



BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email [info.bmjopen@bmj.com](mailto:info.bmjopen@bmj.com)

# BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-023217
Article Type:	Research
Date Submitted by the Author:	04-Apr-2018
Complete List of Authors:	Chamadol, Nittaya; Khon Kaen University Faculty of Medicine Khuntikeo, Narong; Khon Kaen University Faculty of Medicine Thinkhamrop, Bandit; Faculty of Public Health, Khon Kaen University, Department of Biostatistics and Epidemiology Thinkamrop, Kavin; Faculty of Public Health, Khon Kaen University, Department of Biostatistics and Epidemiology Suwannatarai, Apiporn; Khon Kaen University Faculty of Medicine Kelly, Matthew; The Australian National University, Research School of Population Health Promthet, Supanee; Faculty of Public Health, Khon Kaen University, Department of Epidemiology
Keywords:	bile duct dilatation, periductal fibrosis, ULTRASONOGRAPHY, cholangiocarcinoma, screening, Thailand

SCHOLARONE™  
Manuscripts

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1     **TITLE PAGE**

2  
3     **Title:** Periductal fibrosis and bile duct dilatation: pathways to diagnosis for  
4     cholangiocarcinoma in Northeast Thailand

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

8  
9     **Affiliations:**

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

12    <sup>2</sup>Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand.

13    <sup>3</sup>Department of Radiology, Faculty of Medicine, Khon Kaen University, Khon Kaen,  
14    Thailand.

15    <sup>4</sup>Department of Surgery, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

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

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

20    <sup>7</sup>Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen,  
21    Thailand.

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

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

26

**Email address:**

NC: Nittaya Chamadol (nittayachamadol@yahoo.com)

NK: Narong Khuntikeo (nkhuntikeo@gmail.com)

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

KT: Kavin Thinkhamrop (kvinth@gmail.com)

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

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

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

35

**Corresponding authors:**

Name: Supanee Promthet

Address: ASEAN Cancer Epidemiology and Prevention Research Group,  
Khon Kaen University, Khon Kaen 40002, Thailand.

Telephone: +66-82 668 1995

e-Mail: supanee@kku.ac.th

42

**Type of contribution:** Research article**Number of words in the abstract:** 298**Number of words in the text:** 3,086 (excluding references and tables)**Number of tables:** 2**Number of figures:** 3

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

48     **ABSTRACT**

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

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

58     **Design** Cross-sectional study.

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

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

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

**Conclusions** Our findings reveal that there is a relationship between PDF and BDD, which is associated with CCA. Therefore, PDF can also be an indicator for suspected-CCA diagnosis through US.

**Keywords** bile duct dilatation; periductal fibrosis; ultrasonography; cholangiocarcinoma; screening; Thailand

## Article summary

### Strengths and limitations of the study

- The large size of the study population and its geographic distribution across Northeast Thailand are a significant strength.
- This is the first and largest screening program for cholangiocarcinoma (CCA) in an area with the highest incidence in the world.
- CCA risk factors (PDF and BDD) were measured using ultrasonography by skilled radiologists.
- Demographic, and some health, data were self-reported leading to potential bias in measurement of liver fluke infection, praziquantel treatment, and pre-existing medical conditions including HB, HC, and DM.
- Self-report could lead to prevalence underestimates due to the fact that subjects may not have been willing to disclose sensitive or personal information.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

93     **INTRODUCTION**

94     Cholangiocarcinoma (CCA) and hepatocellular carcinoma (HCC), are ranked the most  
95     prevalent cancers in Southeast Asia.<sup>1-3</sup> 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.<sup>4</sup> Other liver disorders: fatty liver disease, cirrhosis, and liver mass are  
98     likewise recognized risk factors for both CCA and HCC.<sup>5-10</sup> 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.<sup>11 12</sup> A previous study  
101    demonstrated that US screening is highly sensitive in identifying CCA through confirmed  
102    incidences of BDD.<sup>13</sup> 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.<sup>14</sup> 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    pre-malignant lesions such as biliary intraepithelial neoplasm (BilIN), and intraductal  
111    papillary neoplasm of the bile duct (IPNB).<sup>15 16</sup> 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*.<sup>17-21</sup> PDF is caused by the  
114    thickening of the bile duct wall, along the periportal space.<sup>22</sup> PDF can be categorized into  
115    three groups (PDF1, 2, and 3), which were first classified by the World Health Organization  
116    (WHO).<sup>23</sup> 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

segmental bile duct wall running parallel with the portal vein, and PDF3 is a high echo along the main bile duct wall running parallel with the portal vein in the periportal space.<sup>19</sup>

The relationship between PDF and CCA is indicated by the regular detection of PDF in confirmed CCA cases, and this has been particularly common in Northeast Thailand where *O. viverrini* is endemic and a leading potential cause of CCA.<sup>8</sup> As a result of this relationship, US detection has been utilized to identify people with PDF as a risk group for CCA development.<sup>8 20 24 25</sup> Importantly, PDF can be identified through US, but does not require the services of a specialist radiologist increasing the potential access to screening, and PDF can be detected earlier than BDD allowing more effective intervention.

The potential to detect the risk of CCA earlier and without the need for specialist radiologists, through the identification of PDF may be an important breakthrough in reducing CCA incidence. So, both PDF and BDD have been recognized as indicators of CCA<sup>8 17</sup>, but their relationship to one another has yet to be established or even studied in depth. Determining their relationship, such as learning if one precedes the other may make a significant change in how we screen for CCA via US. Therefore, this study seeks to determine if there is an association between PDF and BDD among people at a high-risk CCA population in Northeast Thailand. The results of this work will clarify necessary directions toward early screening methodologies and appropriate cancer treatment.

## METHODS

### Study design

This cross-sectional study collected data from the Cholangiocarcinoma Screening and Care Program (CASCAP) in Northeast Thailand. CASCAP is a prospective cohort study that is considered the first project for CCA screening in a high-risk population with a community-based bottom-up approach.<sup>26</sup> This cohort study was conducted at 9 tertiary care hospitals in



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

143 Northeast of Thailand. These hospitals serve as the main source of affordable tertiary care for  
144 local people in the region. The study aims to recruit all people living in Northeast Thailand  
145 and aged 40 years and over, including patients attending screening for CCA and the general  
146 population attending primary health care units. All participants were asked to join the project  
147 by signing a consent form. All CCA patients were diagnosed and treated according to routine,  
148 real world clinical practice by participating hospitals. Patients were followed-up and provided  
149 with either clinical or palliative care depending on the stage of their disease. Treatment  
150 outcomes were recorded. Follow-up took place every 3-6 months depending on the patient's  
151 condition and unless scheduled otherwise.

152  
153 **Study population**

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

160  
161 **Patient and Public Involvement**

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

## **Main outcome and independent variables**

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

## **Statistical analysis**

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

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

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

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

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

## 209 RESULTS

### 210 Descriptive summary

211 The demographic characteristics of subjects were presented as numbers and percentages. A  
212 total of 394 026 subjects who underwent US screenings for CCA were enrolled in our study.  
213 The subjects were all between the ages of 40-100 years old and reported a mean age of  
214 54.92±9.03 years old. Of these, approximately two-thirds were female (61.4%) and the  
215 majority of them completed primary school education level (72.9%) and worked as farmers  
216 (77.9%). About one-third (29.7%) had ever used PZQ treatment, and about one-fourth  
217 (21.3%) reported being a smoker or ex-smoker. The data of PDF diagnosis, 17.6% have

positive diagnosed and the highest percentage was in subjects diagnosed with PDF1 (12.3%) while only 0.6% for PDF3 (table 1).

<Table 1 located here>

### Prevalence of BDD

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

### Associations with BDD

#### Bivariate analysis

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

#### Multivariable analysis

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Through the multivariable analysis using multiple logistic regression, all factors were adjusted and the association of PDF3 subjects having BDD remained significantly high compared with non-PDF subjects (adjusted OR=5.74, 95% CI 4.57 to 7.21, P<0.001) (table 2). Compared to crude OR, the adjusted OR of gender, age, occupations, liver fluke infection, smoking history and alcohol consumption history, and a positive diagnosis of DM remained statistically significant, while a positive diagnosis of HB and HC remained non-significant (figure 3). Our study also found that relatives diagnosed with CCA changed from non-significant in bivariate analysis to significant in multivariable analysis, while education levels and PZQ treatment changed from significant to non-significant.

<Table 2 located here>

<Figure 1 located here>

<Figure 2 located here>

<Figure 3 located here>

**DISCUSSION**

Liver cancer is one of the leading causes of death throughout the world.<sup>27</sup> CCA accounts for more than 60% of these liver cancer cases with Northeast Thailand reporting the highest incidence in the world.<sup>28 29</sup> PDF and BDD have been recognized as the key risk factors of CCA development.<sup>8 17 21</sup> Due to ambiguities in the relationship between PDF and BDD, our study investigated the prevalence of PDF and BDD in a high-risk CCA population to find if there was a presence of a statistically significant relationship between the two factors. Our

study specifically found that the prevalence of BDD was significantly higher (6.6%) among subjects who were diagnosed with PDF3 and it was the most statistically significant associated factor of BDD (adjusted OR=5.74, 95% CI 4.57 to 7.21,  $P<0.001$ ). Although a study conducted in Japan, concluded fibrosis and BDD as being indicators of CCA, they did not mention an association between them.<sup>17</sup> In addition, studies conducted in Thailand report only PDF as a major risk factor of CCA development.<sup>8 21 30</sup>

We conducted a bivariate analysis via a simple logistic regression and found that gender, age, and smoking history were the three most significant factors associated with BDD and remained significant in the multivariable analysis. The factor of relatives diagnosed with CCA became significant in multivariable analysis, but the magnitude of association was still relatively low, while education levels and PZQ treatment became non-significant. The other factors that were statistically significant in the bivariate analysis became less significant after adjusting for all factors in the multivariable analysis included occupations, alcohol consumption history, and being diagnosed with DM.

Our study found that those aged 60-years-old and over are more likely to have BDD than other age groups. Meanwhile, our study also found the association of BDD increased with increasing age. We conclude that age plays a role in BDD development. This result is similar to a study conducted in Israel between 2001-2002 which found that bile duct size increases with age and reported age was positively correlated with bile duct size.<sup>31</sup> A study from Canada in 2014 found that older age was associated with bile duct diameters which increases with age.<sup>32</sup> Therefore, it is not a surprise that those who were in the oldest age group in our study had a strong association with BDD, which causes the bile duct to grow.

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



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

and colleagues in 2012 which found that HB and HC were not related to CCA.<sup>33</sup> However, it is also important to mention contradictory results reported in South Korea which found that HBV infection was a significant risk factor for intrahepatic cholangiocarcinoma (ICC) development with OR=2.3, 95% CI 1.6 to 3.3 P<0.05.<sup>34</sup> HBV infection was also related to a 3.4-fold risk of ICC in China.<sup>35</sup> Another study conducted in Northeast Thailand in 2010, examined the association of HB and HC with CCA and reported a greater risk of CCA for those carrying the virus (OR=4, 95% CI 1.29 to 16.44, P<0.05).<sup>36</sup>

And interestingly, those who had CCA diagnosed relatives, had a higher association to BDD than those who did not have CCA diagnosed relatives only 12% (adjusted OR=1.12, 95% CI 1.02 to 1.24, P=0.018). However, our results were consistent with Zhou et al. (2014), who identified genetic and familial risk factors as significantly contributing to the development of combined HCC-CCA through a bivariate analysis.<sup>37</sup> It is worth mentioning that this significance could not be confirmed through a multivariable analysis. Other studies also demonstrate that having a family history of cancer is a significant associated factor for CCA development.<sup>38 39</sup> A risk factor study of CCA in Northeast Thailand also reported patients who had a family history of cancer were more likely to develop CCA than those without a family history of liver cancer.<sup>40</sup> Death or traumatic incidences influence the decision-making process. This may be the reason behind the lack of association between family history of CCA and BDD in our statistical analysis. Perhaps family members who experience a death of CCA-diagnosed family member are more likely to take measures in preventing the occurrence of a second CCA incidence in the family. A CCA traumatic experience may have served as a warning for family members to avoid this rapid and fatal outcome. These results reveal the complicated nature of understanding the true risk factors of CCA and pathogenesis to hepatic carcinoma in certain Asian societies.

## CONCLUSIONS

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

## Recommendations

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

## List of abbreviations

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



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

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

**Conflict of interest**

All authors declare no conflict of interest.

**Acknowledgements** This study was supported by Khon Kaen University (KKU) through CASCAP project and the National Research Council of Thailand through the Medical Research Network of the Consortium of Thai Medical Schools. The study was also supported by the Data Management and Statistical Analysis Center (DAMASAC), Faculty of Public Health, KKU, Thailand. We would like to acknowledge and thank Dr.Malcolm Anthony Moore for his comments and suggestions over the final version of the manuscript.

**Author contributions** NC, SP, and KT conceived and designed this study. KT and BT performed the analysis. NC, SP, NK, BT, KT, ATS and MK wrote the manuscript. NC, NK, BT and KT collected the data and generated the clinical database. All authors have been involved in revising the manuscript, and all authors have read and approved the final manuscript.

**Funding** This work was supported by Khon Kaen University through CASCAP) Grant No . CASCAP 1/60(, the National Research Council of Thailand through the Medical Research Network of the Consortium of Thai Medical Schools) Grant No .MRF.59-076 (and National Research Council of Thailand )NRCT/2559-134.(

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

368

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

370

371 **Ethics approval** The research protocol was approved by Khon Kaen University Ethics  
372 Committee for Human Research, reference number HE591067.

373

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

375

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

377

378

379

## 380 REFERENCES

- 381 1. Moore MA, Attasara P, Khuhaprema T, et al. Cancer epidemiology in mainland South-  
382 East Asia - past, present and future. *Asian Pacific journal of cancer prevention : APJCP*  
383 2010;11 Suppl 2:67-80.
- 384 2. Moore MA, Manan AA, Chow KY, et al. Cancer epidemiology and control in peninsular  
385 and island South-East Asia - past, present and future. *Asian Pacific journal of cancer*  
386 *prevention : APJCP* 2010;11 Suppl 2:81-98.
- 387 3. National Cancer Institue. Hospital based cancer registry annual report 2012. Bangkok:  
388 Eastern Printing Public Company Limited PCL.157 2012.
- 389 4. Rosen CB, Nagorney DM, Wiesner RH, et al. Cholangiocarcinoma complicating primary  
390 sclerosing cholangitis. *Annals of surgery* 1991;213(1):21-5.
- 391 5. Songserm N, Promthet S, Sithithaworn P, et al. Risk factors for cholangiocarcinoma in  
392 high-risk area of Thailand: role of lifestyle, diet and methylenetetrahydrofolate reductase

1  
2  
3 393 polymorphisms. *Cancer epidemiology* 2012;36(2):e89-94. doi:  
4  
5 394 10.1016/j.canep.2011.11.007  
6  
7 395 6. Tao LY, He XD, Qu Q, et al. Risk factors for intrahepatic and extrahepatic  
8  
9 396 cholangiocarcinoma: a case-control study in China. *Liver international : official journal*  
10  
11 397 *of the International Association for the Study of the Liver* 2010;30(2):215-21. doi:  
12  
13 398 10.1111/j.1478-3231.2009.02149.x  
14  
15 399 7. Shaib YH, El-Serag HB, Nooka AK, et al. Risk factors for intrahepatic and extrahepatic  
16  
17 400 cholangiocarcinoma: a hospital-based case-control study. *The American journal of*  
18  
19 401 *gastroenterology* 2007;102(5):1016-21. doi: 10.1111/j.1572-0241.2007.01104.x  
20  
21 402 8. Chamadol N, Pairojkul C, Khuntikeo N, et al. Histological confirmation of periductal  
22  
23 403 fibrosis from ultrasound diagnosis in cholangiocarcinoma patients. *Journal of hepato-*  
24  
25 404 *biliary-pancreatic sciences* 2014;21(5):316-22. doi: 10.1002/jhbp.64  
26  
27 405 9. Welzel TM, Graubard BI, El-Serag HB, et al. Risk factors for intrahepatic and  
28  
29 406 extrahepatic cholangiocarcinoma in the United States: a population-based case-control  
30  
31 407 study. *Clinical gastroenterology and hepatology : the official clinical practice journal of*  
32  
33 408 *the American Gastroenterological Association* 2007;5(10):1221-8. doi:  
34  
35 409 10.1016/j.cgh.2007.05.020  
36  
37 410 10. Shaib YH, El-Serag HB, Davila JA, et al. Risk factors of intrahepatic cholangiocarcinoma  
38  
39 411 in the United States: a case-control study. *Gastroenterology* 2005;128(3):620-6.  
40  
41 412 11. Hamilton SR, Aaltonen LA. Pathology and genetics of tumours of the digestive system.  
42  
43 413 France: Lyon : Oxford : IARC Press ; Oxford University Press (distributor). 2000.  
44  
45 414 12. Khan SA, Davidson BR, Goldin RD, et al. Guidelines for the diagnosis and treatment of  
46  
47 415 cholangiocarcinoma: an update. *Gut* 2012;61(12):1657-69. doi: 10.1136/gutjnl-2011-  
48  
49 416 301748  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

13. Saini S. Imaging of the hepatobiliary tract. *The New England journal of medicine* 1997;336(26):1889-94. doi: 10.1056/NEJM199706263362607
14. Blechacz B, Gores GJ. Cholangiocarcinoma: advances in pathogenesis, diagnosis, and treatment. *Hepatology* 2008;48(1):308-21. doi: 10.1002/hep.22310
15. Zen Y, Adsay NV, Bardadin K, et al. Biliary intraepithelial neoplasia: an international interobserver agreement study and proposal for diagnostic criteria. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* 2007;20(6):701-9. doi: 10.1038/modpathol.3800788
16. Nakanuma Y, Sasaki M, Sato Y, et al. Multistep carcinogenesis of perihilar cholangiocarcinoma arising in the intrahepatic large bile ducts. *World journal of hepatology* 2009;1(1):35-42. doi: 10.4254/wjh.v1.i1.35
17. Maetani Y, Itoh K, Watanabe C, et al. MR imaging of intrahepatic cholangiocarcinoma with pathologic correlation. *AJR American journal of roentgenology* 2001;176(6):1499-507. doi: 10.2214/ajr.176.6.1761499
18. National Cancer Institute. Guidelines for screening, diagnosis and treatment of liver cancer and cholangiocarcinoma. Bangkok: National Office of Buddhism 2011:81.
19. Nittaya Chamadol. Imaging in Cholangiocarcinoma. Khon Kaen, Thailand: Department of Radiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand 2014.
20. Xu HX, Chen LD, Liu LN, et al. Contrast-enhanced ultrasound of intrahepatic cholangiocarcinoma: correlation with pathological examination. *The British journal of radiology* 2012;85(1016):1029-37. doi: 10.1259/bjr/21653786
21. Sripa B, Mairiang E, Thinkhamrop B, et al. Advanced periductal fibrosis from infection with the carcinogenic human liver fluke *Opisthorchis viverrini* correlates with elevated levels of interleukin-6. *Hepatology* 2009;50(4):1273-81. doi: 10.1002/hep.23134

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

22. Benedetti NJ, Desser TS, Jeffrey RB. Imaging of hepatic infections. *Ultrasound Q* 2008;24(4):267-78. doi: 10.1097/RUQ.0b013e31818e5981

23. Berhe N, Geitung JT, Medhin G, et al. Large scale evaluation of WHO's ultrasonographic staging system of schistosomal periportal fibrosis in Ethiopia. *Tropical Medicine & International Health* 2006;11(8):1286-94. doi: DOI 10.1111/j.1365-3156.2006.01665.x

24. Loria F, Loria G, Basile S, et al. Contrast-enhanced ultrasound appearances of enhancement patterns of intrahepatic cholangiocarcinoma: correlation with pathological findings. *Updates in surgery* 2014;66(2):135-43. doi: 10.1007/s13304-014-0251-6

25. Elkins DB, Mairiang E, Sithithaworn P, et al. Cross-sectional patterns of hepatobiliary abnormalities and possible precursor conditions of cholangiocarcinoma associated with *Opisthorchis viverrini* infection in humans. *The American journal of tropical medicine and hygiene* 1996;55(3):295-301.

26. Khuntikeo N, Chamadol N, Yongvanit P, et al. Cohort profile: cholangiocarcinoma screening and care program (CASCAP). *BMC cancer* 2015;15:459. doi: 10.1186/s12885-015-1475-7

27. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International journal of cancer Journal international du cancer* 2015;136(5):E359-86. doi: 10.1002/ijc.29210

28. Sripa B, Pairojkul C. Cholangiocarcinoma: lessons from Thailand. *Current opinion in gastroenterology* 2008;24(3):349-56. doi: 10.1097/MOG.0b013e3282fbf9b3

29. Srivatanakul P, Sriplung H, Deerasamee S. Epidemiology of liver cancer: an overview. *Asian Pacific journal of cancer prevention : APJCP* 2004;5(2):118-25.

30. Prakobwong S, Yongvanit P, Hiraku Y, et al. Involvement of MMP-9 in peribiliary fibrosis and cholangiocarcinogenesis via Rac1-dependent DNA damage in a hamster

- model. *International journal of cancer Journal international du cancer* 2010;127(11):2576-87. doi: 10.1002/ijc.25266
31. Bachar GN, Cohen M, Belenky A, et al. Effect of aging on the adult extrahepatic bile duct: a sonographic study. *Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine* 2003;22(9):879-82; quiz 83-5.
32. Landry D, Tang A, Murphy-Lavallee J, et al. Dilatation of the bile duct in patients after cholecystectomy: a retrospective study. *Canadian Association of Radiologists journal = Journal l'Association canadienne des radiologistes* 2014;65(1):29-34. doi: 10.1016/j.carj.2012.09.004
33. Barusrux S, Nanok C, Puthisawas W, et al. Viral hepatitis B, C infection and genotype distribution among cholangiocarcinoma patients in northeast Thailand. *Asian Pacific journal of cancer prevention : APJCP* 2012;13 Suppl:83-7.
34. Lee TY, Lee SS, Jung SW, et al. Hepatitis B virus infection and intrahepatic cholangiocarcinoma in Korea: a case-control study. *The American journal of gastroenterology* 2008;103(7):1716-20. doi: 10.1111/j.1572-0241.2008.01796.x
35. Li M, Li J, Li P, et al. Hepatitis B virus infection increases the risk of cholangiocarcinoma: a meta-analysis and systematic review. *Journal of gastroenterology and hepatology* 2012;27(10):1561-8. doi: 10.1111/j.1440-1746.2012.07207.x
36. Srivatanakul P, Honjo S, Kittiwatanachot P, et al. Hepatitis viruses and risk of cholangiocarcinoma in northeast Thailand. *Asian Pacific journal of cancer prevention : APJCP* 2010;11(4):985-8.
37. Zhou YM, Zhang XF, Wu LP, et al. Risk factors for combined hepatocellular-cholangiocarcinoma: a hospital-based case-control study. *World journal of gastroenterology : WJG* 2014;20(35):12615-20. doi: 10.3748/wjg.v20.i35.12615

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

489 38. Kamsa-Ard S, Luvira V, Pugkhem A, et al. Association between praziquantel treatment  
490 and cholangiocarcinoma: a hospital-based matched case-control study. *BMC cancer*  
491 2015;15:776. doi: 10.1186/s12885-015-1788-6

492 39. Liu ZY, Zhou YM, Shi LH, et al. Risk factors of intrahepatic cholangiocarcinoma in  
493 patients with hepatolithiasis: a case-control study. *Hepatobiliary & pancreatic diseases*  
494 *international : HBPD INT* 2011;10(6):626-31.

495 40. Manwong M, Songserm N, Promthet S, et al. Risk factors for cholangiocarcinoma in the  
496 lower part of Northeast Thailand: a hospital-based case-control study. *Asian Pacific*  
497 *journal of cancer prevention : APJCP* 2013;14(10):5953-6.

498

499



## Captions for the figures:

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

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

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

**Table 1** Baseline demographic and clinical characteristics of subjects

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

508

**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	

N/A, Not applicable.

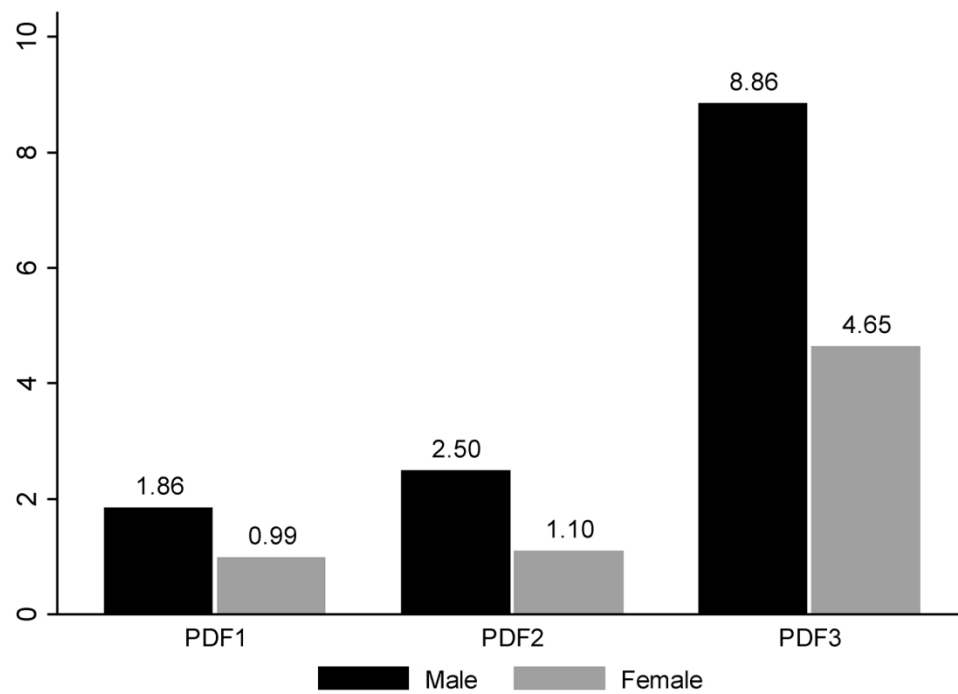


Figure 1 Percentage of BDD between male and female according to PDF1, 2, and 3.

143x104mm (300 x 300 DPI)

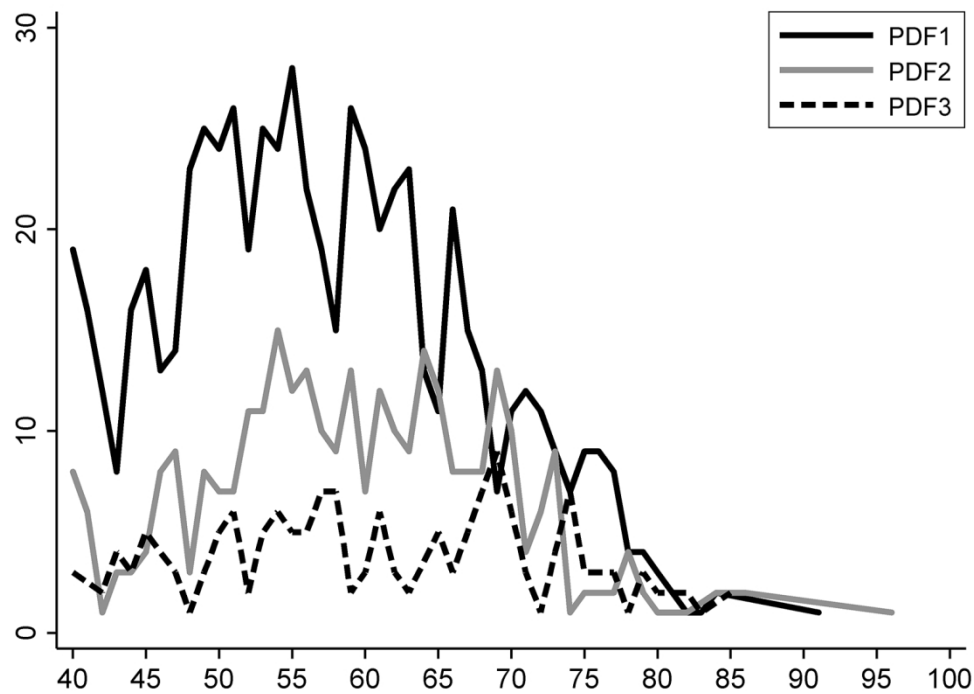


Figure 2 Number of BDD in PDF subjects by age range.

141x103mm (300 x 300 DPI)

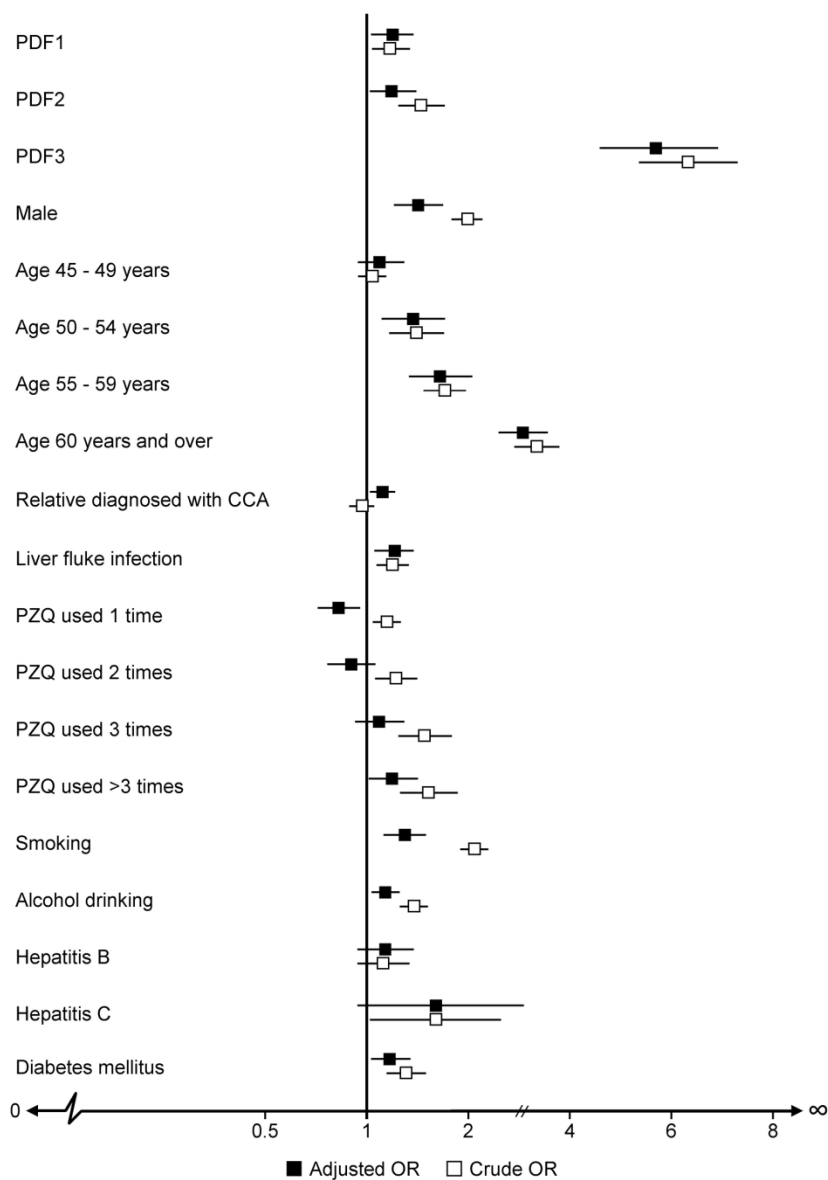


Figure 3 The adjusted OR and crude OR of the associated factors of BDD.

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) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Case-control study—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 Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants
		N/A	(b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed Case-control study—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	(b) Describe any methods used to examine subgroups and interactions
		Pg9	(c) Explain how missing data were addressed
		N/A	(d) Cohort study—If applicable, explain how loss to follow-up was addressed Case-control study—If applicable, explain how matching of cases and controls was addressed Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy
		N/A	(e) Describe any sensitivity analyses

Continued on next page

For peer review only

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](http://www.strobe-statement.org).

# BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-023217.R1
Article Type:	Research
Date Submitted by the Author:	30-Sep-2018
Complete List of Authors:	Chamadol, Nittaya; Khon Kaen University Faculty of Medicine Khuntikeo, Narong; Khon Kaen University Faculty of Medicine Thinkhamrop , Bandit; Faculty of Public Health, Khon Kaen University, Department of Biostatistics and Epidemiology Thinkamrop, Kavin; Faculty of Public Health, Khon Kaen University, Department of Biostatistics and Epidemiology Suwannatrai, Apiporn; Khon Kaen University Faculty of Medicine Kelly, Matthew; The Australian National University, Research School of Population Health Promthet, Supanee; Faculty of Public Health, Khon Kaen University, Department of Epidemiology
<b>Primary Subject Heading</b>:	Public health
Secondary Subject Heading:	Global health
Keywords:	bile duct dilatation, periductal fibrosis, ULTRASONOGRAPHY, cholangiocarcinoma, screening, Thailand

SCHOLARONE™  
Manuscripts

# TITLE PAGE

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

**Authors:** Nittaya Chamadol,<sup>1,2,3</sup> Narong Khuntikeo,<sup>1,2,4</sup> Bandit Thinkhamrop,<sup>1,2,5,6</sup> Kavin Thinkhamrop,<sup>1,2,6</sup> Apiporn T. Suwannatnai,<sup>1,2,7</sup> Matthew Kelly,<sup>8</sup> and Supanee Promthet<sup>1,5,9</sup>

### Affiliations:

<sup>1</sup>Cholangiocarcinoma Screening and Care Program (CASCAP), Khon Kaen University, Khon Kaen, Thailand.

<sup>2</sup>Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand.

<sup>3</sup>Department of Radiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

<sup>4</sup>Department of Surgery, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

<sup>5</sup>Epidemiology and Biostatistics Section, Faculty of Public Health, Khon Kaen University, Khon Kaen, Thailand.

<sup>6</sup>Data Management and Statistical Analysis Center (DAMASAC), Faculty of Public Health, Khon Kaen University, Khon Kaen, Thailand.

<sup>7</sup>Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

<sup>8</sup>Department of Global Health, Research School of Population Health, Australian National University, Canberra, Australia.

<sup>9</sup>ASEAN Cancer Epidemiology and Prevention Research Group, Khon Kaen University, Khon Kaen, Thailand.

26

**Email address:**

NC: Nittaya Chamadol (nittayachamadol@yahoo.com)

NK: Narong Khuntikeo (nkhuntikeo@gmail.com)

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

KT: Kavin Thinkhamrop (kvinth@gmail.com)

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

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

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

35

**Corresponding authors:**

Name: Supanee Promthet

Address: ASEAN Cancer Epidemiology and Prevention Research Group,  
Khon Kaen University, Khon Kaen 40002, Thailand.

Telephone: +66-82 668 1995

e-Mail: supanee@kku.ac.th

42

**Type of contribution:** Research article**Number of words in the abstract:** 298**Number of words in the text:** 3,086 (excluding references and tables)**Number of tables:** 2**Number of figures:** 3

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

48 **ABSTRACT**

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

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

58 **Design** Cross-sectional study.

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

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

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

**Conclusions** Our findings reveal that there is a relationship between PDF and BDD, which is associated with CCA. Therefore, PDF can also be an indicator for suspected-CCA diagnosis through US.

**Keywords** bile duct dilatation; periductal fibrosis; ultrasonography; cholangiocarcinoma; screening; Thailand

## Article summary

### Strengths and limitations of the study

- The large size of the study population and its geographic distribution across Northeast Thailand are a significant strength.
- This is the first and largest screening program for cholangiocarcinoma (CCA) in an area with the highest incidence in the world.
- CCA risk factors (PDF and BDD) were measured using ultrasonography by skilled radiologists.
- Demographic, and some health, data were self-reported leading to potential bias in measurement of liver fluke infection, praziquantel treatment, and pre-existing medical conditions including HB, HC, and DM.
- Self-report could lead to prevalence underestimates due to the fact that subjects may not have been willing to disclose sensitive or personal information.



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

93     **INTRODUCTION**

94     Cholangiocarcinoma (CCA) and hepatocellular carcinoma (HCC), are ranked the most  
95     prevalent cancers in Southeast Asia.<sup>1-3</sup> 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.<sup>4</sup> Other liver disorders: fatty liver disease, cirrhosis, and liver mass are  
98     likewise recognized risk factors for both CCA and HCC.<sup>5-10</sup> 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.<sup>11 12</sup> A previous study  
101    demonstrated that US screening is highly sensitive in identifying CCA through confirmed  
102    incidences of BDD.<sup>13</sup> 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.<sup>14</sup> 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    pre-malignant lesions such as biliary intraepithelial neoplasm (BilIN), and intra-ductal  
111    papillary neoplasm of the bile duct (IPNB).<sup>15 16</sup> 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*.<sup>17-21</sup> 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.<sup>22</sup>

The relationship between PDF and CCA is indicated by the regular detection of PDF in confirmed CCA cases, and this has been particularly common in Northeast Thailand where *O. viverrini* is endemic and a leading potential cause of CCA.<sup>8</sup> As a result of this relationship, US detection has been utilized to identify people with PDF as a risk group for CCA development.<sup>8 20 23 24</sup> Hepatobiliary abnormalities identified through ultrasound have been shown in other studies to correlate well with histopathological confirmation making US a valuable tool in early identification of these health issues.<sup>8</sup> Importantly, PDF can be identified through US, but does not require the services of a specialist radiologist increasing the potential access to screening, and PDF can be detected earlier than BDD allowing more effective intervention.

The potential to detect the risk of CCA earlier and without the need for specialist radiologists, through the identification of PDF may be an important breakthrough in reducing CCA incidence. So, both PDF and BDD have been recognized as indicators of CCA<sup>8 17</sup>, but their relationship to one another has yet to be established or even studied in depth. Determining their relationship, such as learning if one precedes the other may make a significant change in how we screen for CCA via US. Therefore, this study seeks to determine if there is an association between PDF and BDD among people at a high-risk CCA population in Northeast Thailand. The results of this work will clarify necessary directions toward early screening methodologies and appropriate cancer treatment.

## METHODS

### Study design

This study presents data collected from the Cholangiocarcinoma Screening and Care Program (CASCAP) in Northeast Thailand. CASCAP is a prospective cohort study that is considered the first project for CCA screening in a high-risk population with a community-based bottom-

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

up approach.<sup>25</sup> Although this overall project is a prospective cohort study, the results presented here use cross sectional data from the baseline study carried out with participants.

The overall aim of the study is to recruit all adults aged 40 years or over who reside in Northeast Thailand and to screen them for cholangiocarcinoma and its risk factors in terms of hepatobiliary abnormalities and infection with the liver fluke *Opisthorchis viverrini*. As such there are no strict inclusion or exclusion criteria apart from age group and place of residence. Once consent has been obtained, the participants will be enrolled in the program. The primary place of recruitment for this cohort study were 9 tertiary care hospitals in the Northeast of Thailand. These hospitals serve as the main source of affordable tertiary care for local people in the region. Subjects were recruited at these hospitals in two ways. Firstly the screening group comprised individuals who had attended the hospital for other reasons and were invited to receive ultrasound screening without evidencing any symptoms. The second group, the walk-in group, were individuals who were attending the hospital because of CCA symptoms and this group can then receive treatment. All participants were asked to join the project by signing a consent form. All CCA patients were diagnosed and treated according to routine, real world clinical practice by participating hospitals. Patients were followed-up and provided with either clinical or palliative care depending on the stage of their disease. Treatment outcomes were recorded. Follow-up took place every 3-6 months depending on the patient's condition and unless scheduled otherwise.

**Study population**

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

The walk-in group was people who come to the hospital with symptoms indicating CCA which has been diagnosed with US. The subjects included in our study only those enrolled in the CASCAP database from 2013-2017 with a total of 394 026 subjects.

## **Patient and Public Involvement**

The CASCAP project is a comprehensive screening and treatment program for CCA. Members of the public were first involved in the research in two ways. Firstly when members of the public attended a participating hospital for any reason, hospital staff would actively recruit them to the study. Village health volunteers also recruited participants while carrying out their work. A second group were those who already has some suspected symptoms and attended a hospital for screening at which point they were recruited into the study. The study participants were not directly involved in the design of the study. Participants will be contacted at least annually to be screened for CCA risk. Patients identified as having CCA will receive standard care for the condition through the project. For the screening procedures covered by this report participants are informed of the purpose, outcomes and implications of these procedures.

## **Main outcome and independent variables**

The primary outcome for this study was BDD which was categorized into two groups (no/yes). The independent variable of interest was PDF. We classify PDF into 3 categories (PDF1, 2 and 3) using a World Health Organization standard methodology originally developed for use in the assessment of schistosomal periportal fibrosis (PPF) but which is also valid for the study of PDF given that PPF and PDF have the same ultrasound images of Increased Periportal Echo.<sup>26</sup> We only use 3 of the 5 classifications utilized in this methodology since anatomically extra and intra hepatic bile ducts run in parallel to the portal

vein in the periportal space, so the pathology of the bile duct should be detected first in the periportal space. This identification system has been validated by comparing US diagnoses with histopathologically proven cases of PDF with good agreement between the methods.<sup>8</sup> Using this system PDF is categorized based on the anatomical location of the intrahepatic and extrahepatic bile duct. PDF1 is defined as having a high echo in the wall of small bile ducts scattered in the liver as a starry sky pattern, PDF2 is a high echo along the segmental bile duct wall running parallel with the portal vein, and PDF3 is a high echo along the main bile duct wall running parallel with the portal vein in the periportal space.<sup>19</sup> Both BDD and PDF diagnosed via US by radiologists from the CASCAP project all of whom took part in a special training course for ultrasound examination including all criteria to diagnose hepatobiliary abnormalities. A teleconsultation system was also set up to confirm diagnoses from radiologists. Demographic characteristics of PDF and non-PDF subjects were the independent variables includes gender, age, education levels, occupations, having a relative diagnosed with CCA, liver fluke infection, praziquantel (PZQ) treatments, smoking (current or previous), alcohol consumption (current or previous) and diagnosis with hepatitis B (HB), hepatitis C (HC), and diabetes mellitus (DM). All demographic characteristics listed above were collected via face-to-face interview by interviewer from the CASCAP using questionnaire.

211

## 212 **Statistical analysis**

The demographic characteristics that were categorical data were summarized using frequencies and percentages (i.e. gender, age groups, education levels, occupations, having a relative diagnosed with CCA, liver fluke infection, PZQ treatments, smoking history, alcohol consumption history and diagnosis with HB, HC, DM, and PDFs). The continuous data, such

as the age of the subjects, were summarized by their mean, standard deviation, median, minimum and maximum range.

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

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

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

## RESULTS

### Descriptive summary



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

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

251

252 <Table 1 located here>

253

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,

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

### Multivariable analysis

Through the multivariable analysis using multiple logistic regression, all factors were adjusted and the association of PDF3 subjects having BDD remained significantly high compared with non-PDF subjects (adjusted OR=5.74, 95% CI 4.57 to 7.21, P<0.001) (table 2). Compared to crude OR, the adjusted OR of gender, age, occupations, liver fluke infection, smoking history and alcohol consumption history, and a positive diagnosis of DM remained statistically significant, while a positive diagnosis of HB and HC remained non-significant (figure 3). Our study also found that relatives diagnosed with CCA changed from non-significant in bivariate analysis to significant in multivariable analysis, while education levels and PZQ treatment changed from significant to non-significant.

<Table 2 located here>

<Figure 1 located here>

<Figure 2 located here>

<Figure 3 located here>



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**DISCUSSION**

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

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

Our study found that those aged 60-years-old and over are more likely to have BDD than other age groups. Meanwhile, our study also found the association of BDD increased with increasing age. We conclude that age plays a role in BDD development. This result is similar to a study conducted in Israel between 2001-2002 which found that bile duct size increases with age and reported age was positively correlated with bile duct size.<sup>31</sup> A study from Canada in 2014 found that older age was associated with bile duct diameters which increases with age.<sup>32</sup> Therefore, it is not a surprise that those who were in the oldest age group in our study had a strong association with BDD, which causes the bile duct to grow.

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

And interestingly, those who had CCA diagnosed relatives, had a higher association to BDD than those who did not have CCA diagnosed relatives only 12% (adjusted OR=1.12, 95% CI 1.02 to 1.24, P=0.018). However, our results were consistent with Zhou et al. (2014), who identified genetic and familial risk factors as significantly contributing to the development of combined HCC-CCA through a bivariate analysis.<sup>37</sup> It is worth mentioning that this significance could not be confirmed through a multivariable analysis. Other studies also demonstrate that having a family history of cancer is a significant associated factor for

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

CCA development.<sup>38 39</sup> A risk factor study of CCA in Northeast Thailand also reported patients who had a family history of cancer were more likely to develop CCA than those without a family history of liver cancer.<sup>40</sup> Death or traumatic incidences influence the decision-making process. This may be the reason behind the lack of association between family history of CCA and BDD in our statistical analysis. Perhaps family members who experience a death of CCA-diagnosed family member are more likely to take measures in preventing the occurrence of a second CCA incidence in the family. A CCA traumatic experience may have served as a warning for family members to avoid this rapid and fatal outcome. These results reveal the complicated nature of understanding the true risk factors of CCA and pathogenesis to hepatic carcinoma in certain Asian societies.

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

**CONCLUSIONS**

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

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

## Recommendations

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

## List of abbreviations

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

## Conflict of interest

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

392 All authors declare no conflict of interest.

393

394 **Acknowledgements** This study was supported by Khon Kaen University (KKU) through

395 CASCAP project and the National Research Council of Thailand through the Medical

396 Research Network of the Consortium of Thai Medical Schools. The study was also supported

397 by the Data Management and Statistical Analysis Center (DAMASAC), Faculty of Public

398 Health, KKU, Thailand. We would like to acknowledge and thank Dr.Malcolm Anthony

399 Moore for his comments and suggestions over the final version of the manuscript.

400

401 **Author contributions** NC, SP, and KT conceived and designed this study. KT and BT

402 performed the analysis. NC, SP, NK, BT, KT, ATS and MK wrote the manuscript. NC, NK,

403 BT and KT collected the data and generated the clinical database. All authors have been

404 involved in revising the manuscript, and all authors have read and approved the final

405 manuscript.

406

407 **Funding** This work was supported by Khon Kaen University through CASCAP (Grant No .

408 CASCAP 1/60), the National Research Council of Thailand through the Medical Research

409 Network of the Consortium of Thai Medical Schools (Grant No .MRF.59-076) and National

410 Research Council of Thailand (NRCT/2559-134).

411

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

413

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

415

**Ethics approval** The research protocol was approved by Khon Kaen University Ethics Committee for Human Research, reference number HE591067.

418

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

420

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

422

423

## 424 REFERENCES

- 425 1. Moore MA, Attasara P, Khuhaprema T, et al. Cancer epidemiology in mainland South-  
 426 East Asia - past, present and future. *Asian Pacific journal of cancer prevention : APJCP*  
 427 2010;11 Suppl 2:67-80.
- 428 2. Moore MA, Manan AA, Chow KY, et al. Cancer epidemiology and control in peninsular  
 429 and island South-East Asia - past, present and future. *Asian Pacific journal of cancer*  
 430 *prevention : APJCP* 2010;11 Suppl 2:81-98.
- 431 3. National Cancer Institute. Hospital based cancer registry annual report 2012. Bangkok:  
 432 Eastern Printing Public Company Limited PCL.157 2012.
- 433 4. Rosen CB, Nagorney DM, Wiesner RH, et al. Cholangiocarcinoma complicating primary  
 434 sclerosing cholangitis. *Annals of surgery* 1991;213(1):21-5.
- 435 5. Songserm N, Promthet S, Sithithaworn P, et al. Risk factors for cholangiocarcinoma in  
 436 high-risk area of Thailand: role of lifestyle, diet and methylenetetrahydrofolate reductase  
 437 polymorphisms. *Cancer epidemiology* 2012;36(2):e89-94. doi:  
 438 10.1016/j.canep.2011.11.007
- 439 6. Tao LY, He XD, Qu Q, et al. Risk factors for intrahepatic and extrahepatic  
 440 cholangiocarcinoma: a case-control study in China. *Liver international : official journal*



1  
2  
3 441 of the International Association for the Study of the Liver 2010;30(2):215-21. doi:  
4  
5 442 10.1111/j.1478-3231.2009.02149.x  
6  
7 443 7. Shaib YH, El-Serag HB, Nooka AK, et al. Risk factors for intrahepatic and extrahepatic  
8  
9 444 cholangiocarcinoma: a hospital-based case-control study. *The American journal of*  
10  
11 445 *gastroenterology* 2007;102(5):1016-21. doi: 10.1111/j.1572-0241.2007.01104.x  
12  
13 446 8. Chamadol N, Pairojkul C, Khuntikeo N, et al. Histological confirmation of periductal  
14  
15 447 fibrosis from ultrasound diagnosis in cholangiocarcinoma patients. *Journal of hepato-*  
16  
17 448 *biliary-pancreatic sciences* 2014;21(5):316-22. doi: 10.1002/jhbp.64  
18  
19 449 9. Welzel TM, Graubard BI, El-Serag HB, et al. Risk factors for intrahepatic and  
20  
21 450 extrahepatic cholangiocarcinoma in the United States: a population-based case-control  
22  
23 451 study. *Clinical gastroenterology and hepatology : the official clinical practice journal of*  
24  
25 452 *the American Gastroenterological Association* 2007;5(10):1221-8. doi:  
26  
27 453 10.1016/j.cgh.2007.05.020  
28  
29 454 10. Shaib YH, El-Serag HB, Davila JA, et al. Risk factors of intrahepatic cholangiocarcinoma  
30  
31 455 in the United States: a case-control study. *Gastroenterology* 2005;128(3):620-6.  
32  
33 456 11. Hamilton SR, Aaltonen LA. Pathology and genetics of tumours of the digestive system.  
34  
35 457 France: Lyon : Oxford : IARC Press ; Oxford University Press (distributor). 2000.  
36  
37 458 12. Khan SA, Davidson BR, Goldin RD, et al. Guidelines for the diagnosis and treatment of  
38  
39 459 cholangiocarcinoma: an update. *Gut* 2012;61(12):1657-69. doi: 10.1136/gutjnl-2011-  
40  
41 460 301748  
42  
43 461 13. Saini S. Imaging of the hepatobiliary tract. *The New England journal of medicine*  
44  
45 462 1997;336(26):1889-94. doi: 10.1056/NEJM199706263362607  
46  
47 463 14. Blechacz B, Gores GJ. Cholangiocarcinoma: advances in pathogenesis, diagnosis, and  
48  
49 464 treatment. *Hepatology* 2008;48(1):308-21. doi: 10.1002/hep.22310  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

15. Zen Y, Adsay NV, Bardadin K, et al. Biliary intraepithelial neoplasia: an international interobserver agreement study and proposal for diagnostic criteria. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* 2007;20(6):701-9. doi: 10.1038/modpathol.3800788
16. Nakanuma Y, Sasaki M, Sato Y, et al. Multistep carcinogenesis of perihilar cholangiocarcinoma arising in the intrahepatic large bile ducts. *World journal of hepatology* 2009;1(1):35-42. doi: 10.4254/wjh.v1.i1.35
17. Maetani Y, Itoh K, Watanabe C, et al. MR imaging of intrahepatic cholangiocarcinoma with pathologic correlation. *AJR American journal of roentgenology* 2001;176(6):1499-507. doi: 10.2214/ajr.176.6.1761499
18. National Cancer Institute. Guidelines for screening, diagnosis and treatment of liver cancer and cholangiocarcinoma. Bangkok: National Office of Buddhism 2011:81.
19. Nittaya Chamadol. Imaging in Cholangiocarcinoma. Khon Kaen, Thailand: Department of Radiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand 2014.
20. Xu HX, Chen LD, Liu LN, et al. Contrast-enhanced ultrasound of intrahepatic cholangiocarcinoma: correlation with pathological examination. *The British journal of radiology* 2012;85(1016):1029-37. doi: 10.1259/bjr/21653786
21. Sripa B, Mairiang E, Thinkhamrop B, et al. Advanced periductal fibrosis from infection with the carcinogenic human liver fluke *Opisthorchis viverrini* correlates with elevated levels of interleukin-6. *Hepatology* 2009;50(4):1273-81. doi: 10.1002/hep.23134
22. Benedetti NJ, Desser TS, Jeffrey RB. Imaging of hepatic infections. *Ultrasound Q* 2008;24(4):267-78. doi: 10.1097/RUQ.0b013e31818e5981
23. Loria F, Loria G, Basile S, et al. Contrast-enhanced ultrasound appearances of enhancement patterns of intrahepatic cholangiocarcinoma: correlation with pathological findings. *Updates in surgery* 2014;66(2):135-43. doi: 10.1007/s13304-014-0251-6



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

24. Elkins DB, Mairiang E, Sithithaworn P, et al. Cross-sectional patterns of hepatobiliary abnormalities and possible precursor conditions of cholangiocarcinoma associated with *Opisthorchis viverrini* infection in humans. *The American journal of tropical medicine and hygiene* 1996;55(3):295-301.

25. Khuntikeo N, Chamadol N, Yongvanit P, et al. Cohort profile: cholangiocarcinoma screening and care program (CASCAP). *BMC cancer* 2015;15:459. doi: 10.1186/s12885-015-1475-7

26. Berhe N, Geitung JT, Medhin G, et al. Large scale evaluation of WHO's ultrasonographic staging system of schistosomal periportal fibrosis in Ethiopia. *Tropical Medicine & International Health* 2006;11(8):1286-94. doi: DOI 10.1111/j.1365-3156.2006.01665.x

27. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International journal of cancer Journal international du cancer* 2015;136(5):E359-86. doi: 10.1002/ijc.29210

28. Sripa B, Pairojkul C. Cholangiocarcinoma: lessons from Thailand. *Current opinion in gastroenterology* 2008;24(3):349-56. doi: 10.1097/MOG.0b013e3282fb9b3

29. Srivatanakul P, Sriplung H, Deerasamee S. Epidemiology of liver cancer: an overview. *Asian Pacific journal of cancer prevention : APJCP* 2004;5(2):118-25.

30. Prakobwong S, Yongvanit P, Hiraku Y, et al. Involvement of MMP-9 in peribiliary fibrosis and cholangiocarcinogenesis via Rac1-dependent DNA damage in a hamster model. *International journal of cancer Journal international du cancer* 2010;127(11):2576-87. doi: 10.1002/ijc.25266

31. Bachar GN, Cohen M, Belenky A, et al. Effect of aging on the adult extrahepatic bile duct: a sonographic study. *Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine* 2003;22(9):879-82; quiz 83-5.

- 514 32. Landry D, Tang A, Murphy-Lavallee J, et al. Dilatation of the bile duct in patients after  
 515 cholecystectomy: a retrospective study. *Canadian Association of Radiologists journal =*  
 516 *Journal l'Association canadienne des radiologistes* 2014;65(1):29-34. doi:  
 517 10.1016/j.carj.2012.09.004
- 518 33. Barusrux S, Nanok C, Puthisawas W, et al. Viral hepatitis B, C infection and genotype  
 519 distribution among cholangiocarcinoma patients in northeast Thailand. *Asian Pacific*  
 520 *journal of cancer prevention : APJCP* 2012;13 Suppl:83-7.
- 521 34. Lee TY, Lee SS, Jung SW, et al. Hepatitis B virus infection and intrahepatic  
 522 cholangiocarcinoma in Korea: a case-control study. *The American journal of*  
 523 *gastroenterology* 2008;103(7):1716-20. doi: 10.1111/j.1572-0241.2008.01796.x
- 524 35. Li M, Li J, Li P, et al. Hepatitis B virus infection increases the risk of  
 525 cholangiocarcinoma: a meta-analysis and systematic review. *Journal of gastroenterology*  
 526 *and hepatology* 2012;27(10):1561-8. doi: 10.1111/j.1440-1746.2012.07207.x
- 527 36. Srivatanakul P, Honjo S, Kittiwatanachot P, et al. Hepatitis viruses and risk of  
 528 cholangiocarcinoma in northeast Thailand. *Asian Pacific journal of cancer prevention :*  
 529 *APJCP* 2010;11(4):985-8.
- 530 37. Zhou YM, Zhang XF, Wu LP, et al. Risk factors for combined hepatocellular-  
 531 cholangiocarcinoma: a hospital-based case-control study. *World journal of*  
 532 *gastroenterology : WJG* 2014;20(35):12615-20. doi: 10.3748/wjg.v20.i35.12615
- 533 38. Kamsa-Ard S, Luvira V, Pugkhem A, et al. Association between praziquantel treatment  
 534 and cholangiocarcinoma: a hospital-based matched case-control study. *BMC cancer*  
 535 2015;15:776. doi: 10.1186/s12885-015-1788-6
- 536 39. Liu ZY, Zhou YM, Shi LH, et al. Risk factors of intrahepatic cholangiocarcinoma in  
 537 patients with hepatolithiasis: a case-control study. *Hepatobiliary & pancreatic diseases*  
 538 *international : HBPD INT* 2011;10(6):626-31.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

539 40. Manwong M, Songserm N, Promthet S, et al. Risk factors for cholangiocarcinoma in the  
540 lower part of Northeast Thailand: a hospital-based case-control study. *Asian Pacific*  
541 *journal of cancer prevention* : *APJCP* 2013;14(10):5953-6.  
542  
543

For peer review only

## Captions for the figures:

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

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

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

**Table 1** Baseline demographic and clinical characteristics of subjects

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

552

**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	

N/A, Not applicable.

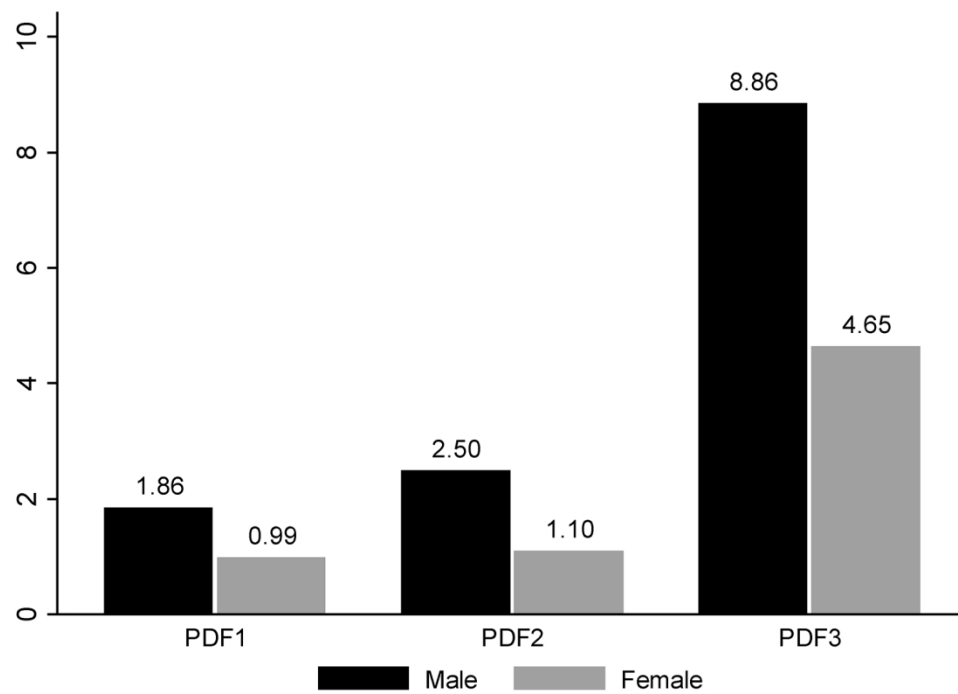


Figure 1 Percentage of BDD between male and female according to PDF1, 2, and 3.

143x104mm (300 x 300 DPI)

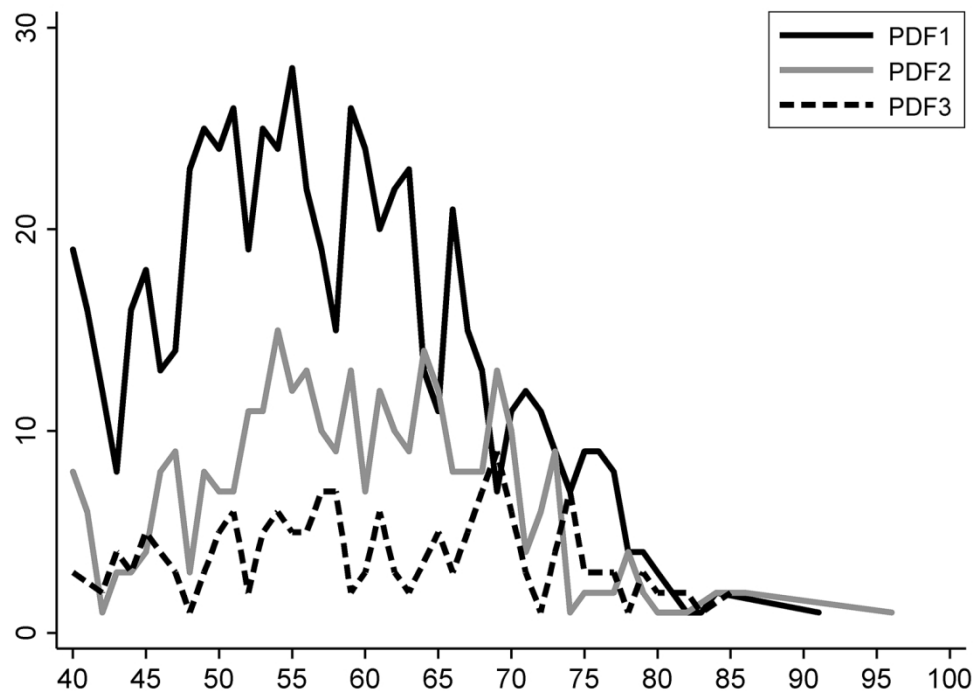


Figure 2 Number of BDD in PDF subjects by age range.

141x103mm (300 x 300 DPI)

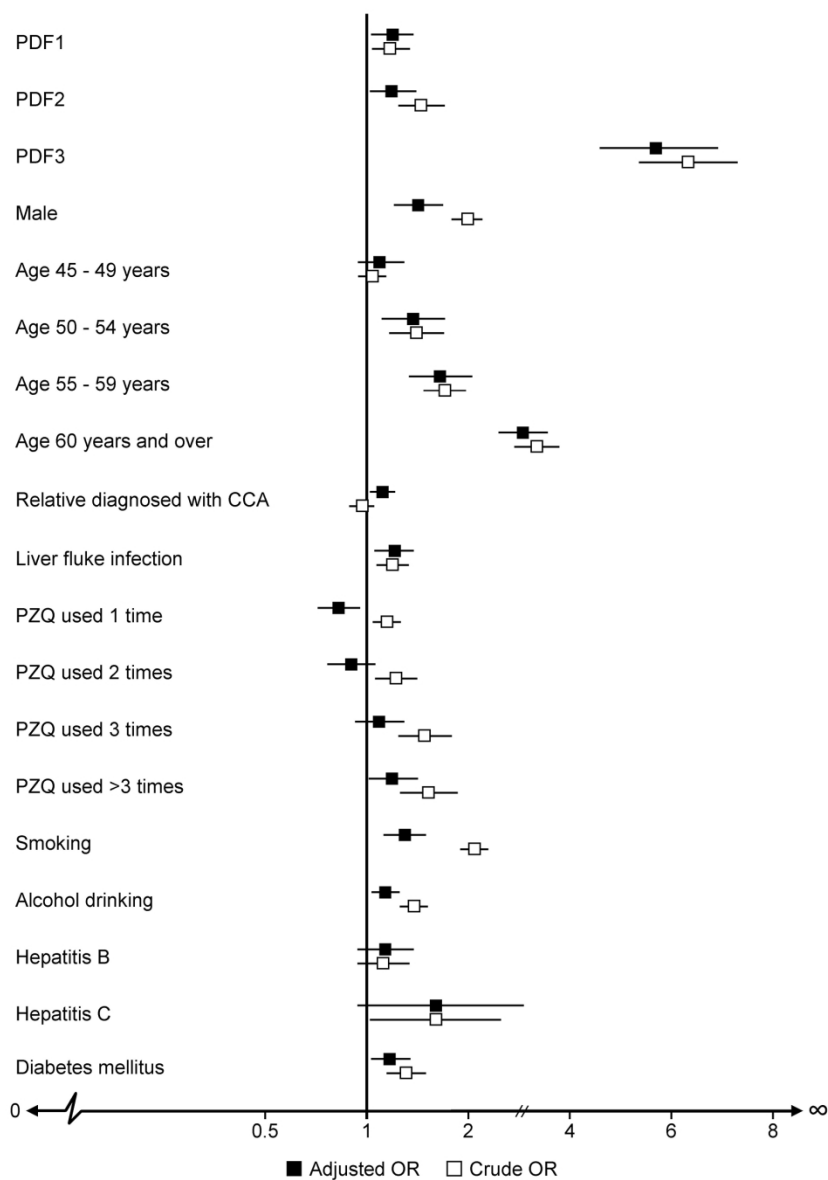


Figure 3 The adjusted OR and crude OR of the associated factors of BDD.

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) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Case-control study—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 Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants
		N/A	(b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed Case-control study—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	(b) Describe any methods used to examine subgroups and interactions
		Pg9	(c) Explain how missing data were addressed
		N/A	(d) Cohort study—If applicable, explain how loss to follow-up was addressed Case-control study—If applicable, explain how matching of cases and controls was addressed Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy
		N/A	(e) Describe any sensitivity analyses

Continued on next page

For peer review only

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](http://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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-023217.R2
Article Type:	Research
Date Submitted by the Author:	24-Jan-2019
Complete List of Authors:	Chamadol, Nittaya; Khon Kaen University Faculty of Medicine Khuntikeo, Narong; Khon Kaen University Faculty of Medicine Thinkhamrop, Bandit; Faculty of Public Health, Khon Kaen University, Department of Biostatistics and Epidemiology Thinkhamrop, Kavin; Khon Kaen University Faculty of Public Health Suwannatrai, Apiporn; Khon Kaen University Faculty of Medicine Kelly, Matthew; The Australian National University, Research School of Population Health Promthet, Supanee; Faculty of Public Health, Khon Kaen University, Department of Epidemiology
<b>Primary Subject Heading</b>:	Public health
Secondary Subject Heading:	Global health
Keywords:	bile duct dilatation, periductal fibrosis, ULTRASONOGRAPHY, cholangiocarcinoma, screening, Thailand

SCHOLARONE™  
Manuscripts



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1   **TITLE PAGE**  
  
2  
  
3   **Title:** Association between periductal fibrosis and bile duct dilatation among a population at  
4   high-risk of cholangiocarcinoma: a cross-sectional study cholangiocarcinoma screening in  
5   Northeast Thailand  
  
6  
  
7   **Authors:** Nittaya Chamadol,<sup>1,2,3</sup> Narong Khuntikeo,<sup>1,2,4</sup> Bandit Thinkhamrop,<sup>1,2,5,6</sup> Kavin  
8   Thinkhamrop,<sup>1,2,6</sup> Apiporn T. Suwannatrai,<sup>1,2,7</sup> Matthew Kelly,<sup>8</sup> and Supanee Promthet<sup>1,5,9</sup>  
  
9  
  
10   **Affiliations:**  
11   <sup>1</sup>Cholangiocarcinoma Screening and Care Program (CASCAP), Khon Kaen University, Khon  
12   Kaen, Thailand.  
13   <sup>2</sup>Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand.  
14   <sup>3</sup>Department of Radiology, Faculty of Medicine, Khon Kaen University, Khon Kaen,  
15   Thailand.  
16   <sup>4</sup>Department of Surgery, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.  
17   <sup>5</sup>Epidemiology and Biostatistics Section, Faculty of Public Health, Khon Kaen University,  
18   Khon Kaen, Thailand.  
19   <sup>6</sup>Data Management and Statistical Analysis Center (DAMASAC), Faculty of Public Health,  
20   Khon Kaen University, Khon Kaen, Thailand.  
21   <sup>7</sup>Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen,  
22   Thailand.  
23   <sup>8</sup>Department of Global Health, Research School of Population Health, Australian National  
24   University, Canberra, Australia.

<sup>9</sup>ASEAN Cancer Epidemiology and Prevention Research Group, Khon Kaen University,  
Khon Kaen, Thailand.

**Email address:**

NC: Nittaya Chamadol (nittayachamadol@yahoo.com)

NK: Narong Khuntikeo (nkhuntikeo@gmail.com)

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

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

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

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

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

**Corresponding authors:**

Name: Kavin Thinkhamrop, Dr.P.H.

Address: Data Management and Statistical Analysis Center, Faculty of Public Health,  
Khon Kaen University, Thailand.

Telephone: +66-97 317 1976

e-Mail: kvinth@gmail.com/ kavith@kku.ac.th

**Type of contribution:** Research article

**Number of words in the abstract:** 298

**Number of words in the text:** 3,086 (excluding references and tables)

**Number of tables:** 2

**Number of figures:** 3

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**ABSTRACT**

**Objectives** To assess associations between periductal fibrosis (PDF) and bile duct dilatation (BDD) in ultrasonography (US) screening of population at risk of cholangiocarcinoma (CCA) due to residence in an endemic area for *Opisthorchis viverrini*. CCA survival rates are low and early identification of risk factors is essential. BDD is one symptom which can identify patients at risk of CCA. Detection of PDF by US can also identify at risk patients, at an earlier stage of CCA development. Identification of association between PDF and BDD will inform screening practices for CCA risk, by increasing the viability of PDF screening for CCA risk.

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

**Design** Cross-sectional study.

**Participants** Study subjects in the Cholangiocarcinoma Screening and Care Program (CASCAP) in Northeast Thailand. CASCAP inclusion criteria are all residents of Northeast Thailand aged 40 years and over. Participants are recruited through CCA screening centers and through primary health care units. So far 394 026 have been enrolled.

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

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

**Conclusions** Our findings reveal that there is a relationship between PDF and BDD, which is associated with CCA. Therefore, PDF can also be an indicator for suspected-CCA diagnosis through US.

**Keywords** bile duct dilatation; periductal fibrosis; ultrasonography; cholangiocarcinoma; screening; Thailand

## Article summary

### Strengths and limitations of the study

- The large size of the study population and its geographic distribution across Northeast Thailand are a significant strength.
- This is the first and largest screening program for cholangiocarcinoma (CCA) in an area with the highest incidence in the world.
- CCA risk factors (PDF and BDD) were measured using ultrasonography by skilled radiologists.
- Demographic, and some health, data were self-reported leading to potential bias in measurement of liver fluke infection, praziquantel treatment, and pre-existing medical conditions including hepatitis B (HB), hepatitis C (HC), and diabetes mellitus (DM).
- Self-report could lead to prevalence underestimates due to the fact that subjects may not have been willing to disclose sensitive or personal information.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**INTRODUCTION**

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

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

The relationship between PDF and CCA is indicated by the regular detection of PDF in confirmed CCA cases, and this has been particularly common in Northeast Thailand where *O. viverrini* is endemic and a leading potential cause of CCA.<sup>8</sup> As a result of this relationship, US detection has been utilized to identify people with PDF as a risk group for CCA development.<sup>8 20 23 24</sup> Hepatobiliary abnormalities identified through ultrasound have been shown in other studies to correlate well with histopathological confirmation making US a valuable tool in early identification of these health issues.<sup>8</sup> Importantly, PDF can be identified through US, but does not require the services of a specialist radiologist increasing the potential access to screening, and PDF can be detected earlier than BDD allowing more effective intervention.

The potential to detect the risk of CCA earlier and without the need for specialist radiologists, through the identification of PDF may be an important breakthrough in reducing CCA incidence. So, both PDF and BDD have been recognized as indicators of CCA<sup>8 17</sup>, but their relationship to one another has yet to be established or even studied in depth. Determining their relationship, such as learning if one precedes the other may make a significant change in how we screen for CCA via US. Therefore, this study seeks to determine if there is an association between PDF and BDD among people at a high-risk CCA population in Northeast Thailand. The results of this work will clarify necessary directions toward early screening methodologies and appropriate cancer treatment.

## METHODS

### Study design

This study presents data collected from the Cholangiocarcinoma Screening and Care Program (CASCAP) in Northeast Thailand. CASCAP is a prospective cohort study that is considered the first project for CCA screening in a high-risk population with a community-based bottom-

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

up approach.<sup>25</sup> Although this overall project is a prospective cohort study, the results presented here use cross sectional data from the baseline study carried out with participants.

The overall aim of the study is to recruit all adults aged 40 years or over who reside in Northeast Thailand and to screen them for cholangiocarcinoma and its risk factors in terms of hepatobiliary abnormalities and infection with the liver fluke *Opisthorchis viverrini*. As such there are no strict inclusion or exclusion criteria apart from age group and place of residence. Once consent has been obtained, the participants will be enrolled in the program. The primary place of recruitment for this cohort study were 9 tertiary care hospitals in the Northeast of Thailand. These hospitals serve as the main source of affordable tertiary care for local people in the region. Subjects were recruited at these hospitals in two ways. Firstly the screening group comprised individuals who had attended the hospital for other reasons and were invited to receive ultrasound screening without evidencing any symptoms. The second group, the walk-in group, were individuals who were attending the hospital because of CCA symptoms and this group can then receive treatment. All participants were asked to join the project by signing a consent form. All CCA patients were diagnosed and treated according to routine, real world clinical practice by participating hospitals. Patients were followed-up and provided with either clinical or palliative care depending on the stage of their disease. Treatment outcomes were recorded. Follow-up took place every 3-6 months depending on the patient's condition and unless scheduled otherwise.

**Study population**

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

Enseignement Supérieur (ABES) .  
Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.



169 The walk-in group was people who come to the hospital with symptoms indicating CCA  
170 which has been diagnosed with US. The subjects included in our study only those enrolled in  
171 the CASCAP database from 2013-2017 with a total of 394 026 subjects.

172

### 173 **Patient and Public Involvement**

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

185

### 186 **Main outcome and independent variables**

187 The primary outcome for this study was BDD which was categorized into two groups  
188 (no/yes). The independent variable of interest was PDF. We classify PDF into 3 categories  
189 (PDF1, 2 and 3) using a World Health Organization standard methodology originally  
190 developed for use in the assessment of schistosomal periportal fibrosis (PPF) but which is  
191 also valid for the study of PDF given that PPF and PDF have the same ultrasound images of  
192 Increased Periportal Echo.<sup>26</sup> We only use 3 of the 5 classifications utilized in this  
193 methodology since anatomically extra and intra hepatic bile ducts run in parallel to the portal



1  
2  
3 194 vein in the periportal space, so the pathology of the bile duct should be detected first in the  
4  
5 195 periportal space. This identification system has been validated by comparing US diagnoses  
6  
7 196 with histopathologically proven cases of PDF with good agreement between the methods.<sup>8</sup>  
8  
9  
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.<sup>19</sup>  
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  
34  
35 209 B (HB), hepatitis C (HC), and diabetes mellitus (DM). All demographic characteristics listed  
36  
37 210 above were collected via face-to-face interview by interviewer from the CASCAP using  
38  
39 211 questionnaire.  
40  
41  
42  
43  
44  
45 212

46  
47 213 **Statistical analysis**

48  
49 214 The demographic characteristics that were categorical data were summarized using  
50  
51 215 frequencies and percentages (i.e. gender, age groups, education levels, occupations, having a  
52  
53 216 relative diagnosed with CCA, liver fluke infection, PZQ treatments, smoking history, alcohol  
54  
55 217 consumption history and diagnosis with HB, HC, DM, and PDFs). The continuous data, such  
56  
57  
58  
59  
60

Enseignement Supérieur (ABES) .  
Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

218 as the age of the subjects, were summarized by their mean, standard deviation, median,  
219 minimum and maximum range.

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

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

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

240

## 241 RESULTS

### 242 Descriptive summary

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

The demographic characteristics of subjects were presented as numbers and percentages. A total of 394 026 subjects who underwent US screenings for CCA were enrolled in our study. The subjects were all between the ages of 40-100 years old and reported a mean age of 54.92±9.03 years old. Of these, approximately two-thirds were female (61.4%) and the majority of them completed primary school education level (72.9%) and worked as farmers (77.9%). About one-third (29.7%) had ever used PZQ treatment, and about one-fourth (21.3%) reported being a smoker or ex-smoker. The data of PDF diagnosis, 17.6% have positive diagnosed and the highest percentage was in subjects diagnosed with PDF1 (12.3%) while only 0.6% for PDF3 (table 1).

<Table 1 located here>

**Prevalence of BDD**

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

**Associations with BDD**

**Bivariate analysis**

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

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

### **Multivariable analysis**

Through the multivariable analysis using multiple logistic regression, all factors were adjusted and the association of PDF3 subjects having BDD remained significantly high compared with non-PDF subjects (adjusted OR=5.74, 95% CI 4.57 to 7.21, P<0.001) (table 2). Compared to crude OR, the adjusted OR of gender, age, occupations, liver fluke infection, smoking history and alcohol consumption history, and a positive diagnosis of DM remained statistically significant, while a positive diagnosis of HB and HC remained non-significant (figure 3). Our study also found that relatives diagnosed with CCA changed from non-significant in bivariate analysis to significant in multivariable analysis, while education levels and PZQ treatment changed from significant to non-significant.

<Table 2 located here>

<Figure 1 located here>

<Figure 2 located here>

<Figure 3 located here>

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**DISCUSSION**

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

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

Enseignement Supérieur (ABES) .  
Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

Our study found that those aged 60-years-old and over are more likely to have BDD than other age groups. Meanwhile, our study also found the association of BDD increased with increasing age. We conclude that age plays a role in BDD development. This result is similar to a study conducted in Israel between 2001-2002 which found that bile duct size increases with age and reported age was positively correlated with bile duct size.<sup>31</sup> A study from Canada in 2014 found that older age was associated with bile duct diameters which increases with age.<sup>32</sup> Therefore, it is not a surprise that those who were in the oldest age group in our study had a strong association with BDD, which causes the bile duct to grow.

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

And interestingly, those who had CCA diagnosed relatives, had a higher association to BDD than those who did not have CCA diagnosed relatives only 12% (adjusted OR=1.12, 95% CI 1.02 to 1.24, P=0.018). However, our results were consistent with Zhou et al. (2014), who identified genetic and familial risk factors as significantly contributing to the development of combined HCC-CCA through a bivariate analysis.<sup>37</sup> It is worth mentioning that this significance could not be confirmed through a multivariable analysis. Other studies also demonstrate that having a family history of cancer is a significant associated factor for

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

CCA development.<sup>38 39</sup> A risk factor study of CCA in Northeast Thailand also reported patients who had a family history of cancer were more likely to develop CCA than those without a family history of liver cancer.<sup>40</sup> Death or traumatic incidences influence the decision-making process. This may be the reason behind the lack of association between family history of CCA and BDD in our statistical analysis. Perhaps family members who experience a death of CCA-diagnosed family member are more likely to take measures in preventing the occurrence of a second CCA incidence in the family. A CCA traumatic experience may have served as a warning for family members to avoid this rapid and fatal outcome. These results reveal the complicated nature of understanding the true risk factors of CCA and pathogenesis to hepatic carcinoma in certain Asian societies.

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

PDF and BDD can be detected by ultrasound screening before any clinical symptom of CCA are evident. Additional further characterization by other advanced imaging and endoscopic examinations is standard for differential diagnosis of CCA from other diseases. Histopathological confirmation is mandatory in the patient with a surgical indication.

Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies. Enseignement Supérieur (ABES).



Longitudinal data collection is necessary for further study of the relationship between PDF and BDD and CCA.

## CONCLUSIONS

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

## Recommendations

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

## List of abbreviations



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

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

**Conflict of interest**

All authors declare no conflict of interest.

**Acknowledgements** This study was supported by Khon Kaen University (KKU) through  
CASCAP project and the National Research Council of Thailand through the Medical  
Research Network of the Consortium of Thai Medical Schools. The study was also supported  
by the Data Management and Statistical Analysis Center (DAMASAC), Faculty of Public  
Health, KKU, Thailand. We would like to acknowledge and thank Dr.Malcolm Anthony  
Moore for his comments and suggestions over the final version of the manuscript.

**Author contributions** NC, SP, and KT conceived and designed this study. KT and BT  
performed the analysis. NC, SP, NK, BT, KT, ATS and MK wrote the manuscript. NC, NK,  
BT and KT collected the data and generated the clinical database. All authors have been  
involved in revising the manuscript, and all authors have read and approved the final  
manuscript.

**Funding** This work was supported by Khon Kaen University through CASCAP (Grant No .  
CASCAP 1/60), the National Research Council of Thailand through the Medical Research

Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.  
Enseignement Supérieur (ABES) .

416 Network of the Consortium of Thai Medical Schools (Grant No .MRF.59-076) and National  
417 Research Council of Thailand (NRCT/2559-134).

418

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

420

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

422

423 **Ethics approval** The research protocol was approved by Khon Kaen University Ethics  
424 Committee for Human Research, reference number HE591067.

425

426 **Provenance and peer review** Not commissioned; externally peer reviewed.

427

428 **Data sharing statement** No additional data are available.

429

430

## 431 REFERENCES

- 432 1. Moore MA, Attasara P, Khuhaprema T, et al. Cancer epidemiology in mainland South-  
433 East Asia - past, present and future. *Asian Pacific journal of cancer prevention : APJCP*  
434 2010;11 Suppl 2:67-80.
- 435 2. Moore MA, Manan AA, Chow KY, et al. Cancer epidemiology and control in peninsular  
436 and island South-East Asia - past, present and future. *Asian Pacific journal of cancer*  
437 *prevention : APJCP* 2010;11 Suppl 2:81-98.
- 438 3. National Cancer Institue. Hospital based cancer registry annual report 2012. Bangkok:  
439 Eastern Printing Public Company Limited PCL.157 2012.

- 440 4. Rosen CB, Nagorney DM, Wiesner RH, et al. Cholangiocarcinoma complicating primary  
441 sclerosing cholangitis. *Annals of surgery* 1991;213(1):21-5.
- 442 5. Songserm N, Promthet S, Sithithaworn P, et al. Risk factors for cholangiocarcinoma in  
443 high-risk area of Thailand: role of lifestyle, diet and methylenetetrahydrofolate reductase  
444 polymorphisms. *Cancer epidemiology* 2012;36(2):e89-94. doi:  
445 10.1016/j.canep.2011.11.007
- 446 6. Tao LY, He XD, Qu Q, et al. Risk factors for intrahepatic and extrahepatic  
447 cholangiocarcinoma: a case-control study in China. *Liver international : official journal*  
448 *of the International Association for the Study of the Liver* 2010;30(2):215-21. doi:  
449 10.1111/j.1478-3231.2009.02149.x
- 450 7. Shaib YH, El-Serag HB, Nooka AK, et al. Risk factors for intrahepatic and extrahepatic  
451 cholangiocarcinoma: a hospital-based case-control study. *The American journal of*  
452 *gastroenterology* 2007;102(5):1016-21. doi: 10.1111/j.1572-0241.2007.01104.x
- 453 8. Chamadol N, Pairojkul C, Khuntikeo N, et al. Histological confirmation of periductal  
454 fibrosis from ultrasound diagnosis in cholangiocarcinoma patients. *Journal of hepato-*  
455 *biliary-pancreatic sciences* 2014;21(5):316-22. doi: 10.1002/jhbp.64
- 456 9. Welzel TM, Graubard BI, El-Serag HB, et al. Risk factors for intrahepatic and  
457 extrahepatic cholangiocarcinoma in the United States: a population-based case-control  
458 study. *Clinical gastroenterology and hepatology : the official clinical practice journal of*  
459 *the American Gastroenterological Association* 2007;5(10):1221-8. doi:  
460 10.1016/j.cgh.2007.05.020
- 461 10. Shaib YH, El-Serag HB, Davila JA, et al. Risk factors of intrahepatic cholangiocarcinoma  
462 in the United States: a case-control study. *Gastroenterology* 2005;128(3):620-6.
- 463 11. Hamilton SR, Aaltonen LA. Pathology and genetics of tumours of the digestive system.  
464 France: Lyon : Oxford : IARC Press ; Oxford University Press (distributor). 2000.

12. Khan SA, Davidson BR, Goldin RD, et al. Guidelines for the diagnosis and treatment of cholangiocarcinoma: an update. *Gut* 2012;61(12):1657-69. doi: 10.1136/gutjnl-2011-301748
13. Saini S. Imaging of the hepatobiliary tract. *The New England journal of medicine* 1997;336(26):1889-94. doi: 10.1056/NEJM199706263362607
14. Blechacz B, Gores GJ. Cholangiocarcinoma: advances in pathogenesis, diagnosis, and treatment. *Hepatology* 2008;48(1):308-21. doi: 10.1002/hep.22310
15. Zen Y, Adsay NV, Bardadin K, et al. Biliary intraepithelial neoplasia: an international interobserver agreement study and proposal for diagnostic criteria. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* 2007;20(6):701-9. doi: 10.1038/modpathol.3800788
16. Nakanuma Y, Sasaki M, Sato Y, et al. Multistep carcinogenesis of perihilar cholangiocarcinoma arising in the intrahepatic large bile ducts. *World journal of hepatology* 2009;1(1):35-42. doi: 10.4254/wjh.v1.i1.35
17. Maetani Y, Itoh K, Watanabe C, et al. MR imaging of intrahepatic cholangiocarcinoma with pathologic correlation. *AJR American journal of roentgenology* 2001;176(6):1499-507. doi: 10.2214/ajr.176.6.1761499
18. National Cancer Institute. Guidelines for screening, diagnosis and treatment of liver cancer and cholangiocarcinoma. Bangkok: National Office of Buddhism 2011:81.
19. Nittaya Chamadol. Imaging in Cholangiocarcinoma. Khon Kaen, Thailand: Department of Radiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand 2014.
20. Xu HX, Chen LD, Liu LN, et al. Contrast-enhanced ultrasound of intrahepatic cholangiocarcinoma: correlation with pathological examination. *The British journal of radiology* 2012;85(1016):1029-37. doi: 10.1259/bjr/21653786

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

21. Sripa B, Mairiang E, Thinkhamrop B, et al. Advanced periductal fibrosis from infection with the carcinogenic human liver fluke *Opisthorchis viverrini* correlates with elevated levels of interleukin-6. *Hepatology* 2009;50(4):1273-81. doi: 10.1002/hep.23134

22. Benedetti NJ, Desser TS, Jeffrey RB. Imaging of hepatic infections. *Ultrasound Q* 2008;24(4):267-78. doi: 10.1097/RUQ.0b013e31818e5981

23. Loria F, Loria G, Basile S, et al. Contrast-enhanced ultrasound appearances of enhancement patterns of intrahepatic cholangiocarcinoma: correlation with pathological findings. *Updates in surgery* 2014;66(2):135-43. doi: 10.1007/s13304-014-0251-6

24. Elkins DB, Mairiang E, Sithithaworn P, et al. Cross-sectional patterns of hepatobiliary abnormalities and possible precursor conditions of cholangiocarcinoma associated with *Opisthorchis viverrini* infection in humans. *The American journal of tropical medicine and hygiene* 1996;55(3):295-301.

25. Khuntikeo N, Chamadol N, Yongvanit P, et al. Cohort profile: cholangiocarcinoma screening and care program (CASCAP). *BMC cancer* 2015;15:459. doi: 10.1186/s12885-015-1475-7

26. Berhe N, Geitung JT, Medhin G, et al. Large scale evaluation of WHO's ultrasonographic staging system of schistosomal periportal fibrosis in Ethiopia. *Tropical Medicine & International Health* 2006;11(8):1286-94. doi: DOI 10.1111/j.1365-3156.2006.01665.x

27. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International journal of cancer Journal international du cancer* 2015;136(5):E359-86. doi: 10.1002/ijc.29210

28. Sripa B, Pairojkul C. Cholangiocarcinoma: lessons from Thailand. *Current opinion in gastroenterology* 2008;24(3):349-56. doi: 10.1097/MOG.0b013e3282fbf9b3

29. Srivatanakul P, Sriplung H, Deerasamee S. Epidemiology of liver cancer: an overview. *Asian Pacific journal of cancer prevention : APJCP* 2004;5(2):118-25.

Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies. Enseignement Supérieur (ABES).

- 514 30. Prakobwong S, Yongvanit P, Hiraku Y, et al. Involvement of MMP-9 in peribiliary  
515 fibrosis and cholangiocarcinogenesis via Rac1-dependent DNA damage in a hamster  
516 model. *International journal of cancer Journal international du cancer*  
517 2010;127(11):2576-87. doi: 10.1002/ijc.25266
- 518 31. Bachar GN, Cohen M, Belenky A, et al. Effect of aging on the adult extrahepatic bile  
519 duct: a sonographic study. *Journal of ultrasound in medicine : official journal of the*  
520 *American Institute of Ultrasound in Medicine* 2003;22(9):879-82; quiz 83-5.
- 521 32. Landry D, Tang A, Murphy-Lavalley J, et al. Dilatation of the bile duct in patients after  
522 cholecystectomy: a retrospective study. *Canadian Association of Radiologists journal =*  
523 *Journal l'Association canadienne des radiologistes* 2014;65(1):29-34. doi:  
524 10.1016/j.carj.2012.09.004
- 525 33. Barusrux S, Nanok C, Puthisawas W, et al. Viral hepatitis B, C infection and genotype  
526 distribution among cholangiocarcinoma patients in northeast Thailand. *Asian Pacific*  
527 *journal of cancer prevention : APJCP* 2012;13 Suppl:83-7.
- 528 34. Lee TY, Lee SS, Jung SW, et al. Hepatitis B virus infection and intrahepatic  
529 cholangiocarcinoma in Korea: a case-control study. *The American journal of*  
530 *gastroenterology* 2008;103(7):1716-20. doi: 10.1111/j.1572-0241.2008.01796.x
- 531 35. Li M, Li J, Li P, et al. Hepatitis B virus infection increases the risk of  
532 cholangiocarcinoma: a meta-analysis and systematic review. *Journal of gastroenterology*  
533 *and hepatology* 2012;27(10):1561-8. doi: 10.1111/j.1440-1746.2012.07207.x
- 534 36. Srivatanakul P, Honjo S, Kittiwatanachot P, et al. Hepatitis viruses and risk of  
535 cholangiocarcinoma in northeast Thailand. *Asian Pacific journal of cancer prevention :*  
536 *APJCP* 2010;11(4):985-8.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

37. Zhou YM, Zhang XF, Wu LP, et al. Risk factors for combined hepatocellular-  
cholangiocarcinoma: a hospital-based case-control study. *World journal of*  
*gastroenterology : WJG* 2014;20(35):12615-20. doi: 10.3748/wjg.v20.i35.12615

38. Kamsa-Ard S, Luvira V, Pugkhem A, et al. Association between praziquantel treatment  
and cholangiocarcinoma: a hospital-based matched case-control study. *BMC cancer*  
2015;15:776. doi: 10.1186/s12885-015-1788-6

39. Liu ZY, Zhou YM, Shi LH, et al. Risk factors of intrahepatic cholangiocarcinoma in  
patients with hepatolithiasis: a case-control study. *Hepatobiliary & pancreatic diseases*  
*international : HBPD INT* 2011;10(6):626-31.

40. Manwong M, Songserm N, Promthet S, et al. Risk factors for cholangiocarcinoma in the  
lower part of Northeast Thailand: a hospital-based case-control study. *Asian Pacific*  
*journal of cancer prevention : APJCP* 2013;14(10):5953-6.

Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.  
Enseignement Supérieur (ABES).



## Captions for the figures:

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

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

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

**Table 1** Baseline demographic and clinical characteristics of subjects

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

Enseignement Supérieur (ABES) .  
Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

**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

**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	

N/A, Not applicable.

Enseignement Supérieur (ABES) .  
Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

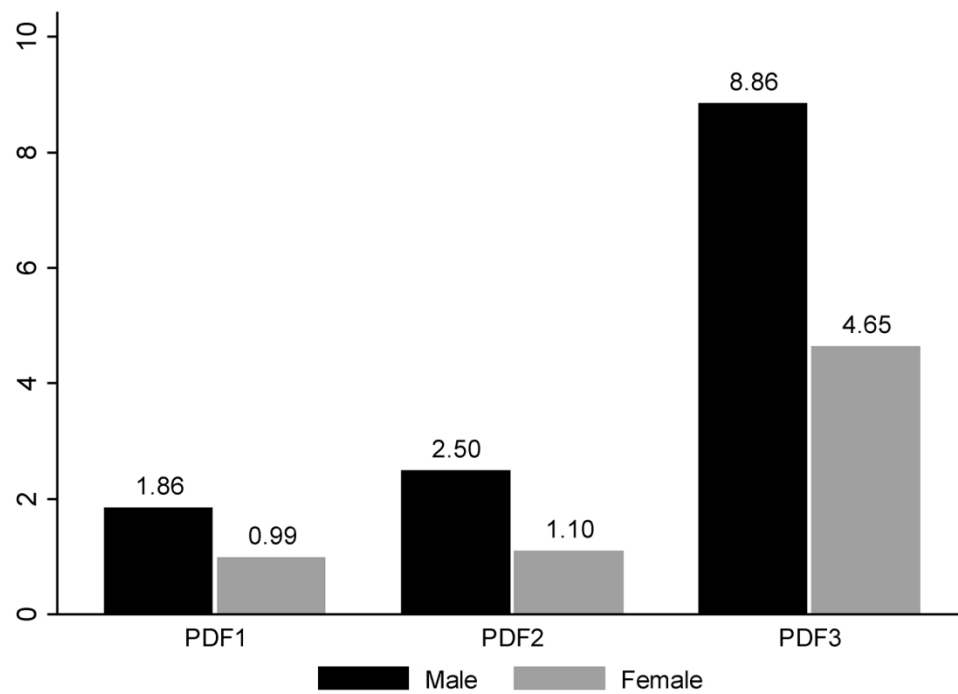


Figure 1 Percentage of BDD between male and female according to PDF1, 2, and 3.

143x104mm (300 x 300 DPI)

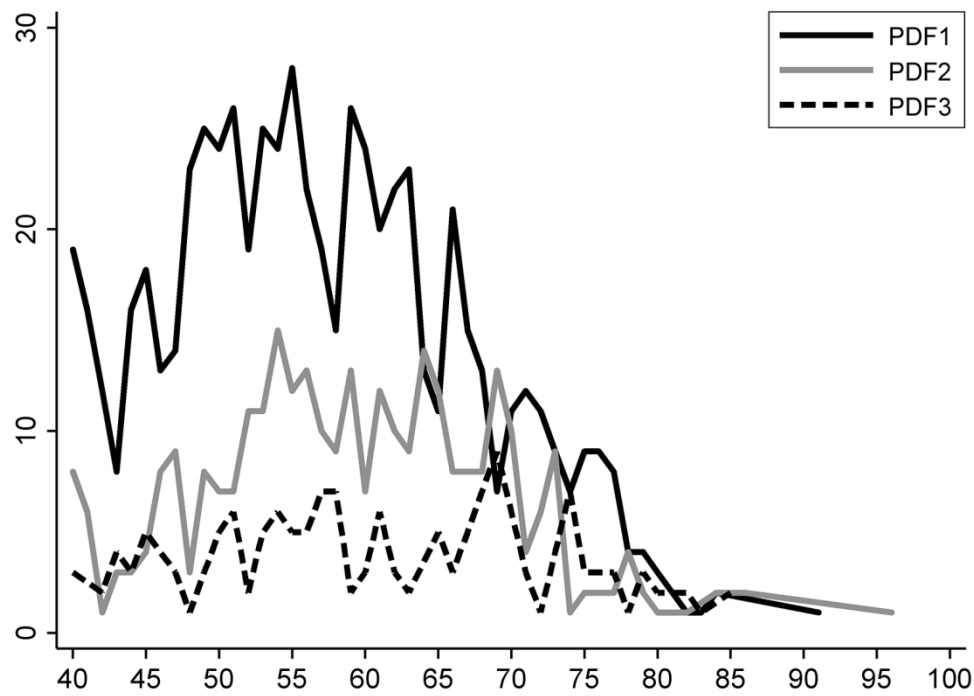


Figure 2 Number of BDD in PDF subjects by age range.

141x103mm (300 x 300 DPI)

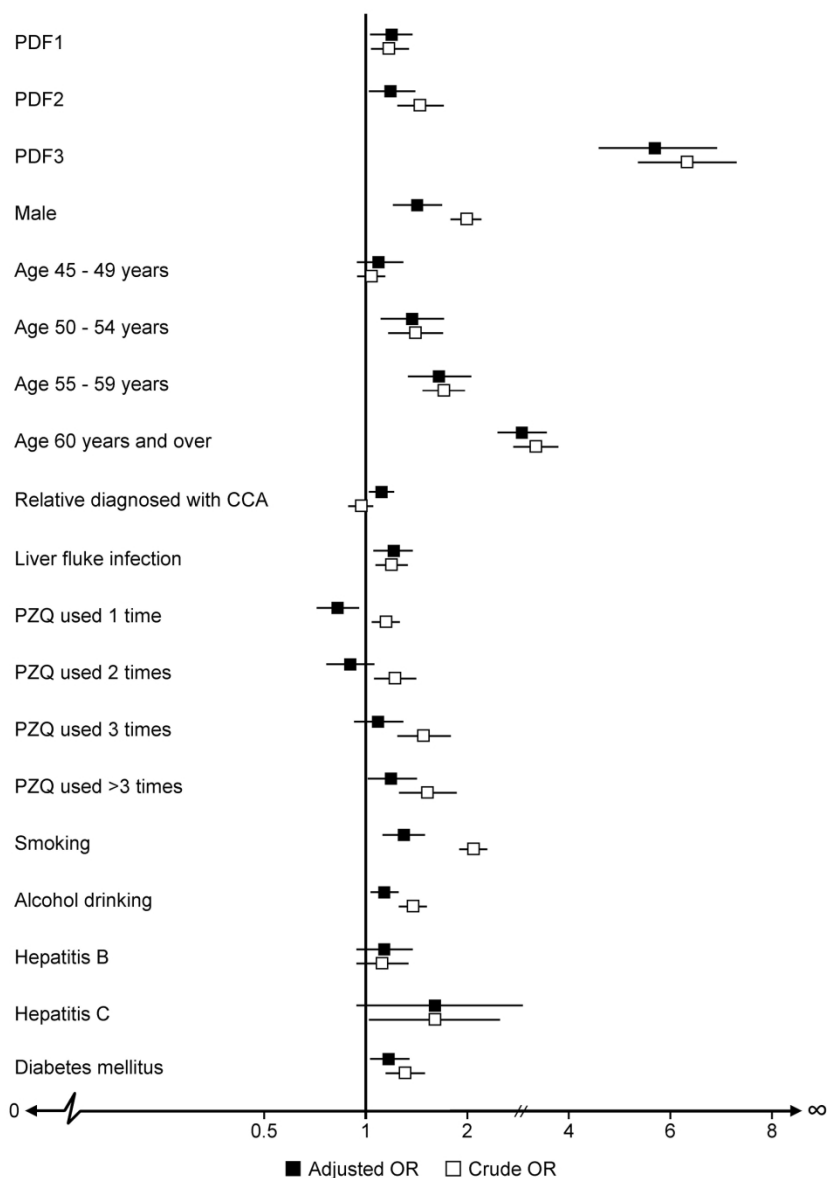


Figure 3 The adjusted OR and crude OR of the associated factors of BDD.

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) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Case-control study—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 Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants
		N/A	(b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed Case-control study—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	(b) Describe any methods used to examine subgroups and interactions
		Pg9	(c) Explain how missing data were addressed
		N/A	(d) Cohort study—If applicable, explain how loss to follow-up was addressed Case-control study—If applicable, explain how matching of cases and controls was addressed Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy
		N/A	(e) Describe any sensitivity analyses

Continued on next page

For peer review only

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](http://www.strobe-statement.org).

## Correction: 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

Chamadol N, Khuntikeo N, Thinkhamrop B, *et al.* 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. *BMJ Open* 2019;9:e023217. doi: 10.1136/bmjopen-2018-023217

This article was previously published with an error.

Ethics reference number was incorrect. The correct number is HE551404.

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

© Author(s) (or their employer(s)) 2019. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

*BMJ Open* 2019;9:e023217corr1. doi:10.1136/bmjopen-2018-023217corr1

