nitrosodimethylamine, which cause granuloma formation and DNA damage in the biliary tract [28, 29]. However, recent study found that liver fluke also causes Bil-IN by oxysterols and N-nitrosodimethylamine. Both eggs and fluke themselves contain oxysterols causing granuloma formation, which damage DNA in biliary tract. These lead to chronic inflammation and DNA mutation which induce carcinogenesis in biliary tracts.

On US, all of the premalignant lesions which presented as nodules in our study were hyperechoic. The increased echogenicity of intraductal lesions may be explained by their submersion in bile duct fluid, with multiple interfaces between bile duct wall, bile fluid, and the lesion causing this increase in echogenicity. These hyperechoic lesions on US are sometimes difficult to distinguish between early CCA and hepatic hemangioma (Fig. 9). However, based on our observations, premalignant lesions (including IPNB) tended to have elongated shapes and were associated with focal bile duct dilatation (Fig. 10). Awareness of these findings on US of early CCA changes may improve surveillance outcome.

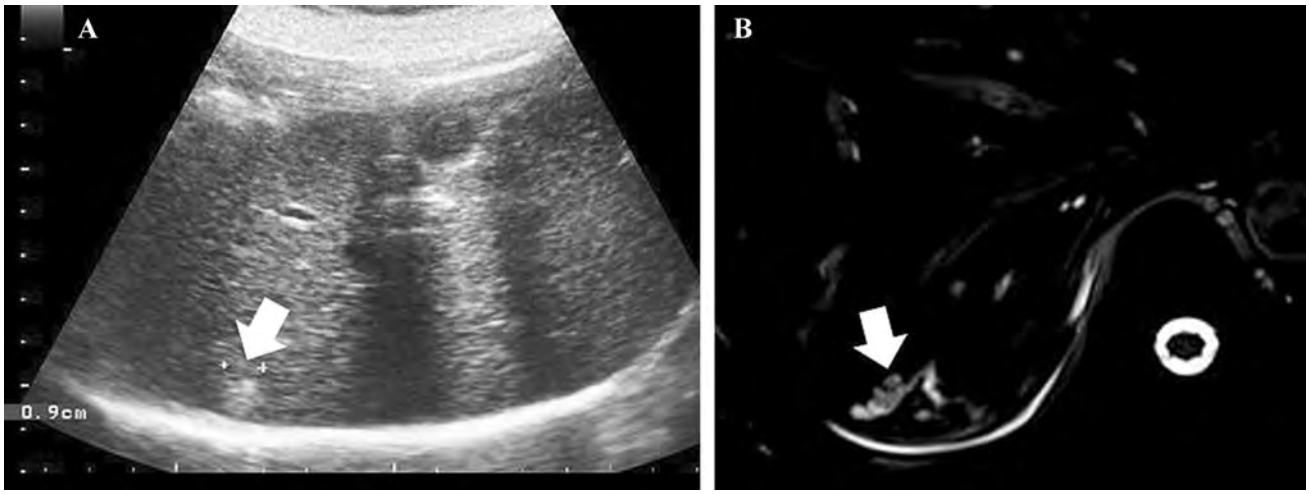


**Fig. 9** A 60-year-old male patient who has hepatic wedge lesion with pathology-proven IPNB. **a** Abdominal sonography shows a 0.8-cm round-shaped hyperechoic nodule (arrow) at hepatic segment 7, resembling hepatic hemangioma. **b** T2-weight image with fat suppression reveals intraductal lesion (arrow) inside peripheral intrahepatic bile duct

MRI is the most sensitive and specific imaging modality for diagnosis of premalignant CCA lesions, as well as for planning surgical resection. The most common and sensitive findings in both IPNB and Bil-IN are focal bile duct dilatation and focal nonbile duct excretion of biliary contrast agent. Bil-IN progresses with periductal thickening and bilateral narrowing leading to obstruction. There is still biliary excretion through nonobstructed, narrow bile ducts. In contrast, the process of obstruction by IPNB is from mucinous excretion and obstruction, which causes nonexcretion of biliary contrast even by small lesions without direct mass obstruction [20].

We observed two interesting findings that could help distinguish between Bil-IN and IPNB on MRI. There were connections of the lesion to the biliary system and intraductal nodules. Both of these findings were associated with IPNB and could be explained by the pathology, since IPNB has tumor growth into the lumen and forms intraductal nodules which are visible on MRI. In addition, 30% of IPNB lesions have been reported to have mucinous excretion and obstruction causing focal bile duct dilatation [5]. However, it is usually not complete obstruction and sometimes connection of the lesion to the biliary system is identified. Such connections to the biliary system are also used to distinguish between IPNB and mucinous cystadenoma/carcinoma [20].

The prior report from Liu et al. supports the finding that nodules in dilated bile ducts and tumors growing along interior walls of bile ducts are useful for distinguishing IPNB from CCA with intraductal papillary growth with the accuracy of 83% to 88% [30].

In order to study premalignant CCA lesions, the lesions with invasive components need to be excluded (especially IPNB). Since invasive components of the IPNB can invade bile duct walls of periductal tissue [31], they may mimic the classic periductal infiltration pattern of Bil-IN.

## Limitations

In this study, there were some limitations due to the retrospective nature and design of the study and relatively small number of cases. However, this is the first report that we are aware of with proven premalignant lesions studied and compared by both US and MRI.

Second, our study was conducted in an endemic area of CCA where the major risk factor was OV infestation. This may not directly apply to other areas where the risk factors for CCA are different.

US is considered operator dependent. Some parts are difficult to evaluate including extrahepatic bile ducts which can be obscured by bowel gas and could result in under-detection of extrahepatic CCA. However, in the CCA surveillance cohort from Nan province, the incidence of extrahepatic CCA occurred only in 15.6% [10].

## Conclusions

US is a primary tool for screening for CCA and is able to detect premalignant CCA lesions. Awareness of common locations, imaging patterns and characteristics on US may help raise suspicion of CCA premalignant lesions, and in selection of subjects for further investigation by MRI.

The findings of focal bile duct dilatation and focal absence of bile duct excretion on MRI are the most sensitive findings in the detection of premalignant CCA lesions.

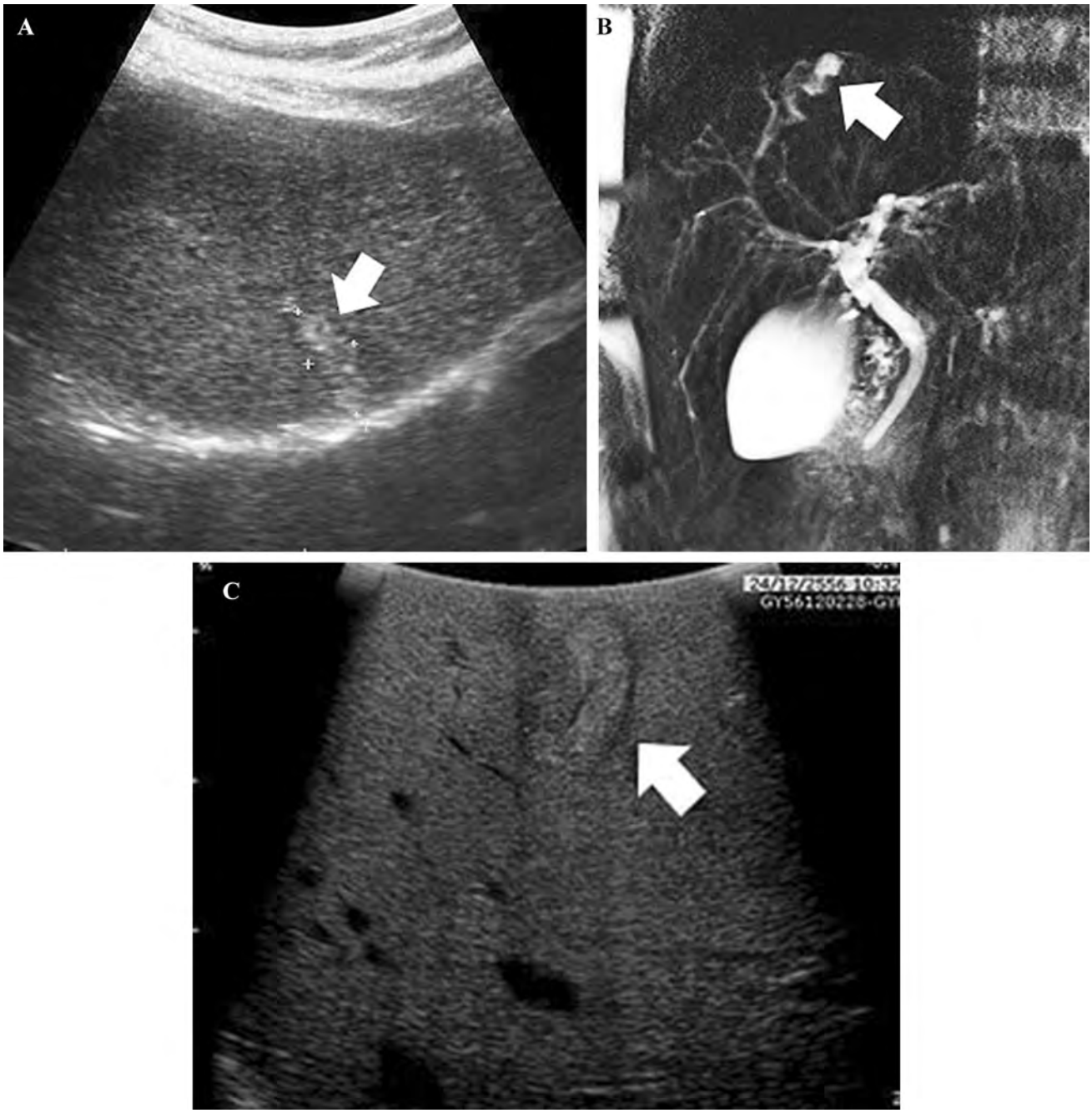


**Fig. 10** A 58-year-old female patient who has hepatic wedge lesion with pathology-proven IPNB. **a** Abdominal sonography shows an elongated shaped hyperechoic nodule at hepatic segment 8. **b** The corresponding MRCP shows focal dilatation of segment 8 bile duct with intraductal nodule (Arrow). Note that the lesion has connection to biliary system. **c** The corresponding intraoperative sonography shows elongated hyperechoic nodule with thin hypoechoicrim which could be intraductal nodule inside the bile duct

The findings of intraductal nodules and connections to the biliary system may be useful in distinguishing IPNB from Bil-IN.

Knowledge of patterns and characteristics of premalignant CCA on MRI may increase confidence in diagnosis and allow more prompt surgical treatment. This may in turn lead to more curative outcomes for CCA.

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**Author contributions** Surachate Siripongsakun: Study concept and design, Acquisition of data, Analysis, and interpretation of data,

Drafting the manuscript, Critical revision of the manuscript; Withawat Sapthanakorn: Acquisition of data, Analysis and interpretation of data, Drafting the manuscript, Critical revision of the manuscript; Poemlarp Mekraksakit: Acquisition of data, Analysis and interpretation of data, Drafting the manuscript, Critical revision of the manuscript; Saruda Vichitpunt: Analysis and interpretation of data, Drafting the manuscript; Saowalak Chonyuen: Analysis and interpretation of data; Jisupa Sritasan: Analysis and interpretation of data; Siwat Bhumiwat: Drafting the manuscript; Thaniya Sricharunrat: Acquisition of data; and Saowanee Srittanapong: Analysis and interpretation of data, Critical revision of the manuscript.

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## Compliance with ethical standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

**Ethical approval** The project was approved by the Ethics Committee for Human Research of Chulabhorn Research Institute (Certificate No. 040/2560). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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