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Periductal fibrosis and bile duct dilatation: pathways to diagnosis for cholangiocarcinoma in Northeast Thailand

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3 **Title:** Periductal fibrosis and bile duct dilatation: pathways to diagnosis for

4 cholangiocarcinoma in Northeast Thailand

6 **Authors:** Nittaya Chamadol,^{1,2,3} Narong Khuntikeo,^{1,2,4} Bandit Thinkhamrop,^{1,2,5,6} Kavin
7 Thinkhamrop,^{1,2,6} Apiporn T. Suwannatrai,^{1,2,7} Matthew Kelly,⁸ and Supanee Promthet^{1,5,9}

9 **Affiliations:**

10 ¹Cholangiocarcinoma Screening and Care Program (CASCAP), Khon Kaen University, Khon
11 Kaen, Thailand.

12 ²Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand.

13 ³Department of Radiology, Faculty of Medicine, Khon Kaen University, Khon Kaen,
14 Thailand.

15 ⁴Department of Surgery, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

16 ⁵Epidemiology and Biostatistics Section, Faculty of Public Health, Khon Kaen University,
17 Khon Kaen, Thailand.

18 ⁶Data Management and Statistical Analysis Center (DAMASAC), Faculty of Public Health,
19 Khon Kaen University, Khon Kaen, Thailand.

20 ⁷Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen,
21 Thailand.

22 ⁸Department of Global Health, Research School of Population Health, Australian National
23 University, Canberra, Australia.

24 ⁹ASEAN Cancer Epidemiology and Prevention Research Group, Khon Kaen University,
25 Khon Kaen, Thailand.

1
2
3 26
4

5 **Email address:**

6
7 NC: Nittaya Chamadol (nittayachamadol@yahoo.com)

8
9 NK: Narong Khuntikeo (nkhuntikeo@gmail.com)

10
11 BT: Bandit Thinkhamrop (bandit@kku.ac.th)

12
13 KT: Kavin Thinkhamrop (kvinth@gmail.com)

14
15 AT: Apiporn T. Suwannatrai (apiporn@kku.ac.th)

16
17 MK: Matthew Kelly (matthew.kelly@anu.edu.au)

18
19 SP: Supanee Promthet (supanee@kku.ac.th)

20
21
22 35
23

24 **Corresponding authors:**

25
26 Name: Supanee Promthet

27
28 Address: ASEAN Cancer Epidemiology and Prevention Research Group,
29
30
31 Khon Kaen University, Khon Kaen 40002, Thailand.

32
33 Telephone: +66-82 668 1995

34
35 e-Mail: supanee@kku.ac.th

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3 48 **ABSTRACT**

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5 49 **Objectives** To assess associations between periductal fibrosis (PDF) and bile duct dilatation
6
7 50 (BDD) in ultrasonography (US) screening of population at risk of cholangiocarcinoma
8
9 51 (CCA) due to residence in an endemic area for *Opisthorchis viverrini*. CCA survival rates
10
11 52 are low and early identification of risk factors is essential. BDD is one symptom which can
12
13 53 identify patients at risk of CCA. Detection of PDF by US can also identify at risk patients, at
14
15 54 an earlier stage of CCA development. Identification of association between PDF and BDD
16
17 55 will inform screening practices for CCA risk, by increasing the viability of PDF screening for
18
19 56 CCA risk.

22 57 **Setting** Nine tertiary care hospitals in Northeast Thailand.

24 58 **Design** Cross-sectional study.

26 59 **Participants** Study subjects in the Cholangiocarcinoma Screening and Care Program
27
28 60 (CASCAP) in Northeast Thailand. CASCAP inclusion criteria are all residents of Northeast
29
30 61 Thailand aged 40 years and over. Participants are recruited through CCA screening centers
31
32 62 and through primary health care units. So far 394 026 have been enrolled.

35 63 **Methods** PDF and BDD were identified through US. PDF was categorized into three groups,
36
37 64 PDF1, 2 and 3, depending on their high echo locality in the peripheral, segmental and main
38
39 65 bile duct, respectively. Associations between PDF and BDD were determined by adjusted
40
41 66 odds ratio (OR) and 95% confidence interval (CI) using multiple logistic regression.

44 67 **Results** BDD was found in 6.6% of PDF3, 1.7% of PDF2, and 1.4% of PDF1 cases. Among
45
46 68 PDF cases, especially in PDF3, BDD was found in male more than female (8.9% and 4.6%,
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48 69 respectively). Compared to non-PDF, the association between PDF3 and BDD was highly
49
50 70 significant (adjusted OR=5.74, 95% CI 4.57 to 7.21, P<0.001).

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3 71 **Conclusions** Our findings reveal that there is a relationship between PDF and BDD, which is
4
5 72 associated with CCA. Therefore, PDF can also be an indicator for suspected-CCA diagnosis
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7 73 through US.
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11 75 **Keywords** bile duct dilatation; periductal fibrosis; ultrasonography; cholangiocarcinoma;
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13 76 screening; Thailand
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18 78 **Article summary**

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20 79 **Strengths and limitations of the study**

- 21
22 80 • The large size of the study population and its geographic distribution across Northeast
23
24 81 Thailand are a significant strength.
25
26 82 • This is the first and largest screening program for cholangiocarcinoma (CCA) in an
27
28 83 area with the highest incidence in the world.
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30 84 • CCA risk factors (PDF and BDD) were measured using ultrasonography by skilled
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32 85 radiologists.
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34 86 • Demographic, and some health, data were self-reported leading to potential bias in
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36 87 measurement of liver fluke infection, praziquantel treatment, and pre-existing medical
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38 88 conditions including HB, HC, and DM.
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40 89 • Self-report could lead to prevalence underestimates due to the fact that subjects may
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42 90 not have been willing to disclose sensitive or personal information.
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93 INTRODUCTION

94 Cholangiocarcinoma (CCA) and hepatocellular carcinoma (HCC), are ranked the most
95 prevalent cancers in Southeast Asia.¹⁻³ The early-stages of CCA can manifest through
96 obstructive jaundice, which is found in 30% of patients who are diagnosed with primary
97 sclerosing cholangitis.⁴ Other liver disorders: fatty liver disease, cirrhosis, and liver mass are
98 likewise recognized risk factors for both CCA and HCC.⁵⁻¹⁰ Suspected CCA cases can also be
99 identified through the presence of bile duct dilatation (BDD), which can be identified in
100 suspected CCA cases through ultrasonography (US) screening.^{11 12} A previous study
101 demonstrated that US screening is highly sensitive in identifying CCA through confirmed
102 incidences of BDD.¹³ However, upon the detection and diagnosis of bile duct and liver
103 disorders, it is often too late to save patients with CCA and HCC due to the rapid progression
104 to advanced stages of hepatic carcinoma.¹⁴ As well, detection of BDD by US requires the
105 services of specialist radiologists, who are generally only available in major hospitals,
106 limiting access to screening. Thus, the best way to save a patient's life and prevent the
107 likelihood of cancer development is through early, easily accessible, screenings to detect the
108 risk factors that may lead to cancer among high-risk populations.

109 As well as BDD there are several other indicators for CCA risk including well-accepted
110 premalignant lesions such as biliary intraepithelial neoplasm (BilIN), and intraductal
111 papillary neoplasm of the bile duct (IPNB).^{15 16} Periductal fibrosis (PDF) is another
112 abnormality of the bile duct which has been used to identify people at risk of developing
113 CCA, especially in those infected with *Opisthorchis viverrini*.¹⁷⁻²¹ PDF is caused by the
114 thickening of the bile duct wall, along the periportal space.²² PDF can be categorized into
115 three groups (PDF1, 2, and 3), which were first classified by the World Health Organization
116 (WHO).²³ Based on certain US findings, PDF1 is defined as having a high echo in the wall of
117 small bile ducts scattered in the liver as a starry sky pattern, PDF2 is a high echo along the

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3 118 segmental bile duct wall running parallel with the portal vein, and PDF3 is a high echo along
4
5 119 the main bile duct wall running parallel with the portal vein in the periportal space.¹⁹
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7 120 The relationship between PDF and CCA is indicated by the regular detection of PDF in
8
9 121 confirmed CCA cases, and this has been particularly common in Northeast Thailand where
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11 122 *O. viverrini* is endemic and a leading potential cause of CCA.⁸ As a result of this relationship,
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13 123 US detection has been utilized to identify people with PDF as a risk group for CCA
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15 124 development.^{8 20 24 25} Importantly, PDF can be identified through US, but does not require the
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17 125 services of a specialist radiologist increasing the potential access to screening, and PDF can
18
19 126 be detected earlier than BDD allowing more effective intervention.
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22 127 The potential to detect the risk of CCA earlier and without the need for specialist
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24 128 radiologists, through the identification of PDF may be an important breakthrough in reducing
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26 129 CCA incidence. So, both PDF and BDD have been recognized as indicators of CCA^{8 17}, but
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28 130 their relationship to one another has yet to be established or even studied in depth.
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30 131 Determining their relationship, such as learning if one precedes the other may make a
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32 132 significant change in how we screen for CCA via US. Therefore, this study seeks to
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34 133 determine if there is an association between PDF and BDD among people at a high-risk CCA
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36 134 population in Northeast Thailand. The results of this work will clarify necessary directions
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38 135 toward early screening methodologies and appropriate cancer treatment.
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43 137 **METHODS**

44 138 **Study design**

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47 139 This cross-sectional study collected data from the Cholangiocarcinoma Screening and Care
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49 140 Program (CASCAP) in Northeast Thailand. CASCAP is a prospective cohort study that is
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51 141 considered the first project for CCA screening in a high-risk population with a community-
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53 142 based bottom-up approach.²⁶ This cohort study was conducted at 9 tertiary care hospitals in
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3 143 Northeast of Thailand. These hospitals serve as the main source of affordable tertiary care for
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5 144 local people in the region. The study aims to recruit all people living in Northeast Thailand
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7 145 and aged 40 years and over, including patients attending screening for CCA and the general
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9 146 population attending primary health care units. All participants were asked to join the project
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11 147 by signing a consent form. All CCA patients were diagnosed and treated according to routine,
12
13 148 real world clinical practice by participating hospitals. Patients were followed-up and provided
14
15 149 with either clinical or palliative care depending on the stage of their disease. Treatment
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17 150 outcomes were recorded. Follow-up took place every 3-6 months depending on the patient's
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19 151 condition and unless scheduled otherwise.
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24 153 **Study population**

26 154 Our study recruited subjects from among people who participated the CASCAP project.
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28 155 These subjects form two groups (screening and walk-in). The screening group was people
29
30 156 who have undergone routine US and who showed no symptoms that could be related to CCA.
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32 157 The walk-in group was people who come to the hospital with symptoms indicating CCA
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34 158 which has been diagnosed with US. The subjects included in our study only those enrolled in
35
36 159 the CASCAP database from 2013-2017 with a total of 394 026 subjects.
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43 161 **Patient and Public Involvement**

45 162 The CASCAP project is a comprehensive screening and treatment program for CCA. Patients
46
47 163 in the screening arm will be contacted at least annually to be screening for CCA risk. Patients
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49 164 identified as having CCA will receive standard care for the condition through the project. For
50
51 165 the screening procedures covered by this report patients are informed of the purpose,
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53 166 outcomes and implications of these procedures.
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168 **Main outcome and independent variables**

169 The primary outcome for this study was BDD which was categorized into two groups
170 (no/yes). The independent variable of interest was PDF which was categorized into three
171 groups (PDF1, 2 and 3) depending on their ultrasound detectable high echo locality in the
172 peripheral, segmental and main bile duct, respectively. Both BDD and PDF diagnosed via US
173 by radiologist from the CASCAP. Demographic characteristics of PDF and non-PDF subjects
174 were the independent variables includes gender, age, education levels, occupations, having a
175 relative diagnosed with CCA, liver fluke infection, praziquantel (PZQ) treatments, smoking
176 (current or previous), alcohol consumption (current or previous) and diagnosis with hepatitis
177 B (HB), hepatitis C (HC), and diabetes mellitus (DM). All demographic characteristics listed
178 above were collected via face-to-face interview by interviewer from the CASCAP using
179 questionnaire.

181 **Statistical analysis**

182 The demographic characteristics that were categorical data were summarized using
183 frequencies and percentages (i.e. gender, age groups, education levels, occupations, having a
184 relative diagnosed with CCA, liver fluke infection, PZQ treatments, smoking history, alcohol
185 consumption history and diagnosis with HB, HC, DM, and PDFs). The continuous data, such
186 as the age of the subjects, were summarized by their mean, standard deviation, median,
187 minimum and maximum range.

188 The prevalence of BDD was calculated and the percentage of the prevalence was
189 computed based on a normal approximation to a binomial distribution. Bivariate analysis
190 using simple logistic regression was performed to investigate the association between the
191 independent factors listed above and BDD. They were determined by crude odds ratio (OR)
192 and their 95% confidence intervals (CI). Then multivariable analysis using multiple logistic

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3 193 regression was carried out to investigate the association between PDF and BDD as
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5 194 determined by the adjusted OR and 95% CI. The final multivariate model was adjusted for all
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7 195 factors which previous studies have reported to be associated with the hepatobiliary disease:
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9 196 PDF, gender, age, education levels, occupations, having a relative diagnosed with CCA, liver
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11 197 fluke infection, PZQ treatments, smoking, alcohol consumption as well as diagnosis with HB,
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13 198 HC, and DM.

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16 199 There were missing values for some variables due to unwillingness of some participants
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18 200 to answer some socio-demographic or health history questions or from errors in data
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20 201 collection. Missing values for most variables were rare with proportions missing less than 3%
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22 202 of participants. The only variable with a significant proportion of missing values was that of
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24 203 previous liver fluke diagnosis (n=211 869), but this number includes those who had reported
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26 204 never having been tested for infection.

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29 205 All test statistics were two-tailed and a p-value of less than 0.05 was considered
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31 206 statistically significant. All analyses were performed by using a statistical package, Stata
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33 207 version 15 (StataCorp, College Station, Texas, USA).

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37 209 **RESULTS**

39 210 **Descriptive summary**

41 211 The demographic characteristics of subjects were presented as numbers and percentages. A
42
43 212 total of 394 026 subjects who underwent US screenings for CCA were enrolled in our study.
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45 213 The subjects were all between the ages of 40-100 years old and reported a mean age of
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47 214 54.92±9.03 years old. Of these, approximately two-thirds were female (61.4%) and the
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49 215 majority of them completed primary school education level (72.9%) and worked as farmers
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51 216 (77.9%). About one-third (29.7%) had ever used PZQ treatment, and about one-fourth
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53 217 (21.3%) reported being a smoker or ex-smoker. The data of PDF diagnosis, 17.6% have
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3 218 positive diagnosed and the highest percentage was in subjects diagnosed with PDF1 (12.3%)
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5 219 while only 0.6% for PDF3 (table 1).
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9 221 <Table 1 located here>
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11 222

12 13 223 **Prevalence of BDD**

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15 224 From this study, the overall prevalence of BDD was reported to be 1.2%. The highest
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17 225 prevalence of BDD was 6.6% from the PDF3 group under periductal fibrosis. PDF1 and
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19 226 PDF2 subjects reported a low prevalence rate of only 1.4% and 1.7%, respectively (table 2).
20
21 227 Our study found that the prevalence of BDD occurring in PDF subjects was high in male
22
23 228 more than female, particularly in PDF3 (8.9% and 4.6%, respectively) (figure 1). Meanwhile,
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25 229 we also found the number of BDD in PDF1 subjects was highest among people aged 55 years
26
27 230 old (figure 2).
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32 33 232 **Associations with BDD**

34 35 233 **Bivariate analysis**

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37 234 The crude analysis using simple logistic regression found the variable with the strongest
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39 235 association to BDD to be PDF3 compared to non-PDF (OR=6.35, 95% CI 5.40 to 7.46,
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41 236 P<0.001). Other factors that were significantly associated with BDD included: gender, with
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43 237 male being more affected by BDD than female; age, with a progressively increasing OR;
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45 238 lower education levels; occupations that was unemployed; infected liver fluke; PZQ used,
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47 239 with a progressively increasing OR; having a history of smoking and alcohol consumption;
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49 240 being positive for DM diagnosis (table 2).
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52 53 242 **Multivariable analysis**

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3 243 Through the multivariable analysis using multiple logistic regression, all factors were
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5 244 adjusted and the association of PDF3 subjects having BDD remained significantly high
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7 245 compared with non-PDF subjects (adjusted OR=5.74, 95% CI 4.57 to 7.21, P<0.001) (table
8
9 246 2). Compared to crude OR, the adjusted OR of gender, age, occupations, liver fluke infection,
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11 247 smoking history and alcohol consumption history, and a positive diagnosis of DM remained
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13 248 statistically significant, while a positive diagnosis of HB and HC remained non-significant
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15 249 (figure 3). Our study also found that relatives diagnosed with CCA changed from non-
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17 250 significant in bivariate analysis to significant in multivariable analysis, while education levels
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19 251 and PZQ treatment changed from significant to non-significant.
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23 253 <Table 2 located here>

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25 255 <Figure 1 located here>

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27 257 <Figure 2 located here>

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29 259 <Figure 3 located here>

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31 261 **DISCUSSION**

32 262 Liver cancer is one of the leading causes of death throughout the world.²⁷ CCA accounts for
33 263 more than 60% of these liver cancer cases with Northeast Thailand reporting the highest
34 264 incidence in the world.^{28 29} PDF and BDD have been recognized as the key risk factors of
35 265 CCA development.^{8 17 21} Due to ambiguities in the relationship between PDF and BDD, our
36 266 study investigated the prevalence of PDF and BDD in a high-risk CCA population to find if
37 267 there was a presence of a statistically significant relationship between the two factors. Our
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3 268 study specifically found that the prevalence of BDD was significantly higher (6.6%) among
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5 269 subjects who were diagnosed with PDF3 and it was the most statistically significant
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7 270 associated factor of BDD (adjusted OR=5.74, 95% CI 4.57 to 7.21, P<0.001). Although a
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9 271 study conducted in Japan, concluded fibrosis and BDD as being indicators of CCA, they did
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11 272 not mention an association between them.¹⁷ In addition, studies conducted in Thailand report
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13 273 only PDF as a major risk factor of CCA development.^{8 21 30}

14
15 274 We conducted a bivariate analysis via a simple logistic regression and found that gender,
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17 275 age, and smoking history were the three most significant factors associated with BDD and
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19 276 remained significant in the multivariable analysis. The factor of relatives diagnosed with
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21 277 CCA became significant in multivariable analysis, but the magnitude of association was still
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23 278 relatively low, while education levels and PZQ treatment became non-significant. The other
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25 279 factors that were statistically significant in the bivariate analysis became less significant after
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27 280 adjusting for all factors in the multivariable analysis included occupations, alcohol
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29 281 consumption history, and being diagnosed with DM.

30
31 282 Our study found that those aged 60-years-old and over are more likely to have BDD than
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33 283 other age groups. Meanwhile, our study also found the association of BDD increased with
34
35 284 increasing age. We conclude that age plays a role in BDD development. This result is similar
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37 285 to a study conducted in Israel between 2001-2002 which found that bile duct size increases
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39 286 with age and reported age was positively correlated with bile duct size.³¹ A study from
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41 287 Canada in 2014 found that older age was associated with bile duct diameters which increases
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43 288 with age.³² Therefore, it is not a surprise that those who were in the oldest age group in our
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45 289 study had a strong association with BDD, which causes the bile duct to grow.

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47 290 Subjects positive for HB and HC diagnosis demonstrated a non-significant association
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49 291 with BDD (adjusted OR=1.16, 95% CI 0.88 to 1.52, P=0.298 and adjusted OR=1.69, 95% CI
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51 292 0.87 to 3.31, P=0.124, respectively). Our findings are close to results reported by Barusrux

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3 293 and colleagues in 2012 which found that HB and HC were not related to CCA.³³ However, it
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5 294 is also important to mention contradictory results reported in South Korea which found that
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7 295 HBV infection was a significant risk factor for intrahepatic cholangiocarcinoma (ICC)
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9 296 development with OR=2.3, 95% CI 1.6 to 3.3 P<0.05.³⁴ HBV infection was also related to a
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11 297 3.4-fold risk of ICC in China.³⁵ Another study conducted in Northeast Thailand in 2010,
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13 298 examined the association of HB and HC with CCA and reported a greater risk of CCA for
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15 299 those carrying the virus (OR=4, 95% CI 1.29 to 16.44, P<0.05).³⁶

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18 300 And interestingly, those who had CCA diagnosed relatives, had a higher association to
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20 301 BDD than those who did not have CCA diagnosed relatives only 12% (adjusted OR=1.12,
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22 302 95% CI 1.02 to 1.24, P=0.018). However, our results were consistent with Zhou et al. (2014),
23
24 303 who identified genetic and familial risk factors as significantly contributing to the
25
26 304 development of combined HCC-CCA through a bivariate analysis.³⁷ It is worth mentioning
27
28 305 that this significance could not be confirmed through a multivariable analysis. Other studies
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30 306 also demonstrate that having a family history of cancer is a significant associated factor for
31
32 307 CCA development.^{38 39} A risk factor study of CCA in Northeast Thailand also reported
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34 308 patients who had a family history of cancer were more likely to develop CCA than those
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36 309 without a family history of liver cancer.⁴⁰ Death or traumatic incidences influence the
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38 310 decision-making process. This may be the reason behind the lack of association between
39
40 311 family history of CCA and BDD in our statistical analysis. Perhaps family members who
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42 312 experience a death of CCA-diagnosed family member are more likely to take measures in
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44 313 preventing the occurrence of a second CCA incidence in the family. A CCA traumatic
45
46 314 experience may have served as a warning for family members to avoid this rapid and fatal
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48 315 outcome. These results reveal the complicated nature of understanding the true risk factors of
49
50 316 CCA and pathogenesis to hepatic carcinoma in certain Asian societies.
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318 **CONCLUSIONS**

319 In conclusion, our key findings included identifying the factors associated with biliary tract
320 disease in a high-risk population for CCA: PDF3, male gender, older age, having CCA-
321 diagnosed relatives, infected liver fluke, and smoking history. Based on our results, patients
322 should be considered suspected-CCA cases during US screenings in high-risk areas through
323 the detection of PDF, old age (50 and over), if they were infected for liver fluke, have CCA-
324 diagnosed relatives, and are current or previous smokers. The interesting results regarding
325 HB and HC diagnoses may need further evaluation and review due to some contradictions in
326 the data. Greater consideration toward CCA and HCC prevention should be aimed at those in
327 older age groups. Despite certain limitations, our data was based on a very large sample size
328 and suggests a statistically robust association between PDF and BDD, specifically the PDF3
329 grouping. Early and routine screening of BDD and PDF may provide a means to reduce the
330 incidence of liver-related diseases and CCA. Future planning of CCA surveillance should
331 focus on early screening for both PDF and BDD.

332

333 **Recommendations**

334 This study was conducted in Northeast Thailand and may not reflect the general population.
335 Further study is necessary in the region to test the generality of our results. Nevertheless, the
336 methodology and results of our study can be used as a guideline in formulating clinical
337 practice and future research priorities.

338

339 **List of abbreviations**

340 BDD, Bile duct dilatation; CASCAP, Cholangiocarcinoma Screening and Care Program;
341 CCA, Cholangiocarcinoma; CI, Confidence interval; DM, Diabetes mellitus, HB, Hepatitis
342 B; HC, Hepatitis C; HCC, Hepatocellular carcinoma; ICC, Intrahepatic cholangiocarcinoma;

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3 343 N/A, Not applicable; OR, Odds ratios; PDF, Periductal fibrosis; PZQ, Praziquantel; US,
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5 344 Ultrasonography; WHO, World Health Organization.

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9 346 **Conflict of interest**

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11 347 All authors declare no conflict of interest.

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27 355

28
29 356 **Author contributions** NC, SP, and KT conceived and designed this study. KT and BT
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31 357 performed the analysis. NC, SP, NK, BT, KT, ATS and MK wrote the manuscript. NC, NK,
32
33 358 BT and KT collected the data and generated the clinical database. All authors have been
34
35 359 involved in revising the manuscript, and all authors have read and approved the final
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37 360 manuscript.

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51 367 **Competing interests** The authors declare that they have no competing interests.

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5 369 **Patient consent** All patients gave written informed consent for the study.

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9 371 **Ethics approval** The research protocol was approved by Khon Kaen University Ethics

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11 372 Committee for Human Research, reference number HE591067.

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15 374 **Provenance and peer review** Not commissioned; externally peer reviewed.

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19 376 **Data sharing statement** No additional data are available.

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3 500 **Captions for the figures:**
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7 502 **Figure 1** Percentage of BDD between male and female according to PDF1, 2, and 3.
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11 504 **Figure 2** Number of BDD in PDF subjects by age range.
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15 506 **Figure 3** The adjusted OR and crude OR of the associated factors of BDD.
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20 **Table 1** Baseline demographic and clinical characteristics of subjects
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Characteristics	Number (n=394 026)	Percentage
Gender		
Female	242 115	61.4
Male	151 866	38.6
Missing data (n=45)		
Age group (years)		
40-44	49 281	12.9
45-49	71 564	18.7
50-54	78 428	20.5
55-59	69 530	18.2
60 years and over	114 305	29.8
Mean±Standard deviation	54.92±9.03	
Median (minimum : maximum)	54 (40 : 100)	
Missing data (n=10 918)		
Education levels		
None	6561	1.7
Primary	286 840	72.9

Table 1 Baseline demographic and clinical characteristics of subjects

Characteristics	Number (n=394 026)	Percentage
Secondary	78 090	19.9
Certificate/Bachelor	18 632	4.7
Higher than bachelor	3055	0.8
Missing data (n=848)		
Occupation		
Unemployed	15 582	4.0
Farmer	306 421	77.9
Labor	32 420	8.2
Own business	13 467	3.4
Government official/State enterprises	13 997	3.6
Others	11 335	2.9
Missing data (n=804)		
Relatives diagnosed with CCA		
No	319 902	81.4
Yes	73 286	18.6
Missing data (n=838)		
Liver fluke infection		
No	113 178	62.1
Yes	68 979	37.9
Missing data (n=211 869)		
Praziquantel treatment		
None	270 183	70.3
One time	84 136	21.9
Two times	18 126	4.7
Three times	5264	1.4

Table 1 Baseline demographic and clinical characteristics of subjects

Characteristics	Number (n=394 026)	Percentage
More than three times	6414	1.7
Missing data (n=9903)		
Smoking history		
No	308 776	78.7
Yes, current or previous	83 754	21.3
Missing data (n=1496)		
Alcohol consumption history		
No	214 495	54.6
Yes, current or previous	178 564	45.4
Missing data (n=967)		
Hepatitis B		
No	382 058	98.2
Yes	6803	1.8
Missing data (n=5165)		
Hepatitis C		
No	388 114	99.8
Yes	747	0.2
Missing data (n=5165)		
Diabetes mellitus		
No	362 296	93.2
Yes	26 565	6.8
Missing data (n=5165)		
Periductal fibrosis		
None	324 482	82.4
PDF1	48 383	12.3

Table 1 Baseline demographic and clinical characteristics of subjects

Characteristics	Number (n=394 026)	Percentage
PDF2	18 686	4.7
PDF3	2475	0.6

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Table 2 Prevalence, and crude and adjusted odd ratios of BDD associated factors and their 95% confidence interval

Factors	Subjects	% BDD	Crude OR	Adjusted OR	95% CI	P value
Over all	394 026	1.2	N/A	N/A	N/A	N/A
Periductal fibrosis						<0.001
None	324 482	1.1	1	1		
PDF1	48 383	1.4	1.23	1.25	1.11 to 1.40	
PDF2	18 686	1.7	1.55	1.24	1.04 to 1.47	
PDF3	2475	6.6	6.35	5.74	4.57 to 7.21	
Gender						<0.001
Female	242 115	0.9	1	1		
Male	151 866	1.7	2.00	1.46	1.31 to 1.63	
Age group (years)						<0.001
40-44	49 281	0.6	1	1		
45-49	71 564	0.6	1.04	1.10	0.88 to 1.38	
50-54	78 428	0.9	1.44	1.42	1.15 to 1.75	
55-59	69 530	1.1	1.77	1.74	1.42 to 2.14	
60 years and over	114 305	2.1	3.46	3.14	2.59 to 3.81	
Education levels						0.472
None	6561	1.6	1	1		
Primary	286 840	1.3	0.82	0.91	0.65 to 1.27	
Secondary	78 090	0.8	0.53	0.72	0.51 to 1.03	
Certificate/Bachelor	18 632	1.1	0.71	0.81	0.53 to 1.24	
Higher than bachelor	3055	1.5	0.98	0.94	0.52 to 1.71	

Table 2 Prevalence, and crude and adjusted odd ratios of BDD associated factors and their 95% confidence interval

Factors	Subjects	% BDD	Crude OR	Adjusted OR	95% CI	P value
Occupations						<0.001
Unemployed	15 582	2.5	1	1		
Farmer	306 421	1.1	0.45	0.47	0.40 to 0.55	
Labor	32 420	1.0	0.39	0.53	0.41 to 0.67	
Own business	13 467	1.0	0.40	0.65	0.48 to 0.87	
Government/State enterprises	13 997	1.5	0.59	0.87	0.63 to 1.20	
Others	11 335	1.4	0.57	0.60	0.44 to 0.80	
Relatives diagnosed with CCA						0.018
No	319 902	1.2	1	1		
Yes	73 286	1.2	0.99	1.12	1.02 to 1.24	
Liver fluke infection						<0.001
No	113 178	1.2	1	1		
Yes	68 979	1.5	1.24	1.25	1.12 to 1.39	
Praziquantel treatment						0.067
None	270 183	1.1	1	1		
One time	84 136	1.3	1.20	0.85	0.75 to 0.95	
Two times	18 126	1.5	1.33	0.93	0.79 to 1.10	
Three times	5264	1.7	1.56	1.10	0.85 to 1.43	
More than three times	6414	1.8	1.63	1.26	1.00 to 1.59	
Smoking history						<0.001
No	308 776	1.0	1	1		
Yes, current or previous	83 754	2.0	2.11	1.31	1.17 to 1.46	
Alcohol consumption history						0.002
No	214 495	1.0	1	1		
Yes, current or previous	178 564	1.4	1.45	1.17	1.06 to 1.29	
Hepatitis B virus						0.298

Table 2 Prevalence, and crude and adjusted odd ratios of BDD associated factors and their 95% confidence interval

Factors	Subjects	% BDD	Crude OR	Adjusted OR	95% CI	P value
No	382 058	1.2	1	1		
Yes	6803	1.4	1.13	1.16	0.88 to 1.52	
Hepatitis C virus						0.124
No	388 114	1.2	1	1		
Yes	747	2.0	1.69	1.69	0.87 to 3.31	
Diabetes mellitus						0.011
No	362 296	1.2	1	1		
Yes	26 565	1.6	1.37	1.20	1.04 to 1.37	

509 N/A, Not applicable.

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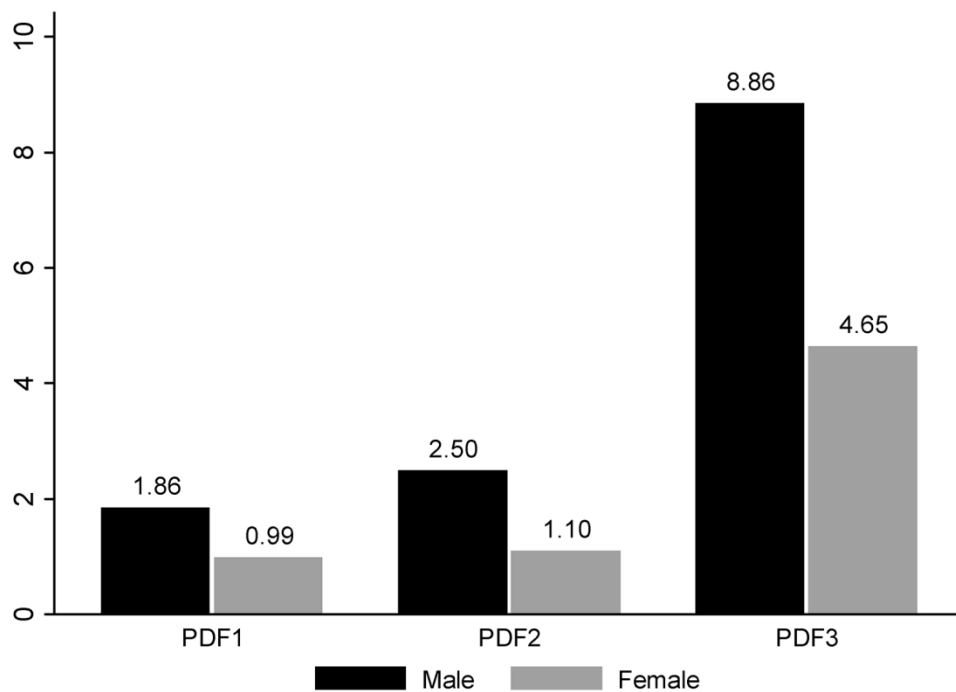


Figure 1 Percentage of BDD between male and female according to PDF1, 2, and 3.

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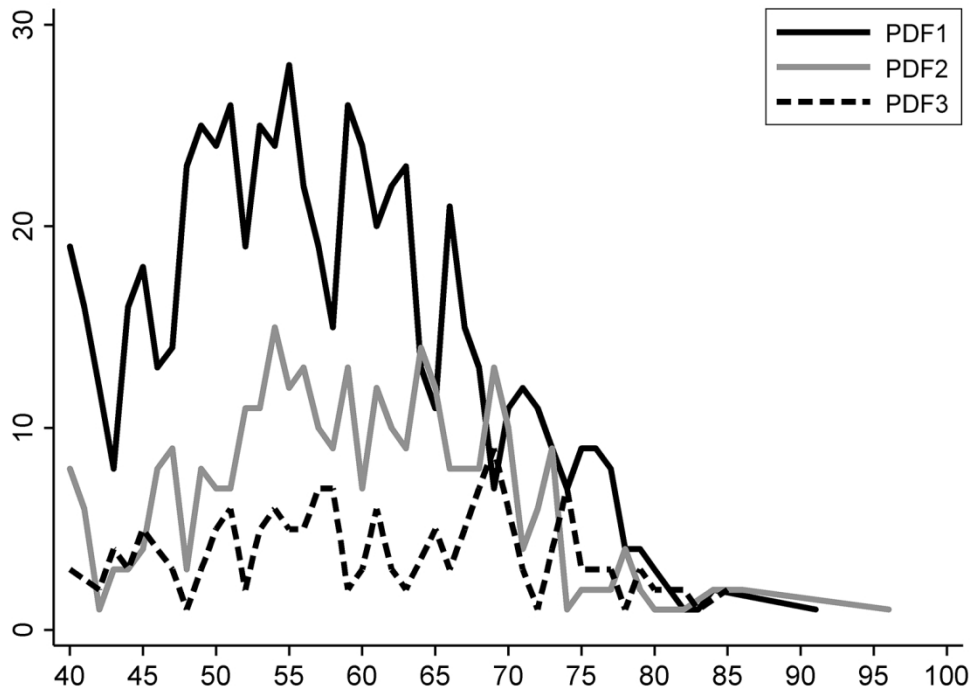


Figure 2 Number of BDD in PDF subjects by age range.
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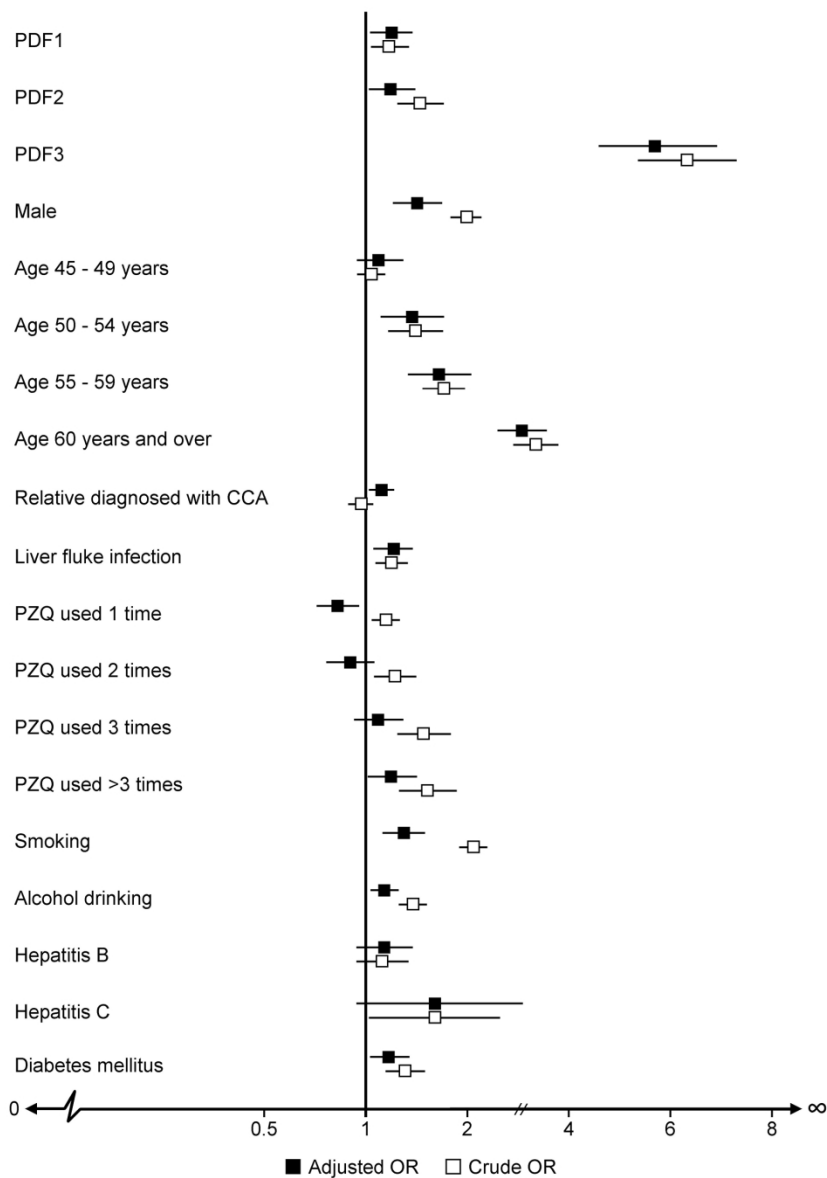


Figure 3 The adjusted OR and crude OR of the associated factors of BDD.

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Location	Recommendation
Title and abstract	1	Pg3	(a) Indicate the study's design with a commonly used term in the title or the abstract
		Pg3	(b) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction			
Background/rationale	2	Pgs5-6	Explain the scientific background and rationale for the investigation being reported
Objectives	3	Pg6	State specific objectives, including any prespecified hypotheses
Methods			
Study design	4	Pgs 6-7	Present key elements of study design early in the paper
Setting	5	Pgs 6-7	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	Pgs6-7	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
			<i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls
		N/A	(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed
			<i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case
Variables	7	Pg8	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	Pgs7-8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Pg4	Describe any efforts to address potential sources of bias
Study size	10	Pg6-7	Explain how the study size was arrived at
Quantitative variables	11	Pg8-9	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	Pg8-9	(a) Describe all statistical methods, including those used to control for confounding
			N/A
		Pg9	(c) Explain how missing data were addressed
		N/A	(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed
			<i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed
	<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy		
	N/A	(e) Describe any sensitivity analyses	

Continued on next page

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Location		Results	
Participants	13*	Pg9	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		N/A	(b) Give reasons for non-participation at each stage
		N/A	(c) Consider use of a flow diagram
Descriptive data	14*	Pg9	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		Pgs22-24	(b) Indicate number of participants with missing data for each variable of interest
		N/A	(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Pgs9-10	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time
		N/A	<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure
		Pg25	<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures
Main results	16	Pgs9-10	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		N/a	(b) Report category boundaries when continuous variables were categorized
		N/a	(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	N/A	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
		Discussion	
Key results	18	Pg11-12	Summarise key results with reference to study objectives
Limitations	19	Pg4	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Pg14	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Pg14	Discuss the generalisability (external validity) of the study results
		Other information	
Funding	22	Pg15	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Periductal fibrosis and bile duct dilatation: pathways to diagnosis for cholangiocarcinoma in Northeast Thailand

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3 **1 TITLE PAGE**
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4 cholangiocarcinoma in Northeast Thailand

5
6 **6 Authors:** Nittaya Chamadol,^{1,2,3} Narong Khuntikeo,^{1,2,4} Bandit Thinkhamrop,^{1,2,5,6} Kavin
7 Thinkhamrop,^{1,2,6} Apiporn T. Suwannatrai,^{1,2,7} Matthew Kelly,⁸ and Supanee Promthet^{1,5,9}
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57
58
59
60
9 Affiliations:

10 ¹Cholangiocarcinoma Screening and Care Program (CASCAP), Khon Kaen University, Khon
11 Kaen, Thailand.

12 ²Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand.

13 ³Department of Radiology, Faculty of Medicine, Khon Kaen University, Khon Kaen,
14 Thailand.

15 ⁴Department of Surgery, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

16 ⁵Epidemiology and Biostatistics Section, Faculty of Public Health, Khon Kaen University,
17 Khon Kaen, Thailand.

18 ⁶Data Management and Statistical Analysis Center (DAMASAC), Faculty of Public Health,
19 Khon Kaen University, Khon Kaen, Thailand.

20 ⁷Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen,
21 Thailand.

22 ⁸Department of Global Health, Research School of Population Health, Australian National
23 University, Canberra, Australia.

24 ⁹ASEAN Cancer Epidemiology and Prevention Research Group, Khon Kaen University,
25 Khon Kaen, Thailand.

1
2
3 26
4

5 27 **Email address:**

6
7 28 NC: Nittaya Chamadol (nittayachamadol@yahoo.com)

8
9 29 NK: Narong Khuntikeo (nkhuntikeo@gmail.com)

10
11 30 BT: Bandit Thinkhamrop (bandit@kku.ac.th)

12
13 31 KT: Kavin Thinkhamrop (kvinth@gmail.com)

14
15 32 ATS: Apiporn T. Suwannatrai (apiporn@kku.ac.th)

16
17 33 MK: Matthew Kelly (matthew.kelly@anu.edu.au)

18
19 34 SP: Supanee Promthet (supanee@kku.ac.th)

20
21
22 35
23

24 36 **Corresponding authors:**

25
26 37 Name: Supanee Promthet

27
28 38 Address: ASEAN Cancer Epidemiology and Prevention Research Group,

29
30
31 39 Khon Kaen University, Khon Kaen 40002, Thailand.

32
33 40 Telephone: +66-82 668 1995

34
35 41 e-Mail: supanee@kku.ac.th

36
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1
2
3 48 **ABSTRACT**

4
5 49 **Objectives** To assess associations between periductal fibrosis (PDF) and bile duct dilatation
6
7 50 (BDD) in ultrasonography (US) screening of population at risk of cholangiocarcinoma (CCA)
8
9 51 due to residence in an endemic area for *Opisthorchis viverrini*. CCA survival rates are low
10
11 52 and early identification of risk factors is essential. BDD is one symptom which can identify
12
13 53 patients at risk of CCA. Detection of PDF by US can also identify at risk patients, at an
14
15 54 earlier stage of CCA development. Identification of association between PDF and BDD will
16
17 55 inform screening practices for CCA risk, by increasing the viability of PDF screening for
18
19 56 CCA risk.

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21
22 57 **Setting** Nine tertiary care hospitals in Northeast Thailand.

23
24 58 **Design** Cross-sectional study.

25
26 59 **Participants** Study subjects in the Cholangiocarcinoma Screening and Care Program
27
28 60 (CASCAP) in Northeast Thailand. CASCAP inclusion criteria are all residents of Northeast
29
30 61 Thailand aged 40 years and over. Participants are recruited through CCA screening centers
31
32 62 and through primary health care units. So far 394 026 have been enrolled.

33
34 63 **Methods** PDF and BDD were identified through US. PDF was categorized into three groups,
35
36 64 PDF1, 2 and 3, depending on their high echo locality in the peripheral, segmental and main
37
38 65 bile duct, respectively. Associations between PDF and BDD were determined by adjusted
39
40 66 odds ratio (OR) and 95% confidence interval (CI) using multiple logistic regression.

41
42 67 **Results** BDD was found in 6.6% of PDF3, 1.7% of PDF2, and 1.4% of PDF1 cases. Among
43
44 68 PDF cases, especially in PDF3, BDD was found in male more than female (8.9% and 4.6%,
45
46 69 respectively). Compared to non-PDF, the association between PDF3 and BDD was highly
47
48 70 significant (adjusted OR=5.74, 95% CI 4.57 to 7.21, P<0.001).

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3 71 **Conclusions** Our findings reveal that there is a relationship between PDF and BDD, which is
4
5 72 associated with CCA. Therefore, PDF can also be an indicator for suspected-CCA diagnosis
6
7 73 through US.
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10
11 75 **Keywords** bile duct dilatation; periductal fibrosis; ultrasonography; cholangiocarcinoma;
12
13 76 screening; Thailand
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18 78 **Article summary**

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20 79 **Strengths and limitations of the study**

- 21
22 80 • The large size of the study population and its geographic distribution across Northeast
23
24 81 Thailand are a significant strength.
25
26 82 • This is the first and largest screening program for cholangiocarcinoma (CCA) in an
27
28 83 area with the highest incidence in the world.
29
30 84 • CCA risk factors (PDF and BDD) were measured using ultrasonography by skilled
31
32 85 radiologists.
33
34 86 • Demographic, and some health, data were self-reported leading to potential bias in
35
36 87 measurement of liver fluke infection, praziquantel treatment, and pre-existing medical
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38 88 conditions including HB, HC, and DM.
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40 89 • Self-report could lead to prevalence underestimates due to the fact that subjects may
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42 90 not have been willing to disclose sensitive or personal information.
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93 INTRODUCTION

94 Cholangiocarcinoma (CCA) and hepatocellular carcinoma (HCC), are ranked the most
95 prevalent cancers in Southeast Asia.¹⁻³ The early-stages of CCA can manifest through
96 obstructive jaundice, which is found in 30% of patients who are diagnosed with primary
97 sclerosing cholangitis.⁴ Other liver disorders: fatty liver disease, cirrhosis, and liver mass are
98 likewise recognized risk factors for both CCA and HCC.⁵⁻¹⁰ Suspected CCA cases can also be
99 identified through the presence of bile duct dilatation (BDD), which can be identified in
100 suspected CCA cases through ultrasonography (US) screening.^{11 12} A previous study
101 demonstrated that US screening is highly sensitive in identifying CCA through confirmed
102 incidences of BDD.¹³ However, upon the detection and diagnosis of bile duct and liver
103 disorders, it is often too late to save patients with CCA and HCC due to the rapid progression
104 to advanced stages of hepatic carcinoma.¹⁴ As well, detection of BDD by US requires the
105 services of specialist radiologists, who are generally only available in major hospitals,
106 limiting access to screening. Thus, the best way to save a patient's life and prevent the
107 likelihood of cancer development is through early, easily accessible, screenings to detect the
108 risk factors that may lead to cancer among high-risk populations.

109 As well as BDD there are several other indicators for CCA risk including well-accepted
110 premalignant lesions such as biliary intraepithelial neoplasm (BilIN), and intraductal
111 papillary neoplasm of the bile duct (IPNB).^{15 16} Periductal fibrosis (PDF) is another
112 abnormality of the bile duct which has been used to identify people at risk of developing
113 CCA. This hepatobiliary abnormality is particularly prominent among people infected with
114 the liver fluke, *Opisthorchis viverrini*.¹⁷⁻²¹ This infection is caused by the consumption of raw
115 or lightly fermented fish products and is one of the key risk factors for development of CCA
116 in the region. PDF is caused by the thickening of the bile duct wall, along the periportal
117 space.²²

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3 118 The relationship between PDF and CCA is indicated by the regular detection of PDF in
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5 119 confirmed CCA cases, and this has been particularly common in Northeast Thailand where
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7 120 *O. viverrini* is endemic and a leading potential cause of CCA.⁸ As a result of this relationship,
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9 121 US detection has been utilized to identify people with PDF as a risk group for CCA
10
11 122 development.^{8 20 23 24} Hepatobiliary abnormalities identified through ultrasound have been
12
13 123 shown in other studies to correlate well with histopathological confirmation making US a
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15 124 valuable tool in early identification of these health issues.⁸ Importantly, PDF can be identified
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17 125 through US, but does not require the services of a specialist radiologist increasing the
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19 126 potential access to screening, and PDF can be detected earlier than BDD allowing more
20
21 127 effective intervention.
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24 128 The potential to detect the risk of CCA earlier and without the need for specialist
25
26 129 radiologists, through the identification of PDF may be an important breakthrough in reducing
27
28 130 CCA incidence. So, both PDF and BDD have been recognized as indicators of CCA^{8 17}, but
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30 131 their relationship to one another has yet to be established or even studied in depth.
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32 132 Determining their relationship, such as learning if one precedes the other may make a
33
34 133 significant change in how we screen for CCA via US. Therefore, this study seeks to
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36 134 determine if there is an association between PDF and BDD among people at a high-risk CCA
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38 135 population in Northeast Thailand. The results of this work will clarify necessary directions
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40 136 toward early screening methodologies and appropriate cancer treatment.
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45 138 **METHODS**

46 139 **Study design**

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48 140 This study presents data collected from the Cholangiocarcinoma Screening and Care Program
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50 141 (CASCAP) in Northeast Thailand. CASCAP is a prospective cohort study that is considered
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52 142 the first project for CCA screening in a high-risk population with a community-based bottom-
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3 143 up approach.²⁵ Although this overall project is a prospective cohort study, the results
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5 144 presented here use cross sectional data from the baseline study carried out with participants.

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8
9 146 The overall aim of the study is to recruit all adults aged 40 years or over who reside in
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11 147 Northeast Thailand and to screen them for cholangiocarcinoma and its risk factors in terms of
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13 148 hepatobiliary abnormalities and infection with the liver fluke *Opisthorchis viverrini*. As such
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15
16 149 there are no strict inclusion or exclusion criteria apart from age group and place of residence.
17
18 150 Once consent has been obtained, the participants will be enrolled in the program. The
19
20 151 primary place of recruitment for this cohort study were 9 tertiary care hospitals in the
21
22 152 Northeast of Thailand. These hospitals serve as the main source of affordable tertiary care for
23
24 153 local people in the region. Subjects were recruited at these hospitals in two ways. Firstly the
25
26 154 screening group comprised individuals who had attended the hospital for other reasons and
27
28 155 were invited to receive ultrasound screening without evidencing any symptoms. The second
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30 156 group, the walk-in group, were individuals who were attending the hospital because of CCA
31
32 157 symptoms and this group can then receive treatment. All participants were asked to join the
33
34 158 project by signing a consent form. All CCA patients were diagnosed and treated according to
35
36 159 routine, real world clinical practice by participating hospitals. Patients were followed-up and
37
38 160 provided with either clinical or palliative care depending on the stage of their disease.
39
40 161 Treatment outcomes were recorded. Follow-up took place every 3-6 months depending on the
41
42 162 patient's condition and unless scheduled otherwise.

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46 47 48 164 **Study population**

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50 165 Our study recruited subjects from among people who participated the CASCAP project.
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52 166 These subjects form two groups (screening and walk-in). The screening group was people
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54 167 who have undergone routine US and who showed no symptoms that could be related to CCA.
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3 168 The walk-in group was people who come to the hospital with symptoms indicating CCA
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5 169 which has been diagnosed with US. The subjects included in our study only those enrolled in
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7 170 the CASCAP database from 2013-2017 with a total of 394 026 subjects.
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12 172 **Patient and Public Involvement**

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15 173 The CASCAP project is a comprehensive screening and treatment program for CCA.
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17 174 Members of the public were first involved in the research in two ways. Firstly when members
18
19 175 of the public attended a participating hospital for any reason, hospital staff would actively
20
21 176 recruit them to the study. Village health volunteers also recruited participants while carrying
22
23 177 out their work. A second group were those who already has some suspected symptoms and
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25 178 attended a hospital for screening at which point they were recruited into the study. The study
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27 179 participants were not directly involved in the design of the study. Participants will be
28
29 180 contacted at least annually to be screened for CCA risk. Patients identified as having CCA
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31 181 will receive standard care for the condition through the project. For the screening procedures
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33 182 covered by this report participants are informed of the purpose, outcomes and implications of
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35 183 these procedures.
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41 185 **Main outcome and independent variables**

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43 186 The primary outcome for this study was BDD which was categorized into two groups
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45 187 (no/yes). The independent variable of interest was PDF. We classify PDF into 3 categories
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47 188 (PDF1, 2 and 3) using a World Health Organization standard methodology originally
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49 189 developed for use in the assessment of schistosomal periportal fibrosis (PPF) but which is
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51 190 also valid for the study of PDF given that PPF and PDF have the same ultrasound images of
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53 191 Increased Periportal Echo.²⁶ We only use 3 of the 5 classifications utilized in this
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55 192 methodology since anatomically extra and intra hepatic bile ducts run in parallel to the portal
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3 193 vein in the periportal space, so the pathology of the bile duct should be detected first in the
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5 194 periportal space. This identification system has been validated by comparing US diagnoses
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7 195 with histopathologically proven cases of PDF with good agreement between the methods.⁸
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9 196 Using this system PDF is categorized based on the anatomical location of the intrahepatic and
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11 197 extrahepatic bile duct. PDF1 is defined as having a high echo in the wall of small bile ducts
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13 198 scattered in the liver as a starry sky pattern, PDF2 is a high echo along the segmental bile
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15 199 duct wall running parallel with the portal vein, and PDF3 is a high echo along the main bile
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17 200 duct wall running parallel with the portal vein in the periportal space.¹⁹
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20 201 Both BDD and PDF diagnosed via US by radiologists from the CASCAP project all of whom
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22 202 took part in a special training course for ultrasound examination including all criteria to
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24 203 diagnose hepatobiliary abnormalities. A teleconsultation system was also set up to confirm
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26 204 diagnoses from radiologists. Demographic characteristics of PDF and non-PDF subjects were
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28 205 the independent variables includes gender, age, education levels, occupations, having a
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30 206 relative diagnosed with CCA, liver fluke infection, praziquantel (PZQ) treatments, smoking
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32 207 (current or previous), alcohol consumption (current or previous) and diagnosis with hepatitis
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34 208 B (HB), hepatitis C (HC), and diabetes mellitus (DM). All demographic characteristics listed
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36 209 above were collected via face-to-face interview by interviewer from the CASCAP using
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38 210 questionnaire.
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43 212 **Statistical analysis**

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46 213 The demographic characteristics that were categorical data were summarized using
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48 214 frequencies and percentages (i.e. gender, age groups, education levels, occupations, having a
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50 215 relative diagnosed with CCA, liver fluke infection, PZQ treatments, smoking history, alcohol
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52 216 consumption history and diagnosis with HB, HC, DM, and PDFs). The continuous data, such
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3 217 as the age of the subjects, were summarized by their mean, standard deviation, median,
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5 218 minimum and maximum range.

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7 219 The prevalence of BDD was calculated and the percentage of the prevalence was
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9 220 computed based on a normal approximation to a binomial distribution. Bivariate analysis
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11 221 using simple logistic regression was performed to investigate the association between the
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13 222 independent factors listed above and BDD. They were determined by crude odds ratio (OR)
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15 223 and their 95% confidence intervals (CI). Then multivariable analysis using multiple logistic
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17 224 regression was carried out to investigate the association between PDF and BDD as
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19 225 determined by the adjusted OR and 95% CI. The final multivariate model was adjusted for all
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21 226 factors which previous studies have reported to be associated with the hepatobiliary disease:
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23 227 PDF, gender, age, education levels, occupations, having a relative diagnosed with CCA, liver
24
25 228 fluke infection, PZQ treatments, smoking, alcohol consumption as well as diagnosis with HB,
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27 229 HC, and DM.

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30
31 230 There were missing values for some variables due to unwillingness of some participants
32
33 231 to answer some socio-demographic or health history questions or from errors in data
34
35 232 collection. Missing values for most variables were rare with proportions missing less than 3%
36
37 233 of participants. The only variable with a significant proportion of missing values was that of
38
39 234 previous liver fluke diagnosis (n=211 869), but this number includes those who had reported
40
41 235 never having been tested for infection.

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44 236 All test statistics were two-tailed and a p-value of less than 0.05 was considered
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46 237 statistically significant. All analyses were performed by using a statistical package, Stata
47
48 238 version 15 (StataCorp, College Station, Texas, USA).

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51 52 240 **RESULTS**

53 54 241 **Descriptive summary**

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3 242 The demographic characteristics of subjects were presented as numbers and percentages. A
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5 243 total of 394 026 subjects who underwent US screenings for CCA were enrolled in our study.
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7 244 The subjects were all between the ages of 40-100 years old and reported a mean age of
8
9 245 54.92±9.03 years old. Of these, approximately two-thirds were female (61.4%) and the
10
11 246 majority of them completed primary school education level (72.9%) and worked as farmers
12
13 247 (77.9%). About one-third (29.7%) had ever used PZQ treatment, and about one-fourth
14
15 248 (21.3%) reported being a smoker or ex-smoker. The data of PDF diagnosis, 17.6% have
16
17 249 positive diagnosed and the highest percentage was in subjects diagnosed with PDF1 (12.3%)
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19 250 while only 0.6% for PDF3 (table 1).
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252 <Table 1 located here>

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254 **Prevalence of BDD**

255 From this study, the overall prevalence of BDD was reported to be 1.2%. The highest
256 prevalence of BDD was 6.6% from the PDF3 group under periductal fibrosis. PDF1 and
257 PDF2 subjects reported a low prevalence rate of only 1.4% and 1.7%, respectively (table 2).
258 Our study found that the prevalence of BDD occurring in PDF subjects was high in male
259 more than female, particularly in PDF3 (8.9% and 4.6%, respectively) (figure 1). Meanwhile,
260 we also found the number of BDD in PDF1 subjects was highest among people aged 55 years
261 old (figure 2).

262

263 **Associations with BDD**

264 **Bivariate analysis**

265 The crude analysis using simple logistic regression found the variable with the strongest
266 association to BDD to be PDF3 compared to non-PDF (OR=6.35, 95% CI 5.40 to 7.46,

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3 267 P<0.001). Other factors that were significantly associated with BDD included: gender, with
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5 268 male being more affected by BDD than female; age, with a progressively increasing OR;
6
7 269 lower education levels; occupations that was unemployed; infected liver fluke; PZQ used,
8
9 270 with a progressively increasing OR; having a history of smoking and alcohol consumption;
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11 271 being positive for DM diagnosis (table 2).

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15 273 **Multivariable analysis**

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18 274 Through the multivariable analysis using multiple logistic regression, all factors were
19
20 275 adjusted and the association of PDF3 subjects having BDD remained significantly high
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22 276 compared with non-PDF subjects (adjusted OR=5.74, 95% CI 4.57 to 7.21, P<0.001) (table
23
24 277 2). Compared to crude OR, the adjusted OR of gender, age, occupations, liver fluke infection,
25
26 278 smoking history and alcohol consumption history, and a positive diagnosis of DM remained
27
28 279 statistically significant, while a positive diagnosis of HB and HC remained non-significant
29
30 280 (figure 3). Our study also found that relatives diagnosed with CCA changed from non-
31
32 281 significant in bivariate analysis to significant in multivariable analysis, while education levels
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34 282 and PZQ treatment changed from significant to non-significant.

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44 286 <Figure 1 located here>

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48 288 <Figure 2 located here>

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52 290 <Figure 3 located here>

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3 292 **DISCUSSION**

4
5 293 Liver cancer is one of the leading causes of death throughout the world.²⁷ CCA accounts for
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7 294 more than 60% of these liver cancer cases with Northeast Thailand reporting the highest
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9 295 incidence in the world.^{28 29} PDF and BDD have been recognized as the key risk factors of
10
11 296 CCA development.^{8 17 21} Due to ambiguities in the relationship between PDF and BDD, our
12
13 297 study investigated the prevalence of PDF and BDD in a high-risk CCA population to find if
14
15 298 there was a presence of a statistically significant relationship between the two factors. Our
16
17 299 study specifically found that the prevalence of BDD was significantly higher (6.6%) among
18
19 300 subjects who were diagnosed with PDF3 and it was the most statistically significant
20
21 301 associated factor of BDD (adjusted OR=5.74, 95% CI 4.57 to 7.21, P<0.001). Although a
22
23 302 study conducted in Japan, concluded fibrosis and BDD as being indicators of CCA, they did
24
25 303 not mention an association between them.¹⁷ In addition, studies conducted in Thailand report
26
27 304 only PDF as a major risk factor of CCA development.^{8 21 30}

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30
31 305 We conducted a bivariate analysis via a simple logistic regression and found that gender,
32
33 306 age, and smoking history were the three most significant factors associated with BDD and
34
35 307 remained significant in the multivariable analysis. The factor of relatives diagnosed with
36
37 308 CCA became significant in multivariable analysis, but the magnitude of association was still
38
39 309 relatively low, while education levels and PZQ treatment became non-significant. The other
40
41 310 factors that were statistically significant in the bivariate analysis became less significant after
42
43 311 adjusting for all factors in the multivariable analysis included occupations, alcohol
44
45 312 consumption history, and being diagnosed with DM. Consistent with other studies,¹⁷⁻²¹ our
46
47 313 results also found a significant association between current liver fluke infection and BDD.
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49 314 Liver fluke infection in Northeast Thailand mainly results from the consumption of raw or
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51 315 insufficiently fermented fish and is one of the main established risk factors for BDD and
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53 316 CCA development.
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3 317 Our study found that those aged 60-years-old and over are more likely to have BDD than
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5 318 other age groups. Meanwhile, our study also found the association of BDD increased with
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7 319 increasing age. We conclude that age plays a role in BDD development. This result is similar
8
9 320 to a study conducted in Israel between 2001-2002 which found that bile duct size increases
10
11 321 with age and reported age was positively correlated with bile duct size.³¹ A study from
12
13 322 Canada in 2014 found that older age was associated with bile duct diameters which increases
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15 323 with age.³² Therefore, it is not a surprise that those who were in the oldest age group in our
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17 324 study had a strong association with BDD, which causes the bile duct to grow.

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20 325 Subjects positive for HB and HC diagnosis demonstrated a non-significant association
21
22 326 with BDD (adjusted OR=1.16, 95% CI 0.88 to 1.52, P=0.298 and adjusted OR=1.69, 95% CI
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24 327 0.87 to 3.31, P=0.124, respectively). Our findings are close to results reported by Barusrux
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26 328 and colleagues in 2012 which found that HB and HC were not related to CCA.³³ However, it
27
28 329 is also important to mention contradictory results reported in South Korea which found that
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30 330 HBV infection was a significant risk factor for intrahepatic cholangiocarcinoma (ICC)
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32 331 development with OR=2.3, 95% CI 1.6 to 3.3 P<0.05.³⁴ HBV infection was also related to a
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34 332 3.4-fold risk of ICC in China.³⁵ Another study conducted in Northeast Thailand in 2010,
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36 333 examined the association of HB and HC with CCA and reported a greater risk of CCA for
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38 334 those carrying the virus (OR=4, 95% CI 1.29 to 16.44, P<0.05).³⁶

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41 335 And interestingly, those who had CCA diagnosed relatives, had a higher association to
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43 336 BDD than those who did not have CCA diagnosed relatives only 12% (adjusted OR=1.12,
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45 337 95% CI 1.02 to 1.24, P=0.018). However, our results were consistent with Zhou et al. (2014),
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47 338 who identified genetic and familial risk factors as significantly contributing to the
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49 339 development of combined HCC-CCA through a bivariate analysis.³⁷ It is worth mentioning
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51 340 that this significance could not be confirmed through a multivariable analysis. Other studies
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53 341 also demonstrate that having a family history of cancer is a significant associated factor for
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3 342 CCA development.^{38 39} A risk factor study of CCA in Northeast Thailand also reported
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5 343 patients who had a family history of cancer were more likely to develop CCA than those
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7 344 without a family history of liver cancer.⁴⁰ Death or traumatic incidences influence the
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9 345 decision-making process. This may be the reason behind the lack of association between
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11 346 family history of CCA and BDD in our statistical analysis. Perhaps family members who
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13 347 experience a death of CCA-diagnosed family member are more likely to take measures in
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15 348 preventing the occurrence of a second CCA incidence in the family. A CCA traumatic
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17 349 experience may have served as a warning for family members to avoid this rapid and fatal
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19 350 outcome. These results reveal the complicated nature of understanding the true risk factors of
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21 351 CCA and pathogenesis to hepatic carcinoma in certain Asian societies.

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24 352 This study has some limitations. Firstly, although large, the study population is not
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26 353 representative of the overall population of Northeast Thailand. The recruitment method,
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28 354 through tertiary hospitals, may mean that the study population has some underlying
29
30 355 differences in health status from the general population. In particular the prevalence of BDD
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32 356 and PDF in the study group is likely to vary from overall population prevalence. However,
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34 357 the study has internal validity meaning relationships found between the various hepatobiliary
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36 358 abnormalities and other predictive factors are still important and useful. Also, many of the
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38 359 risk factors including history of previous liver fluke infection (and PZQ treatment) as well as
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40 360 health behaviors in terms of smoking and alcohol consumption were self-reported leading to
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42 361 some potential bias in their measurements.

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46 47 48 363 **CONCLUSIONS**

49
50 364 In conclusion, our key findings included identifying the factors associated with biliary tract
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52 365 disease in a high-risk population for CCA: PDF3, male gender, older age, having CCA-
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54 366 diagnosed relatives, infected liver fluke, and smoking history. Based on our results, patients

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3 367 should be considered suspected-CCA cases during US screenings in high-risk areas through
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5 368 the detection of PDF, old age (50 and over), if they were infected for liver fluke, have CCA-
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7 369 diagnosed relatives, and are current or previous smokers. The interesting results regarding
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9 370 HB and HC diagnoses may need further evaluation and review due to some contradictions in
10
11 371 the data. Greater consideration toward CCA and HCC prevention should be aimed at those in
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13 372 older age groups. Despite certain limitations, our data was based on a very large sample size
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15 373 and suggests a statistically robust association between PDF and BDD, specifically the PDF3
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17 374 grouping. Early and routine screening of BDD and PDF may provide a means to reduce the
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19 375 incidence of liver-related diseases and CCA. Future planning of CCA surveillance should
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21 376 focus on early screening for both PDF and BDD.
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378 **Recommendations**

379 This study was conducted in Northeast Thailand and may not reflect the general population.
380 Further study is necessary in the region to test the generality of our results. Nevertheless, the
381 methodology and results of our study can be used as a guideline in formulating clinical
382 practice and future research priorities.
383

383

384 **List of abbreviations**

385 BDD, Bile duct dilatation; CASCAP, Cholangiocarcinoma Screening and Care Program;
386 CCA, Cholangiocarcinoma; CI, Confidence interval; DM, Diabetes mellitus, HB, Hepatitis
387 B; HC, Hepatitis C; HCC, Hepatocellular carcinoma; ICC, Intrahepatic cholangiocarcinoma;
388 N/A, Not applicable; OR, Odds ratios; PDF, Periductal fibrosis; PZQ, Praziquantel; US,
389 Ultrasonography; WHO, World Health Organization.
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391 **Conflict of interest**

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3 392 All authors declare no conflict of interest.
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21
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23
24 402 performed the analysis. NC, SP, NK, BT, KT, ATS and MK wrote the manuscript. NC, NK,
25
26 403 BT and KT collected the data and generated the clinical database. All authors have been
27
28 404 involved in revising the manuscript, and all authors have read and approved the final
29
30 405 manuscript.
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39
40 409 Network of the Consortium of Thai Medical Schools (Grant No .MRF.59-076) and National
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42 410 Research Council of Thailand (NRCT/2559-134).
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47 412 **Competing interests** The authors declare that they have no competing interests.
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51 414 **Patient consent** All patients gave written informed consent for the study.
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3 416 **Ethics approval** The research protocol was approved by Khon Kaen University Ethics
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5 417 Committee for Human Research, reference number HE591067.

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9 419 **Provenance and peer review** Not commissioned; externally peer reviewed.
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13 421 **Data sharing statement** No additional data are available.
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For peer review only

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3 544 **Captions for the figures:**
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7 546 **Figure 1** Percentage of BDD between male and female according to PDF1, 2, and 3.
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11 548 **Figure 2** Number of BDD in PDF subjects by age range.
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15 550 **Figure 3** The adjusted OR and crude OR of the associated factors of BDD.
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20 **Table 1** Baseline demographic and clinical characteristics of subjects
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Characteristics	Number (n=394 026)	Percentage
Gender		
Female	242 115	61.4
Male	151 866	38.6
Missing data (n=45)		
Age group (years)		
40-44	49 281	12.9
45-49	71 564	18.7
50-54	78 428	20.5
55-59	69 530	18.2
60 years and over	114 305	29.8
Mean±Standard deviation	54.92±9.03	
Median (minimum : maximum)	54 (40 : 100)	
Missing data (n=10 918)		
Education levels		
None	6561	1.7
Primary	286 840	72.9

Table 1 Baseline demographic and clinical characteristics of subjects

Characteristics	Number (n=394 026)	Percentage
Secondary	78 090	19.9
Certificate/Bachelor	18 632	4.7
Higher than bachelor	3055	0.8
Missing data (n=848)		
Occupation		
Unemployed	15 582	4.0
Farmer	306 421	77.9
Labor	32 420	8.2
Own business	13 467	3.4
Government official/State enterprises	13 997	3.6
Others	11 335	2.9
Missing data (n=804)		
Relatives diagnosed with CCA		
No	319 902	81.4
Yes	73 286	18.6
Missing data (n=838)		
Liver fluke infection		
No	113 178	62.1
Yes	68 979	37.9
Missing data (n=211 869)		
Praziquantel treatment		
None	270 183	70.3
One time	84 136	21.9
Two times	18 126	4.7
Three times	5264	1.4

Table 1 Baseline demographic and clinical characteristics of subjects

Characteristics	Number (n=394 026)	Percentage
More than three times	6414	1.7
Missing data (n=9903)		
Smoking history		
No	308 776	78.7
Yes, current or previous	83 754	21.3
Missing data (n=1496)		
Alcohol consumption history		
No	214 495	54.6
Yes, current or previous	178 564	45.4
Missing data (n=967)		
Hepatitis B		
No	382 058	98.2
Yes	6803	1.8
Missing data (n=5165)		
Hepatitis C		
No	388 114	99.8
Yes	747	0.2
Missing data (n=5165)		
Diabetes mellitus		
No	362 296	93.2
Yes	26 565	6.8
Missing data (n=5165)		
Periductal fibrosis		
None	324 482	82.4
PDF1	48 383	12.3

Table 1 Baseline demographic and clinical characteristics of subjects

Characteristics	Number (n=394 026)	Percentage
PDF2	18 686	4.7
PDF3	2475	0.6

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Table 2 Prevalence, and crude and adjusted odd ratios of BDD associated factors and their 95% confidence interval

Factors	Subjects	% BDD	Crude OR	Adjusted OR	95% CI	P value
Over all	394 026	1.2	N/A	N/A	N/A	N/A
Periductal fibrosis						<0.001
None	324 482	1.1	1	1		
PDF1	48 383	1.4	1.23	1.25	1.11 to 1.40	
PDF2	18 686	1.7	1.55	1.24	1.04 to 1.47	
PDF3	2475	6.6	6.35	5.74	4.57 to 7.21	
Gender						<0.001
Female	242 115	0.9	1	1		
Male	151 866	1.7	2.00	1.46	1.31 to 1.63	
Age group (years)						<0.001
40-44	49 281	0.6	1	1		
45-49	71 564	0.6	1.04	1.10	0.88 to 1.38	
50-54	78 428	0.9	1.44	1.42	1.15 to 1.75	
55-59	69 530	1.1	1.77	1.74	1.42 to 2.14	
60 years and over	114 305	2.1	3.46	3.14	2.59 to 3.81	
Education levels						0.472
None	6561	1.6	1	1		
Primary	286 840	1.3	0.82	0.91	0.65 to 1.27	
Secondary	78 090	0.8	0.53	0.72	0.51 to 1.03	
Certificate/Bachelor	18 632	1.1	0.71	0.81	0.53 to 1.24	
Higher than bachelor	3055	1.5	0.98	0.94	0.52 to 1.71	

Table 2 Prevalence, and crude and adjusted odd ratios of BDD associated factors and their 95% confidence interval

Factors	Subjects	% BDD	Crude OR	Adjusted OR	95% CI	P value
Occupations						<0.001
Unemployed	15 582	2.5	1	1		
Farmer	306 421	1.1	0.45	0.47	0.40 to 0.55	
Labor	32 420	1.0	0.39	0.53	0.41 to 0.67	
Own business	13 467	1.0	0.40	0.65	0.48 to 0.87	
Government/State enterprises	13 997	1.5	0.59	0.87	0.63 to 1.20	
Others	11 335	1.4	0.57	0.60	0.44 to 0.80	
Relatives diagnosed with CCA						0.018
No	319 902	1.2	1	1		
Yes	73 286	1.2	0.99	1.12	1.02 to 1.24	
Liver fluke infection						<0.001
No	113 178	1.2	1	1		
Yes	68 979	1.5	1.24	1.25	1.12 to 1.39	
Praziquantel treatment						0.067
None	270 183	1.1	1	1		
One time	84 136	1.3	1.20	0.85	0.75 to 0.95	
Two times	18 126	1.5	1.33	0.93	0.79 to 1.10	
Three times	5264	1.7	1.56	1.10	0.85 to 1.43	
More than three times	6414	1.8	1.63	1.26	1.00 to 1.59	
Smoking history						<0.001
No	308 776	1.0	1	1		
Yes, current or previous	83 754	2.0	2.11	1.31	1.17 to 1.46	
Alcohol consumption history						0.002
No	214 495	1.0	1	1		
Yes, current or previous	178 564	1.4	1.45	1.17	1.06 to 1.29	
Hepatitis B virus						0.298

Table 2 Prevalence, and crude and adjusted odd ratios of BDD associated factors and their 95% confidence interval

Factors	Subjects	% BDD	Crude OR	Adjusted OR	95% CI	P value
No	382 058	1.2	1	1		
Yes	6803	1.4	1.13	1.16	0.88 to 1.52	
Hepatitis C virus						0.124
No	388 114	1.2	1	1		
Yes	747	2.0	1.69	1.69	0.87 to 3.31	
Diabetes mellitus						0.011
No	362 296	1.2	1	1		
Yes	26 565	1.6	1.37	1.20	1.04 to 1.37	

553 N/A, Not applicable.

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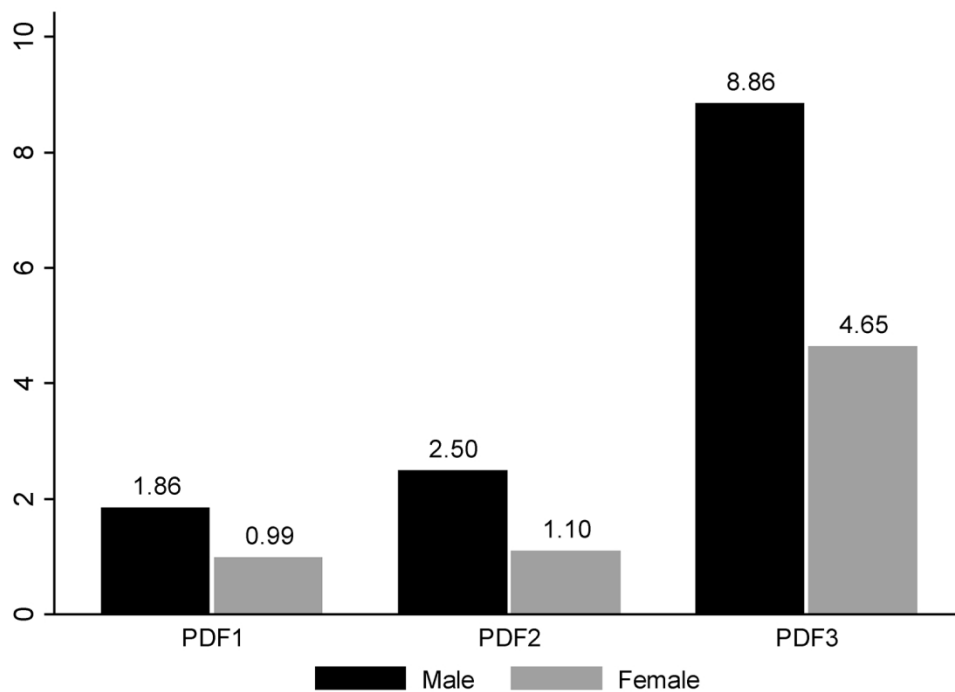


Figure 1 Percentage of BDD between male and female according to PDF1, 2, and 3.

143x104mm (300 x 300 DPI)

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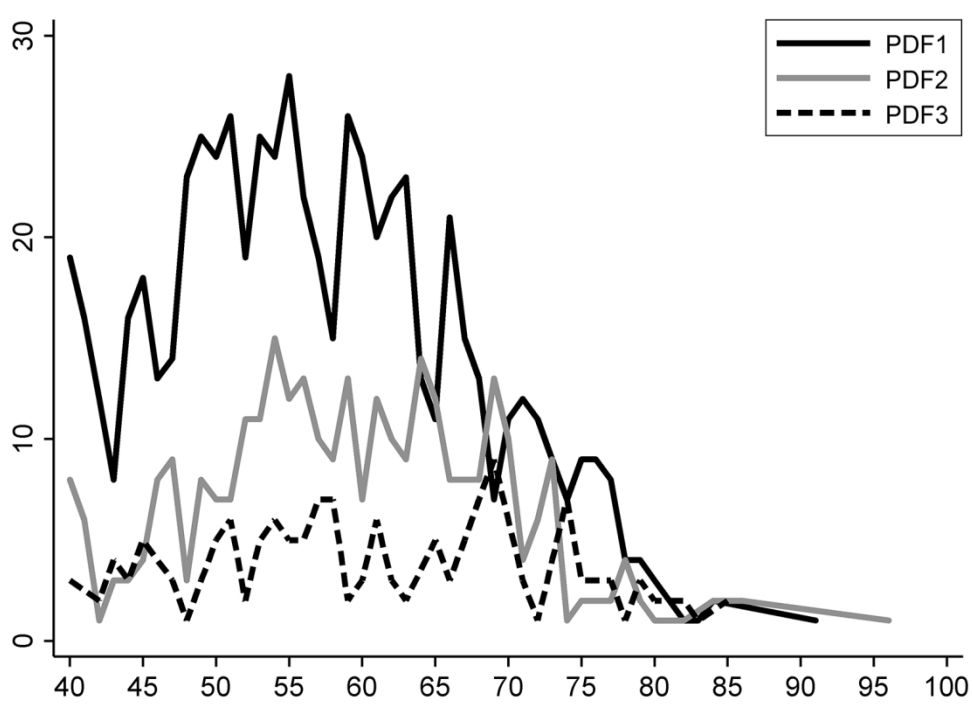


Figure 2 Number of BDD in PDF subjects by age range.

141x103mm (300 x 300 DPI)

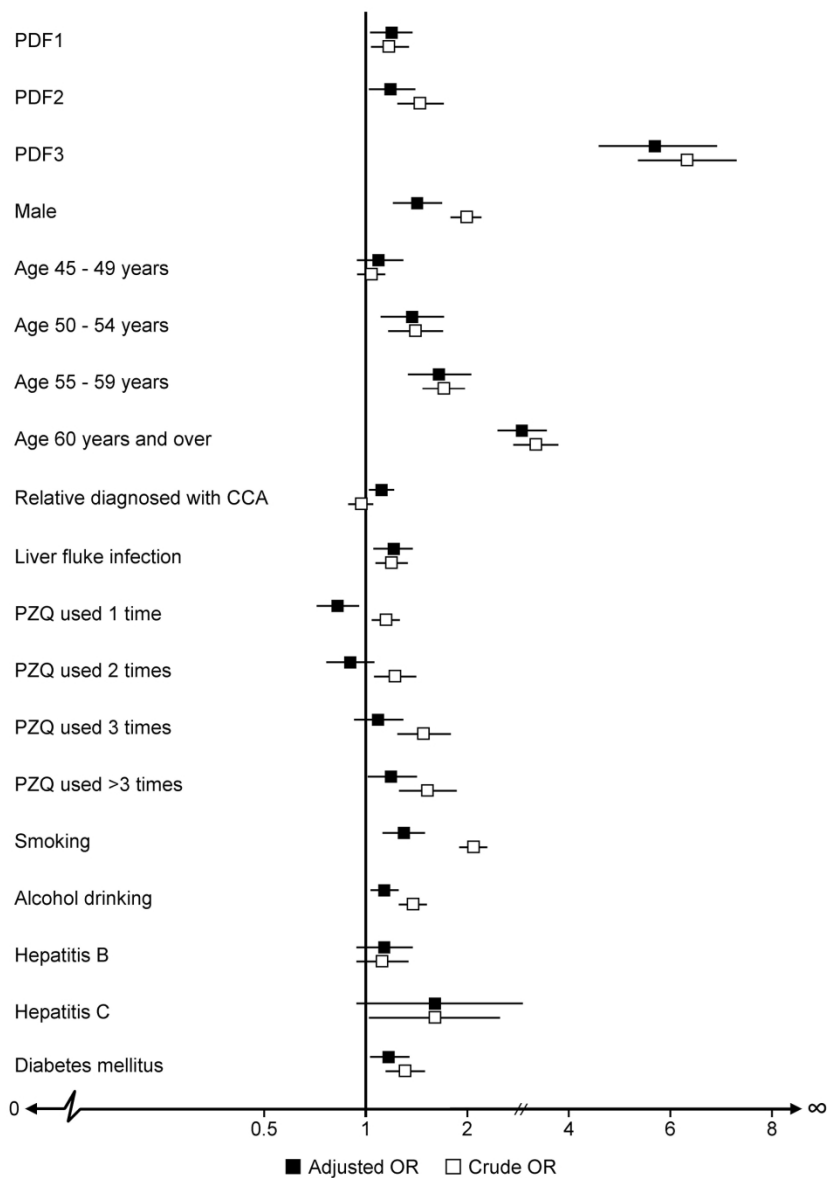


Figure 3 The adjusted OR and crude OR of the associated factors of BDD.

154x215mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Location	Recommendation	
Title and abstract	1	Pg3	(a) Indicate the study's design with a commonly used term in the title or the abstract	
		Pg3	(b) Provide in the abstract an informative and balanced summary of what was done and what was found	
Introduction				
Background/rationale	2	Pgs5-6	Explain the scientific background and rationale for the investigation being reported	
Objectives	3	Pg6	State specific objectives, including any prespecified hypotheses	
Methods				
Study design	4	Pgs 6-7	Present key elements of study design early in the paper	
Setting	5	Pgs 6-7	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	
Participants	6	Pgs6-7	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	
			<i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	
			<i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	
Variables	7	Pg8	(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed	
			<i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	
			Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	
Data sources/ measurement	8*	Pgs7-8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	
Bias	9	Pg4	Describe any efforts to address potential sources of bias	
Study size	10	Pg6-7	Explain how the study size was arrived at	
Quantitative variables	11	Pg8-9	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	Pg8-9	(a) Describe all statistical methods, including those used to control for confounding	
			N/A	(b) Describe any methods used to examine subgroups and interactions
			Pg9	(c) Explain how missing data were addressed
			N/A	(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed
				<i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed
N/A	<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy			
	(e) Describe any sensitivity analyses			

Continued on next page

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Location		Results	
Participants	13*	Pg9	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		N/A	(b) Give reasons for non-participation at each stage
		N/A	(c) Consider use of a flow diagram
Descriptive data	14*	Pg9	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		Pgs22-24	(b) Indicate number of participants with missing data for each variable of interest
		N/A	(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Pgs9-10	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time
		N/A	<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure
		Pg25	<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures
Main results	16	Pgs9-10	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		N/a	(b) Report category boundaries when continuous variables were categorized
		N/a	(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	N/A	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
		Discussion	
Key results	18	Pg11-12	Summarise key results with reference to study objectives
Limitations	19	Pg4	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Pg14	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Pg14	Discuss the generalisability (external validity) of the study results
		Other information	
Funding	22	Pg15	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Association between periductal fibrosis and bile duct dilatation among a population at high-risk of cholangiocarcinoma: a cross-sectional study of cholangiocarcinoma screening in Northeast Thailand

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Primary Subject Heading:	Public health
Secondary Subject Heading:	Global health
Keywords:	bile duct dilatation, periductal fibrosis, ULTRASONOGRAPHY, cholangiocarcinoma, screening, Thailand

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3 **1 TITLE PAGE**
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8 **Title:** Association between periductal fibrosis and bile duct dilatation among a population at
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10 high-risk of cholangiocarcinoma: a cross-sectional study cholangiocarcinoma screening in
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12 Northeast Thailand
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15 **6**

16
17 **Authors:** Nittaya Chamadol,^{1,2,3} Narong Khuntikeo,^{1,2,4} Bandit Thinkhamrop,^{1,2,5,6} Kavin
18
19 Thinkhamrop,^{1,2,6} Apiporn T. Suwannatrai,^{1,2,7} Matthew Kelly,⁸ and Supanee Promthet^{1,5,9}
20

21
22 **9**

23
24 **Affiliations:**

25
26 ¹Cholangiocarcinoma Screening and Care Program (CASCAP), Khon Kaen University, Khon
27
28 Kaen, Thailand.
29

30
31 ²Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand.
32

33
34 ³Department of Radiology, Faculty of Medicine, Khon Kaen University, Khon Kaen,
35
36 Thailand.
37

38
39 ⁴Department of Surgery, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.
40

41
42 ⁵Epidemiology and Biostatistics Section, Faculty of Public Health, Khon Kaen University,
43
44 Khon Kaen, Thailand.
45

46
47 ⁶Data Management and Statistical Analysis Center (DAMASAC), Faculty of Public Health,
48
49 Khon Kaen University, Khon Kaen, Thailand.
50

51
52 ⁷Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen,
53
54 Thailand.
55

56
57 ⁸Department of Global Health, Research School of Population Health, Australian National
58
59 University, Canberra, Australia.
60

1
2
3 25 ⁹ASEAN Cancer Epidemiology and Prevention Research Group, Khon Kaen University,
4
5 26 Khon Kaen, Thailand.
6
7
8 27
9

10 28 **Email address:**

11
12 29 NC: Nittaya Chamadol (nittayachamadol@yahoo.com)

13
14 30 NK: Narong Khuntikeo (nkhuntikeo@gmail.com)

15
16 31 BT: Bandit Thinkhamrop (bandit@kku.ac.th)

17
18 32 KT: Kavin Thinkhamrop (kvinth@gmail.com/ kavith@kku.ac.th)

19
20 33 ATS: Apiporn T. Suwannatrai (apiporn@kku.ac.th)

21
22 34 MK: Matthew Kelly (matthew.kelly@anu.edu.au)

23
24 35 SP: Supanee Promthet (supanee@kku.ac.th)
25
26
27
28
29 36

30
31 37 **Corresponding authors:**

32
33 38 Name: Kavin Thinkhamrop, Dr.P.H.

34
35 39 Address: Data Management and Statistical Analysis Center, Faculty of Public Health,
36
37 Khon Kaen University, Thailand.
38
39

40 41 Telephone: +66-97 317 1976

41
42 42 e-Mail: kvinth@gmail.com/ kavith@kku.ac.th
43
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53 47 **Number of tables:** 2

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55 48 **Number of figures:** 3
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1
2
3 49 **ABSTRACT**

4
5 50 **Objectives** To assess associations between periductal fibrosis (PDF) and bile duct dilatation
6
7 51 (BDD) in ultrasonography (US) screening of population at risk of cholangiocarcinoma (CCA)
8
9 52 due to residence in an endemic area for *Opisthorchis viverrini*. CCA survival rates are low
10
11 53 and early identification of risk factors is essential. BDD is one symptom which can identify
12
13 54 patients at risk of CCA. Detection of PDF by US can also identify at risk patients, at an
14
15 55 earlier stage of CCA development. Identification of association between PDF and BDD will
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17 56 inform screening practices for CCA risk, by increasing the viability of PDF screening for
18
19 57 CCA risk.

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23 58 **Setting** Nine tertiary care hospitals in Northeast Thailand.

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25
26 59 **Design** Cross-sectional study.

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28 60 **Participants** Study subjects in the Cholangiocarcinoma Screening and Care Program
29
30 61 (CASCAP) in Northeast Thailand. CASCAP inclusion criteria are all residents of Northeast
31
32 62 Thailand aged 40 years and over. Participants are recruited through CCA screening centers
33
34 63 and through primary health care units. So far 394 026 have been enrolled.

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37 64 **Methods** PDF and BDD were identified through US. PDF was categorized into three groups,
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39 65 PDF1, 2 and 3, depending on their high echo locality in the peripheral, segmental and main
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41 66 bile duct, respectively. Associations between PDF and BDD were determined by adjusted
42
43 67 odds ratio (OR) and 95% confidence interval (CI) using multiple logistic regression.

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46 68 **Results** BDD was found in 6.6% of PDF3, 1.7% of PDF2, and 1.4% of PDF1 cases. Among
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48 69 PDF cases, especially in PDF3, BDD was found in male more than female (8.9% and 4.6%,
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50 70 respectively). Compared to non-PDF, the association between PDF3 and BDD was highly
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52 71 significant (adjusted OR=5.74, 95% CI 4.57 to 7.21, P<0.001).
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3 72 **Conclusions** Our findings reveal that there is a relationship between PDF and BDD, which is
4
5 73 associated with CCA. Therefore, PDF can also be an indicator for suspected-CCA diagnosis
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7 74 through US.
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11
12 76 **Keywords** bile duct dilatation; periductal fibrosis; ultrasonography; cholangiocarcinoma;
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14 77 screening; Thailand
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19 79 **Article summary**

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21 80 **Strengths and limitations of the study**

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24 81 • The large size of the study population and its geographic distribution across Northeast
25
26 82 Thailand are a significant strength.
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28 83 • This is the first and largest screening program for cholangiocarcinoma (CCA) in an area
29
30 84 with the highest incidence in the world.
31
32 85 • CCA risk factors (PDF and BDD) were measured using ultrasonography by skilled
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34 86 radiologists.
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36 87 • Demographic, and some health, data were self-reported leading to potential bias in
37
38 88 measurement of liver fluke infection, praziquantel treatment, and pre-existing medical
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40 89 conditions including hepatitis B (HB), hepatitis C (HC), and diabetes mellitus (DM).
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42 90 • Self-report could lead to prevalence underestimates due to the fact that subjects may
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44 91 not have been willing to disclose sensitive or personal information.
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94 INTRODUCTION

95 Cholangiocarcinoma (CCA) and hepatocellular carcinoma (HCC), are ranked the most
96 prevalent cancers in Southeast Asia.¹⁻³ The early-stages of CCA can manifest through
97 obstructive jaundice, which is found in 30% of patients who are diagnosed with primary
98 sclerosing cholangitis.⁴ Other liver disorders: fatty liver disease, cirrhosis, and liver mass are
99 likewise recognized risk factors for both CCA and HCC.⁵⁻¹⁰ Suspected CCA cases can also be
100 identified through the presence of bile duct dilatation (BDD), which can be identified in
101 suspected CCA cases through ultrasonography (US) screening.^{11 12} A previous study
102 demonstrated that US screening is highly sensitive in identifying CCA through confirmed
103 incidences of BDD.¹³ However, upon the detection and diagnosis of bile duct and liver
104 disorders, it is often too late to save patients with CCA and HCC due to the rapid progression
105 to advanced stages of hepatic carcinoma.¹⁴ As well, detection of BDD by US requires the
106 services of specialist radiologists, who are generally only available in major hospitals,
107 limiting access to screening. Thus, the best way to save a patient's life and prevent the
108 likelihood of cancer development is through early, easily accessible, screenings to detect the
109 risk factors that may lead to cancer among high-risk populations.

110 As well as BDD there are several other indicators for CCA risk including well-accepted
111 premalignant lesions such as biliary intraepithelial neoplasm (BillIN), and intraductal
112 papillary neoplasm of the bile duct (IPNB).^{15 16} Periductal fibrosis (PDF) is another
113 abnormality of the bile duct which has been used to identify people at risk of developing
114 CCA. This hepatobiliary abnormality is particularly prominent among people infected with
115 the liver fluke, *Opisthorchis viverrini*.¹⁷⁻²¹ This infection is caused by the consumption of raw
116 or lightly fermented fish products and is one of the key risk factors for development of CCA
117 in the region. PDF is caused by the thickening of the bile duct wall, along the periportal
118 space.²²

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3 119 The relationship between PDF and CCA is indicated by the regular detection of PDF in
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5 120 confirmed CCA cases, and this has been particularly common in Northeast Thailand where
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8 121 *O. viverrini* is endemic and a leading potential cause of CCA.⁸ As a result of this relationship,
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10 122 US detection has been utilized to identify people with PDF as a risk group for CCA
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12 123 development.^{8 20 23 24} Hepatobiliary abnormalities identified through ultrasound have been
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14 124 shown in other studies to correlate well with histopathological confirmation making US a
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16 125 valuable tool in early identification of these health issues.⁸ Importantly, PDF can be identified
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18 126 through US, but does not require the services of a specialist radiologist increasing the
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20 127 potential access to screening, and PDF can be detected earlier than BDD allowing more
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22 128 effective intervention.

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26 129 The potential to detect the risk of CCA earlier and without the need for specialist
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28 130 radiologists, through the identification of PDF may be an important breakthrough in reducing
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30 131 CCA incidence. So, both PDF and BDD have been recognized as indicators of CCA^{8 17}, but
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32 132 their relationship to one another has yet to be established or even studied in depth.
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34 133 Determining their relationship, such as learning if one precedes the other may make a
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36 134 significant change in how we screen for CCA via US. Therefore, this study seeks to
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38 135 determine if there is an association between PDF and BDD among people at a high-risk CCA
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40 136 population in Northeast Thailand. The results of this work will clarify necessary directions
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42 137 toward early screening methodologies and appropriate cancer treatment.
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49 139 **METHODS**

51 140 **Study design**

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53 141 This study presents data collected from the Cholangiocarcinoma Screening and Care Program
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55 142 (CASCAP) in Northeast Thailand. CASCAP is a prospective cohort study that is considered
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57 143 the first project for CCA screening in a high-risk population with a community-based bottom-
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3 144 up approach.²⁵ Although this overall project is a prospective cohort study, the results
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5 145 presented here use cross sectional data from the baseline study carried out with participants.
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10 147 The overall aim of the study is to recruit all adults aged 40 years or over who reside in
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12 148 Northeast Thailand and to screen them for cholangiocarcinoma and its risk factors in terms of
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14 149 hepatobiliary abnormalities and infection with the liver fluke *Opisthorchis viverrini*. As such
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16
17 150 there are no strict inclusion or exclusion criteria apart from age group and place of residence.
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19 151 Once consent has been obtained, the participants will be enrolled in the program. The
20
21 152 primary place of recruitment for this cohort study were 9 tertiary care hospitals in the
22
23 153 Northeast of Thailand. These hospitals serve as the main source of affordable tertiary care for
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25
26 154 local people in the region. Subjects were recruited at these hospitals in two ways. Firstly the
27
28 155 screening group comprised individuals who had attended the hospital for other reasons and
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31 156 were invited to receive ultrasound screening without evidencing any symptoms. The second
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33 157 group, the walk-in group, were individuals who were attending the hospital because of CCA
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35 158 symptoms and this group can then receive treatment. All participants were asked to join the
36
37 159 project by signing a consent form. All CCA patients were diagnosed and treated according to
38
39 160 routine, real world clinical practice by participating hospitals. Patients were followed-up and
40
41 161 provided with either clinical or palliative care depending on the stage of their disease.
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44 162 Treatment outcomes were recorded. Follow-up took place every 3-6 months depending on the
45
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47 163 patient's condition and unless scheduled otherwise.
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165 **Study population**

166 Our study recruited subjects from among people who participated the CASCAP project.
167 These subjects form two groups (screening and walk-in). The screening group was people
168 who have undergone routine US and who showed no symptoms that could be related to CCA.

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3 169 The walk-in group was people who come to the hospital with symptoms indicating CCA
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6 170 which has been diagnosed with US. The subjects included in our study only those enrolled in
7
8 171 the CASCAP database from 2013-2017 with a total of 394 026 subjects.
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14 173 **Patient and Public Involvement**

16 174 The CASCAP project is a comprehensive screening and treatment program for CCA.
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18 175 Members of the public were first involved in the research in two ways. Firstly when members
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20 176 of the public attended a participating hospital for any reason, hospital staff would actively
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22 177 recruit them to the study. Village health volunteers also recruited participants while carrying
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24 178 out their work. A second group were those who already has some suspected symptoms and
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26 179 attended a hospital for screening at which point they were recruited into the study. The study
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28 180 participants were not directly involved in the design of the study. Participants will be
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30 181 contacted at least annually to be screened for CCA risk. Patients identified as having CCA
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32 182 will receive standard care for the condition through the project. For the screening procedures
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34 183 covered by this report participants are informed of the purpose, outcomes and implications of
35
36 184 these procedures.
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44 186 **Main outcome and independent variables**

46 187 The primary outcome for this study was BDD which was categorized into two groups
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48 188 (no/yes). The independent variable of interest was PDF. We classify PDF into 3 categories
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50 189 (PDF1, 2 and 3) using a World Health Organization standard methodology originally
51
52 190 developed for use in the assessment of schistosomal periportal fibrosis (PPF) but which is
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54 191 also valid for the study of PDF given that PPF and PDF have the same ultrasound images of
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56 192 Increased Periportal Echo.²⁶ We only use 3 of the 5 classifications utilized in this
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58 193 methodology since anatomically extra and intra hepatic bile ducts run in parallel to the portal
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3 194 vein in the periportal space, so the pathology of the bile duct should be detected first in the
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5 195 periportal space. This identification system has been validated by comparing US diagnoses
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7 196 with histopathologically proven cases of PDF with good agreement between the methods.⁸
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10 197 Using this system PDF is categorized based on the anatomical location of the intrahepatic and
11
12 198 extrahepatic bile duct. PDF1 is defined as having a high echo in the wall of small bile ducts
13
14 199 scattered in the liver as a starry sky pattern, PDF2 is a high echo along the segmental bile
15
16 200 duct wall running parallel with the portal vein, and PDF3 is a high echo along the main bile
17
18 201 duct wall running parallel with the portal vein in the periportal space.¹⁹
19
20
21 202 Both BDD and PDF diagnosed via US by radiologists from the CASCAP project all of whom
22
23 203 took part in a special training course for ultrasound examination including all criteria to
24
25 204 diagnose hepatobiliary abnormalities. A teleconsultation system was also set up to confirm
26
27 205 diagnoses from radiologists. Demographic characteristics of PDF and non-PDF subjects were
28
29 206 the independent variables includes gender, age, education levels, occupations, having a
30
31 207 relative diagnosed with CCA, liver fluke infection, praziquantel (PZQ) treatments, smoking
32
33 208 (current or previous), alcohol consumption (current or previous) and diagnosis with hepatitis
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35 209 B (HB), hepatitis C (HC), and diabetes mellitus (DM). All demographic characteristics listed
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37 210 above were collected via face-to-face interview by interviewer from the CASCAP using
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39 211 questionnaire.
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213 **Statistical analysis**

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49 214 The demographic characteristics that were categorical data were summarized using
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51 215 frequencies and percentages (i.e. gender, age groups, education levels, occupations, having a
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53 216 relative diagnosed with CCA, liver fluke infection, PZQ treatments, smoking history, alcohol
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55 217 consumption history and diagnosis with HB, HC, DM, and PDFs). The continuous data, such
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3 218 as the age of the subjects, were summarized by their mean, standard deviation, median,
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5 219 minimum and maximum range.

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7 220 The prevalence of BDD was calculated and the percentage of the prevalence was
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9 221 computed based on a normal approximation to a binomial distribution. Bivariate analysis
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11 222 using simple logistic regression was performed to investigate the association between the
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13 223 independent factors listed above and BDD. They were determined by crude odds ratio (OR)
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15 224 and their 95% confidence intervals (CI). Then multivariable analysis using multiple logistic
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17 225 regression was carried out to investigate the association between PDF and BDD as
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19 226 determined by the adjusted OR and 95% CI. The final multivariate model was adjusted for all
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21 227 factors which previous studies have reported to be associated with the hepatobiliary disease:
22
23 228 PDF, gender, age, education levels, occupations, having a relative diagnosed with CCA, liver
24
25 229 fluke infection, PZQ treatments, smoking, alcohol consumption as well as diagnosis with HB,
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27 230 HC, and DM.

28
29 231 There were missing values for some variables due to unwillingness of some participants
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31 232 to answer some socio-demographic or health history questions or from errors in data
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33 233 collection. Missing values for most variables were rare with proportions missing less than 3%
34
35 234 of participants. The only variable with a significant proportion of missing values was that of
36
37 235 previous liver fluke diagnosis (n=211 869), but this number includes those who had reported
38
39 236 never having been tested for infection.

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41 237 All test statistics were two-tailed and a p-value of less than 0.05 was considered
42
43 238 statistically significant. All analyses were performed by using a statistical package, Stata
44
45 239 version 15 (StataCorp, College Station, Texas, USA).

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48 241 **RESULTS**

49 242 **Descriptive summary**

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3 243 The demographic characteristics of subjects were presented as numbers and percentages. A
4
5 244 total of 394 026 subjects who underwent US screenings for CCA were enrolled in our study.
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7
8 245 The subjects were all between the ages of 40-100 years old and reported a mean age of
9
10 246 54.92±9.03 years old. Of these, approximately two-thirds were female (61.4%) and the
11
12 247 majority of them completed primary school education level (72.9%) and worked as farmers
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14 248 (77.9%). About one-third (29.7%) had ever used PZQ treatment, and about one-fourth
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16 249 (21.3%) reported being a smoker or ex-smoker. The data of PDF diagnosis, 17.6% have
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18 250 positive diagnosed and the highest percentage was in subjects diagnosed with PDF1 (12.3%)
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20 251 while only 0.6% for PDF3 (table 1).
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26 253 <Table 1 located here>
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30 255 **Prevalence of BDD**

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32 256 From this study, the overall prevalence of BDD was reported to be 1.2%. The highest
33
34 257 prevalence of BDD was 6.6% from the PDF3 group under periductal fibrosis. PDF1 and
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36 258 PDF2 subjects reported a low prevalence rate of only 1.4% and 1.7%, respectively (table 2).
37
38 259 Our study found that the prevalence of BDD occurring in PDF subjects was high in male
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40 260 more than female, particularly in PDF3 (8.9% and 4.6%, respectively) (figure 1). Meanwhile,
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42 261 we also found the number of BDD in PDF1 subjects was highest among people aged 55 years
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44 262 old (figure 2).
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50 263 51 264 **Associations with BDD**

52 265 **Bivariate analysis**

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54 266 The crude analysis using simple logistic regression found the variable with the strongest
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56 267 association to BDD to be PDF3 compared to non-PDF (OR=6.35, 95% CI 5.40 to 7.46,
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3 268 P<0.001). Other factors that were significantly associated with BDD included: gender, with
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5 269 male being more affected by BDD than female; age, with a progressively increasing OR;
6
7 270 lower education levels; occupations that was unemployed; infected liver fluke; PZQ used,
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9
10 271 with a progressively increasing OR; having a history of smoking and alcohol consumption;
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12 272 being positive for DM diagnosis (table 2).
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15 273

17 274 **Multivariable analysis**

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19 275 Through the multivariable analysis using multiple logistic regression, all factors were
20
21 276 adjusted and the association of PDF3 subjects having BDD remained significantly high
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23 277 compared with non-PDF subjects (adjusted OR=5.74, 95% CI 4.57 to 7.21, P<0.001) (table
24
25 278 2). Compared to crude OR, the adjusted OR of gender, age, occupations, liver fluke infection,
26
27 279 smoking history and alcohol consumption history, and a positive diagnosis of DM remained
28
29 280 statistically significant, while a positive diagnosis of HB and HC remained non-significant
30
31 281 (figure 3). Our study also found that relatives diagnosed with CCA changed from non-
32
33 282 significant in bivariate analysis to significant in multivariable analysis, while education levels
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35 283 and PZQ treatment changed from significant to non-significant.
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42 285 <Table 2 located here>

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47 287 <Figure 1 located here>

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51 289 <Figure 2 located here>

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56 291 <Figure 3 located here>

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293 DISCUSSION

294 Liver cancer is one of the leading causes of death throughout the world.²⁷ CCA accounts for
295 more than 60% of these liver cancer cases with Northeast Thailand reporting the highest
296 incidence in the world.^{28 29} PDF and BDD have been recognized as the key risk factors of
297 CCA development.^{8 17 21} Due to ambiguities in the relationship between PDF and BDD, our
298 study investigated the prevalence of PDF and BDD in a high-risk CCA population to find if
299 there was a presence of a statistically significant relationship between the two factors. Our
300 study specifically found that the prevalence of BDD was significantly higher (6.6%) among
301 subjects who were diagnosed with PDF3 and it was the most statistically significant
302 associated factor of BDD (adjusted OR=5.74, 95% CI 4.57 to 7.21, P<0.001). Although a
303 study conducted in Japan, concluded fibrosis and BDD as being indicators of CCA, they did
304 not mention an association between them.¹⁷ In addition, studies conducted in Thailand report
305 only PDF as a major risk factor of CCA development.^{8 21 30}

306 We conducted a bivariate analysis via a simple logistic regression and found that gender,
307 age, and smoking history were the three most significant factors associated with BDD and
308 remained significant in the multivariable analysis. The factor of relatives diagnosed with
309 CCA became significant in multivariable analysis, but the magnitude of association was still
310 relatively low, while education levels and PZQ treatment became non-significant. The other
311 factors that were statistically significant in the bivariate analysis became less significant after
312 adjusting for all factors in the multivariable analysis included occupations, alcohol
313 consumption history, and being diagnosed with DM. Consistent with other studies,¹⁷⁻²¹ our
314 results also found a significant association between current liver fluke infection and BDD.
315 Liver fluke infection in Northeast Thailand mainly results from the consumption of raw or
316 insufficiently fermented fish and is one of the main established risk factors for BDD and
317 CCA development.

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3 318 Our study found that those aged 60-years-old and over are more likely to have BDD than
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5 319 other age groups. Meanwhile, our study also found the association of BDD increased with
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7 320 increasing age. We conclude that age plays a role in BDD development. This result is similar
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10 321 to a study conducted in Israel between 2001-2002 which found that bile duct size increases
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12 322 with age and reported age was positively correlated with bile duct size.³¹ A study from
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14 323 Canada in 2014 found that older age was associated with bile duct diameters which increases
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16 324 with age.³² Therefore, it is not a surprise that those who were in the oldest age group in our
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18 325 study had a strong association with BDD, which causes the bile duct to grow.

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20
21 326 Subjects positive for HB and HC diagnosis demonstrated a non-significant association
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23 327 with BDD (adjusted OR=1.16, 95% CI 0.88 to 1.52, P=0.298 and adjusted OR=1.69, 95% CI
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25 328 0.87 to 3.31, P=0.124, respectively). Our findings are close to results reported by Barusrux
26
27 329 and colleagues in 2012 which found that HB and HC were not related to CCA.³³ However, it
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29 330 is also important to mention contradictory results reported in South Korea which found that
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31 331 HBV infection was a significant risk factor for intrahepatic cholangiocarcinoma (ICC)
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33 332 development with OR=2.3, 95% CI 1.6 to 3.3 P<0.05.³⁴ HBV infection was also related to a
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35 333 3.4-fold risk of ICC in China.³⁵ Another study conducted in Northeast Thailand in 2010,
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37 334 examined the association of HB and HC with CCA and reported a greater risk of CCA for
38
39 335 those carrying the virus (OR=4, 95% CI 1.29 to 16.44, P<0.05).³⁶

40
41 336 And interestingly, those who had CCA diagnosed relatives, had a higher association to
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43 337 BDD than those who did not have CCA diagnosed relatives only 12% (adjusted OR=1.12,
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45 338 95% CI 1.02 to 1.24, P=0.018). However, our results were consistent with Zhou et al. (2014),
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47 339 who identified genetic and familial risk factors as significantly contributing to the
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49 340 development of combined HCC-CCA through a bivariate analysis.³⁷ It is worth mentioning
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51 341 that this significance could not be confirmed through a multivariable analysis. Other studies
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53 342 also demonstrate that having a family history of cancer is a significant associated factor for
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3 343 CCA development.^{38 39} A risk factor study of CCA in Northeast Thailand also reported
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5 344 patients who had a family history of cancer were more likely to develop CCA than those
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7 345 without a family history of liver cancer.⁴⁰ Death or traumatic incidences influence the
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10 346 decision-making process. This may be the reason behind the lack of association between
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12 347 family history of CCA and BDD in our statistical analysis. Perhaps family members who
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14 348 experience a death of CCA-diagnosed family member are more likely to take measures in
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16 349 preventing the occurrence of a second CCA incidence in the family. A CCA traumatic
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18 350 experience may have served as a warning for family members to avoid this rapid and fatal
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20 351 outcome. These results reveal the complicated nature of understanding the true risk factors of
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22 352 CCA and pathogenesis to hepatic carcinoma in certain Asian societies.
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25
26 353 This study has some limitations. Firstly, although large, the study population is not
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28 354 representative of the overall population of Northeast Thailand. The recruitment method,
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30 355 through tertiary hospitals, may mean that the study population has some underlying
31
32 356 differences in health status from the general population. In particular the prevalence of BDD
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34 357 and PDF in the study group is likely to vary from overall population prevalence. However,
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36 358 the study has internal validity meaning relationships found between the various hepatobiliary
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38 359 abnormalities and other predictive factors are still important and useful. Also, many of the
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40 360 risk factors including history of previous liver fluke infection (and PZQ treatment) as well as
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42 361 health behaviors in terms of smoking and alcohol consumption were self-reported leading to
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44 362 some potential bias in their measurements.
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49 363 PDF and BDD can be detected by ultrasound screening before any clinical symptom of
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51 364 CCA are evident. Additional further characterization by other advanced imaging and
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53 365 endoscopic examinations is standard for differential diagnosis of CCA from other diseases.
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55 366 Histopathological confirmation is mandatory in the patient with a surgical indication.
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3 367 Longitudinal data collection is necessary for further study of the relationship between PDF
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5 368 and BDD and CCA.
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10 370 **CONCLUSIONS**

11
12 371 In conclusion, our key findings included identifying the factors associated with biliary tract
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14 372 disease in a high-risk population for CCA: PDF3, male gender, older age, having CCA-
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16 373 diagnosed relatives, infected liver fluke, and smoking history. Based on our results, patients
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18 374 should be considered suspected-CCA cases during US screenings in high-risk areas through
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20 375 the detection of PDF, old age (50 and over), if they were infected for liver fluke, have CCA-
21
22 376 diagnosed relatives, and are current or previous smokers. The interesting results regarding
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24 377 HB and HC diagnoses may need further evaluation and review due to some contradictions in
25
26 378 the data. Greater consideration toward CCA and HCC prevention should be aimed at those in
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28 379 older age groups. Despite certain limitations, our data was based on a very large sample size
29
30 380 and suggests a statistically robust association between PDF and BDD, specifically the PDF3
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32 381 grouping. Early and routine screening of BDD and PDF may provide a means to reduce the
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34 382 incidence of liver-related diseases and CCA. Future planning of CCA surveillance should
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36 383 focus on early screening for both PDF and BDD.
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385 **Recommendations**

386 This study was conducted in Northeast Thailand and may not reflect the general population.
387 Further study is necessary in the region to test the generality of our results. Nevertheless, the
388 methodology and results of our study can be used as a guideline in formulating clinical
389 practice and future research priorities.

391 **List of abbreviations**

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3 392 BDD, Bile duct dilatation; CASCAP, Cholangiocarcinoma Screening and Care Program;
4
5 393 CCA, Cholangiocarcinoma; CI, Confidence interval; DM, Diabetes mellitus, HB, Hepatitis
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7 394 B; HC, Hepatitis C; HCC, Hepatocellular carcinoma; ICC, Intrahepatic cholangiocarcinoma;
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9 395 N/A, Not applicable; OR, Odds ratios; PDF, Periductal fibrosis; PZQ, Praziquantel; US,
10
11 396 Ultrasonography; WHO, World Health Organization.
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17 398 **Conflict of interest**

18
19 399 All authors declare no conflict of interest.
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23
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33
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39
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41
42 409 performed the analysis. NC, SP, NK, BT, KT, ATS and MK wrote the manuscript. NC, NK,
43
44 410 BT and KT collected the data and generated the clinical database. All authors have been
45
46 411 involved in revising the manuscript, and all authors have read and approved the final
47
48 412 manuscript.
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53
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4
5 417 Research Council of Thailand (NRCT/2559-134).
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10 419 **Competing interests** The authors declare that they have no competing interests.
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14
15 421 **Patient consent** All patients gave written informed consent for the study.
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18 422

19
20 423 **Ethics approval** The research protocol was approved by Khon Kaen University Ethics
21
22

23 424 Committee for Human Research, reference number HE591067.
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26 425

27 426 **Provenance and peer review** Not commissioned; externally peer reviewed.
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31 428 **Data sharing statement** No additional data are available.
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3 551 **Captions for the figures:**
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8 553 **Figure 1** Percentage of BDD between male and female according to PDF1, 2, and 3.
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12 555 **Figure 2** Number of BDD in PDF subjects by age range.
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17 557 **Figure 3** The adjusted OR and crude OR of the associated factors of BDD.
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21
22 **Table 1** Baseline demographic and clinical characteristics of subjects
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Characteristics	Number (n=394 026)	Percentage
Gender		
Female	242 115	61.4
Male	151 866	38.6
Missing data (n=45)		
Age group (years)		
40-44	49 281	12.9
45-49	71 564	18.7
50-54	78 428	20.5
55-59	69 530	18.2
60 years and over	114 305	29.8
Mean±Standard deviation	54.92±9.03	
Median (minimum : maximum)	54 (40 : 100)	
Missing data (n=10 918)		
Education levels		
None	6561	1.7
Primary	286 840	72.9

Table 1 Baseline demographic and clinical characteristics of subjects

Characteristics	Number (n=394 026)	Percentage
Secondary	78 090	19.9
Certificate/Bachelor	18 632	4.7
Higher than bachelor	3055	0.8
Missing data (n=848)		
Occupation		
Unemployed	15 582	4.0
Farmer	306 421	77.9
Labor	32 420	8.2
Own business	13 467	3.4
Government official/State enterprises	13 997	3.6
Others	11 335	2.9
Missing data (n=804)		
Relatives diagnosed with CCA		
No	319 902	81.4
Yes	73 286	18.6
Missing data (n=838)		
Liver fluke infection		
No	113 178	62.1
Yes	68 979	37.9
Missing data (n=211 869)		
Praziquantel treatment		
None	270 183	70.3
One time	84 136	21.9
Two times	18 126	4.7
Three times	5264	1.4

Table 1 Baseline demographic and clinical characteristics of subjects

Characteristics	Number (n=394 026)	Percentage
More than three times	6414	1.7
Missing data (n=9903)		
Smoking history		
No	308 776	78.7
Yes, current or previous	83 754	21.3
Missing data (n=1496)		
Alcohol consumption history		
No	214 495	54.6
Yes, current or previous	178 564	45.4
Missing data (n=967)		
Hepatitis B		
No	382 058	98.2
Yes	6803	1.8
Missing data (n=5165)		
Hepatitis C		
No	388 114	99.8
Yes	747	0.2
Missing data (n=5165)		
Diabetes mellitus		
No	362 296	93.2
Yes	26 565	6.8
Missing data (n=5165)		
Periductal fibrosis		
None	324 482	82.4
PDF1	48 383	12.3

Table 1 Baseline demographic and clinical characteristics of subjects

Characteristics	Number (n=394 026)	Percentage
PDF2	18 686	4.7
PDF3	2475	0.6

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Table 2 Prevalence, and crude and adjusted odd ratios of BDD associated factors and their 95% confidence interval

Factors	Subjects	% BDD	Crude OR	Adjusted OR	95% CI	P value
Over all	394 026	1.2	N/A	N/A	N/A	N/A
Periductal fibrosis						<0.001
None	324 482	1.1	1	1		
PDF1	48 383	1.4	1.23	1.25	1.11 to 1.40	
PDF2	18 686	1.7	1.55	1.24	1.04 to 1.47	
PDF3	2475	6.6	6.35	5.74	4.57 to 7.21	
Gender						<0.001
Female	242 115	0.9	1	1		
Male	151 866	1.7	2.00	1.46	1.31 to 1.63	
Age group (years)						<0.001
40-44	49 281	0.6	1	1		
45-49	71 564	0.6	1.04	1.10	0.88 to 1.38	
50-54	78 428	0.9	1.44	1.42	1.15 to 1.75	
55-59	69 530	1.1	1.77	1.74	1.42 to 2.14	
60 years and over	114 305	2.1	3.46	3.14	2.59 to 3.81	
Education levels						0.472
None	6561	1.6	1	1		
Primary	286 840	1.3	0.82	0.91	0.65 to 1.27	
Secondary	78 090	0.8	0.53	0.72	0.51 to 1.03	
Certificate/Bachelor	18 632	1.1	0.71	0.81	0.53 to 1.24	
Higher than bachelor	3055	1.5	0.98	0.94	0.52 to 1.71	

Table 2 Prevalence, and crude and adjusted odd ratios of BDD associated factors and their 95% confidence interval

Factors	Subjects	% BDD	Crude OR	Adjusted OR	95% CI	P value
Occupations						<0.001
Unemployed	15 582	2.5	1	1		
Farmer	306 421	1.1	0.45	0.47	0.40 to 0.55	
Labor	32 420	1.0	0.39	0.53	0.41 to 0.67	
Own business	13 467	1.0	0.40	0.65	0.48 to 0.87	
Government/State enterprises	13 997	1.5	0.59	0.87	0.63 to 1.20	
Others	11 335	1.4	0.57	0.60	0.44 to 0.80	
Relatives diagnosed with CCA						0.018
No	319 902	1.2	1	1		
Yes	73 286	1.2	0.99	1.12	1.02 to 1.24	
Liver fluke infection						<0.001
No	113 178	1.2	1	1		
Yes	68 979	1.5	1.24	1.25	1.12 to 1.39	
Praziquantel treatment						0.067
None	270 183	1.1	1	1		
One time	84 136	1.3	1.20	0.85	0.75 to 0.95	
Two times	18 126	1.5	1.33	0.93	0.79 to 1.10	
Three times	5264	1.7	1.56	1.10	0.85 to 1.43	
More than three times	6414	1.8	1.63	1.26	1.00 to 1.59	
Smoking history						<0.001
No	308 776	1.0	1	1		
Yes, current or previous	83 754	2.0	2.11	1.31	1.17 to 1.46	
Alcohol consumption history						0.002
No	214 495	1.0	1	1		
Yes, current or previous	178 564	1.4	1.45	1.17	1.06 to 1.29	
Hepatitis B virus						0.298

Table 2 Prevalence, and crude and adjusted odd ratios of BDD associated factors and their 95% confidence interval

Factors	Subjects	% BDD	Crude OR	Adjusted OR	95% CI	P value
No	382 058	1.2	1	1		
Yes	6803	1.4	1.13	1.16	0.88 to 1.52	
Hepatitis C virus						0.124
No	388 114	1.2	1	1		
Yes	747	2.0	1.69	1.69	0.87 to 3.31	
Diabetes mellitus						0.011
No	362 296	1.2	1	1		
Yes	26 565	1.6	1.37	1.20	1.04 to 1.37	

560 N/A, Not applicable.

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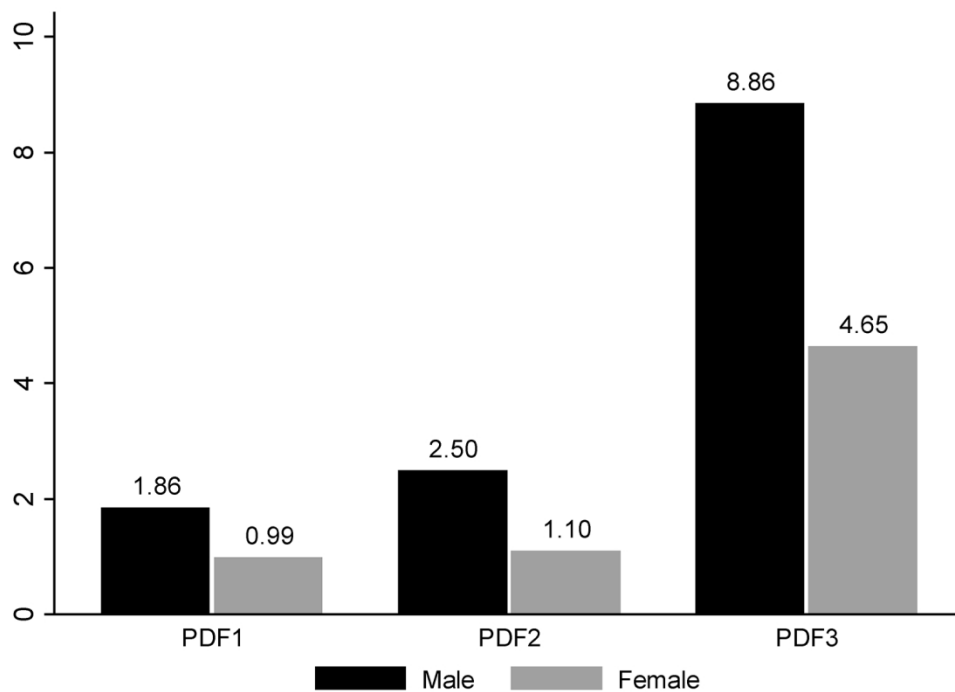


Figure 1 Percentage of BDD between male and female according to PDF1, 2, and 3.

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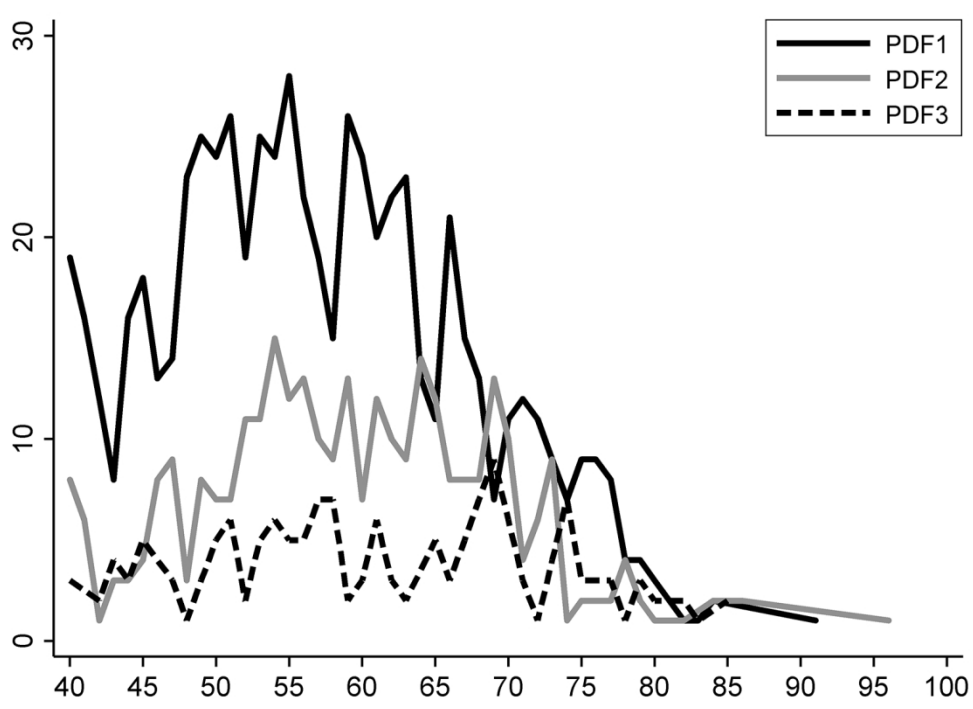


Figure 2 Number of BDD in PDF subjects by age range.

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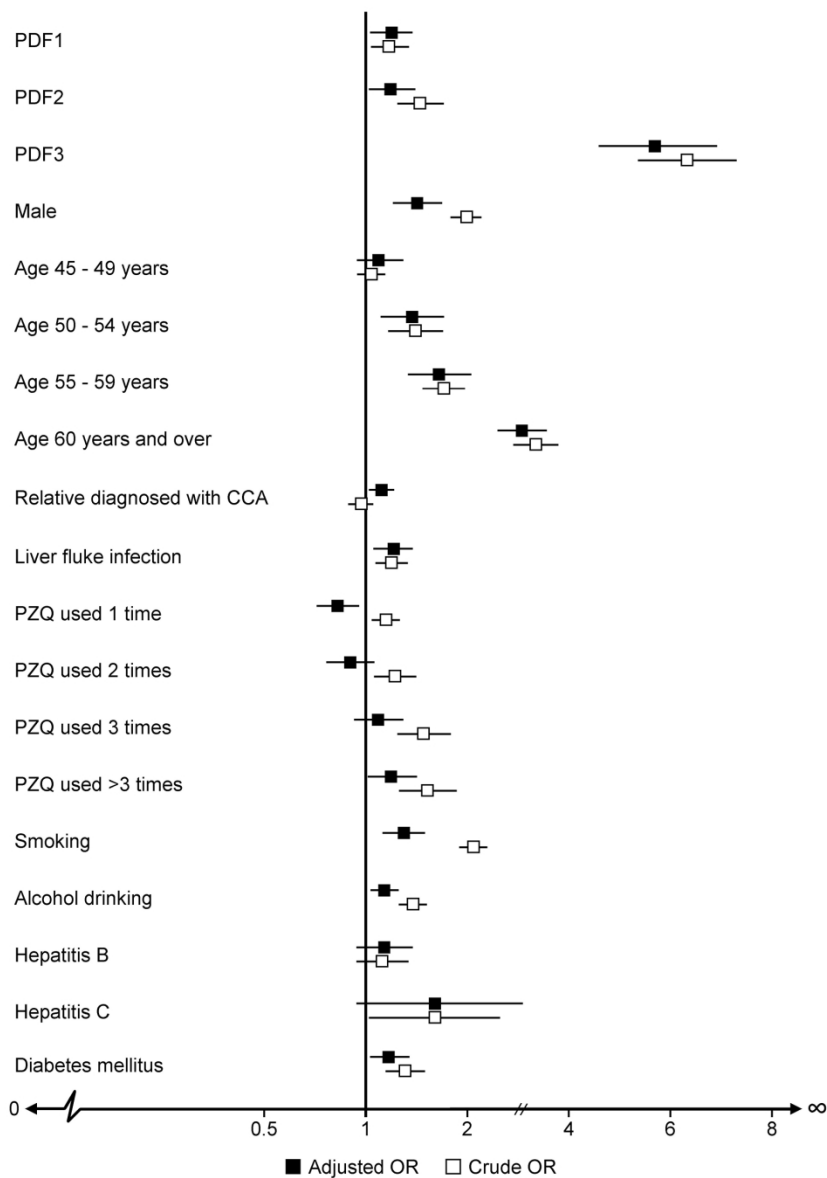


Figure 3 The adjusted OR and crude OR of the associated factors of BDD.

154x215mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Location	Recommendation
Title and abstract	1	Pg3	(a) Indicate the study's design with a commonly used term in the title or the abstract
		Pg3	(b) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction			
Background/rationale	2	Pgs5-6	Explain the scientific background and rationale for the investigation being reported
Objectives	3	Pg6	State specific objectives, including any prespecified hypotheses
Methods			
Study design	4	Pgs 6-7	Present key elements of study design early in the paper
Setting	5	Pgs 6-7	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	Pgs6-7	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
			<i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls
		N/A	(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed
			<i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case
Variables	7	Pg8	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	Pgs7-8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Pg4	Describe any efforts to address potential sources of bias
Study size	10	Pg6-7	Explain how the study size was arrived at
Quantitative variables	11	Pg8-9	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	Pg8-9	(a) Describe all statistical methods, including those used to control for confounding
			N/A
		Pg9	(c) Explain how missing data were addressed
		N/A	(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed
			<i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed
	<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy		
	N/A	(e) Describe any sensitivity analyses	

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Location		Results	
Participants	13*	Pg9	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		N/A	(b) Give reasons for non-participation at each stage
		N/A	(c) Consider use of a flow diagram
Descriptive data	14*	Pg9	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		Pgs22-24	(b) Indicate number of participants with missing data for each variable of interest
		N/A	(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Pgs9-10	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time
		N/A	<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure
		Pg25	<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures
Main results	16	Pgs9-10	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		N/a	(b) Report category boundaries when continuous variables were categorized
		N/a	(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	N/A	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
		Discussion	
Key results	18	Pg11-12	Summarise key results with reference to study objectives
Limitations	19	Pg4	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Pg14	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Pg14	Discuss the generalisability (external validity) of the study results
		Other information	
Funding	22	Pg15	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.