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

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TITLE PAGE

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ABSTRACT

Objectives To assess associations between periductal fibrosis (PDF) and bile duct dilatation (BDD) in ultrasonography (US) screening of population at risk of cholangioncarcinoma (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 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.

INTRODUCTION

Cholangiocarcinoma (CCA) and hepatocellular carcinoma (HCC), are ranked the most prevalent cancers in Southeast Asia.[1-3] The early-stages of CCA can manifest through obstructive jaundice, which is found in 30% of patients who are diagnosed with primary sclerosing cholangitis.[4] Other liver disorders: fatty liver disease, cirrhosis, and liver mass are likewise recognized risk factors for both CCA and HCC.[5-10] 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.[11 12] A previous study demonstrated that US screening is highly sensitive in identifying CCA through confirmed incidences of BDD.[13] 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.[14] 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 (BilIN), and intraductal papillary neoplasm of the bile duct (IPNB).[15 16] Periductal fibrosis (PDF) is another abnormality of the bile duct which has been used to identify people at risk of developing CCA, especially in those infected with *Opisthorchis viverrini*.[17-21] PDF is caused by the thickening of the bile duct wall, along the periportal space.[22] PDF can be categorized into three groups (PDF1, 2, and 3), which were first classified by the World Health Organization (WHO).[23] Based on certain US findings, 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.[19]

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.[8] As a result of this relationship, US detection has been utilized to identify people with PDF as a risk group for CCA development.[8 20 24 25] 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[8 17], 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.[26] This cohort study was conducted at 9 tertiary care hospitals in

Northeast of Thailand. These hospitals serve as the main source of affordable tertiary care for local people in the region. The study aims to recruit all people living in Northeast Thailand and aged 40 years and over, including patients attending screening for CCA and the general population attending primary health care units. 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. Patients in the screening arm will be contacted at least annually to be screening 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 patients 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 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

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

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.

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DISCUSSION

Liver cancer is one of the leading causes of death throughout the world.[27] CCA accounts for more than 60% of these liver cancer cases with Northeast Thailand reporting the highest incidence in the world.[28 29] PDF and BDD have been recognized as the key risk factors of CCA development.[8 17 21] 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.[17] In addition, studies conducted in Thailand report only PDF as a major risk factor of CCA development.[8 21 30]

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.[31] A study from Canada in 2014 found that older age was associated with bile duct diameters which increases with age.[32] 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.[33] 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.[34] HBV infection was also related to a 3.4-fold risk of ICC in China.[35] 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).[36]

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.[37] 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.[38 39] 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.[40] 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;

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.

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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.

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

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

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

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REFERENCES

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

polymorphisms. *Cancer epidemiology* 2012;36(2):e89-94. doi: 10.1016/j.canep.2011.11.007

6. Tao LY, He XD, Qu Q, et al. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma: a case-control study in China. *Liver international : official journal of the International Association for the Study of the Liver* 2010;30(2):215-21. doi: 10.1111/j.1478-3231.2009.02149.x
7. Shaib YH, El-Serag HB, Nooka AK, et al. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma: a hospital-based case-control study. *The American journal of gastroenterology* 2007;102(5):1016-21. doi: 10.1111/j.1572-0241.2007.01104.x
8. Chamadol N, Pairojkul C, Khuntikeo N, et al. Histological confirmation of periductal fibrosis from ultrasound diagnosis in cholangiocarcinoma patients. *Journal of hepato-biliary-pancreatic sciences* 2014;21(5):316-22. doi: 10.1002/jhbp.64
9. Welzel TM, Graubard BI, El-Serag HB, et al. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma in the United States: a population-based case-control study. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* 2007;5(10):1221-8. doi: 10.1016/j.cgh.2007.05.020
10. Shaib YH, El-Serag HB, Davila JA, et al. Risk factors of intrahepatic cholangiocarcinoma in the United States: a case-control study. *Gastroenterology* 2005;128(3):620-6.
11. Hamilton SR, Aaltonen LA. Pathology and genetics of tumours of the digestive system. 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 Institue. Guidelines for screening, diagnosis and treatment of liver cancer and cholangiocarcinoma. Bankok: 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. 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

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.

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.

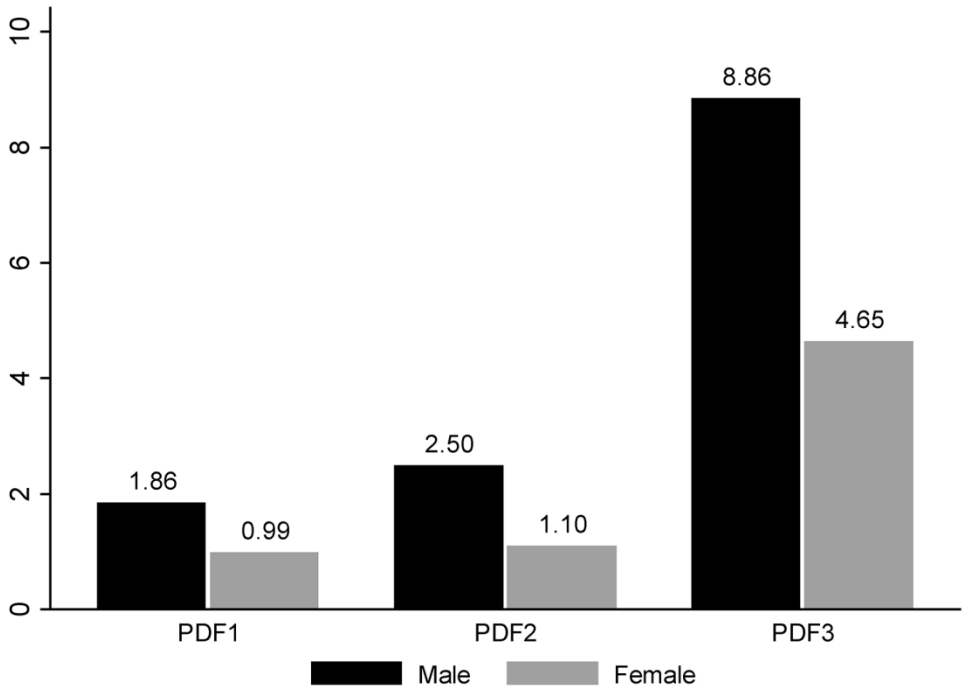

Figure 1 Percentage of BDD between male and female according to PDF1, 2, and 3.

143x104mm (300 x 300 DPI)

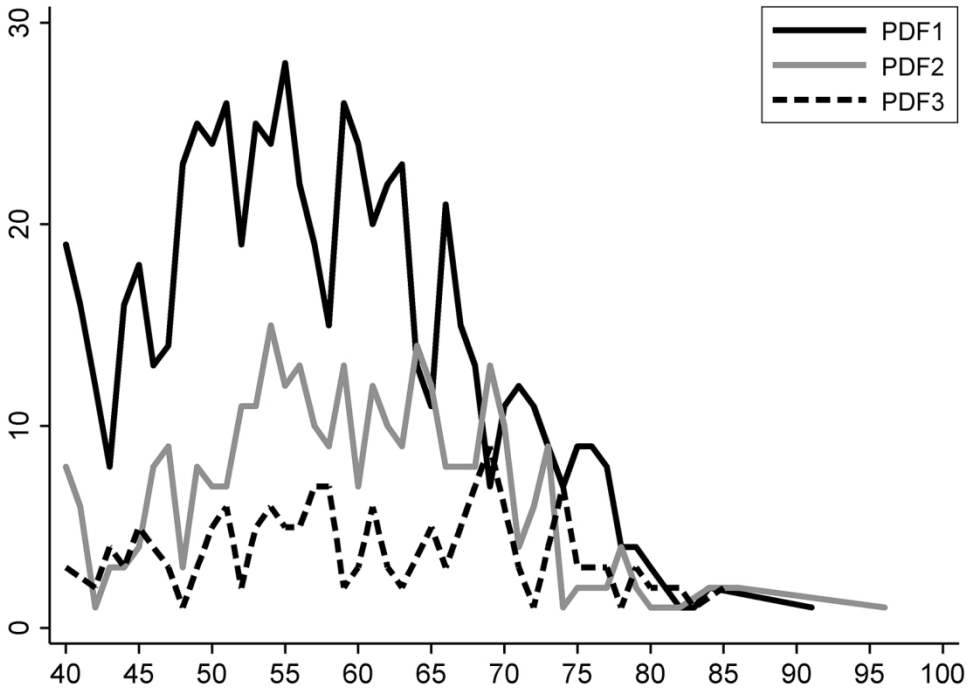

Figure 2 Number of BDD in PDF subjects by age range.

141x103mm (300 x 300 DPI)

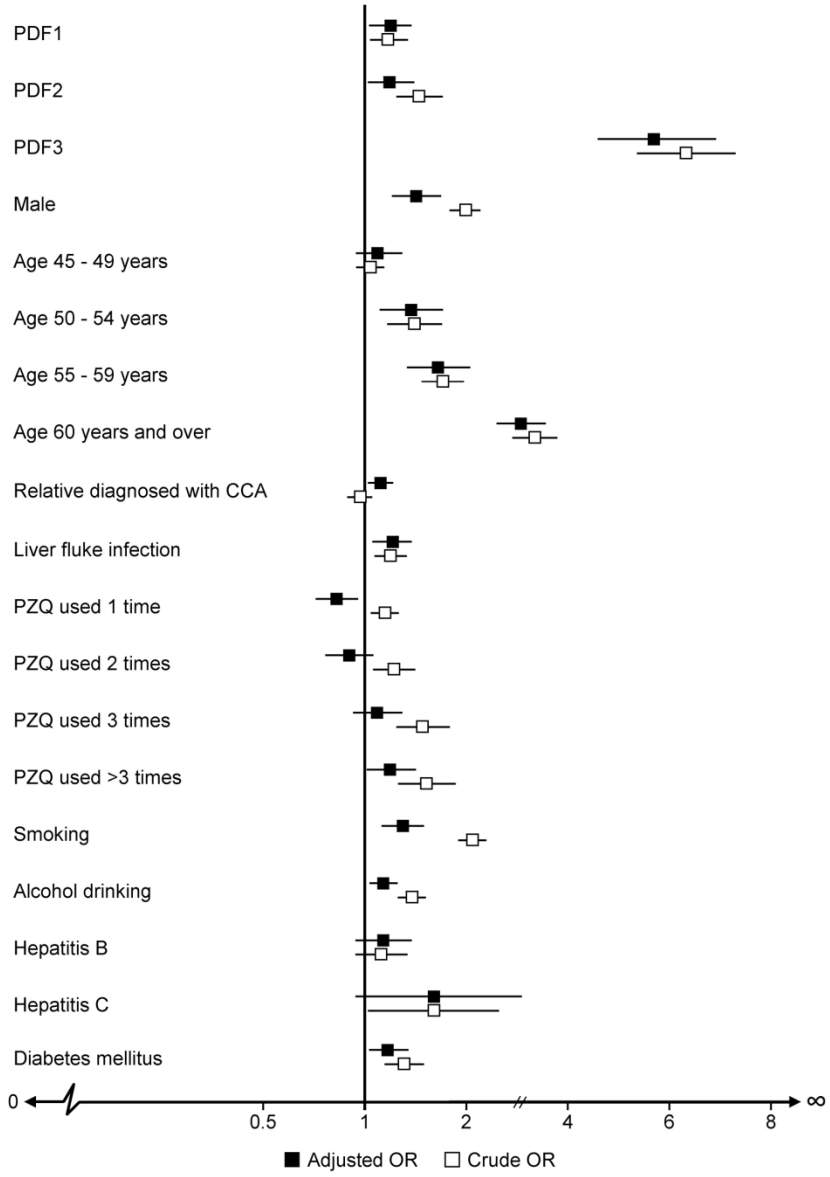

Figure 3 The adjusted OR and crude OR of the associated factors of BDD.

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

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) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Pgs9-10	*Cohort study*—Report numbers of outcome events or summary measures over time
		N/A	*Case-control study*—Report numbers in each exposure category, or summary measures of exposure
		Pg25	*Cross-sectional study*—Report numbers of outcome events or summary measures
Main results	16	Pgs9-10	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		N/a	(*b*) Report category boundaries when continuous variables were categorized
		N/a	(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	N/A	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
		Discussion	
Key results	18	Pg11-12	Summarise key results with reference to study objectives
Limitations	19	Pg4	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Pg14	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Pg14	Discuss the generalisability (external validity) of the study results
		Other information	
Funding	22	Pg15	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

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

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

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

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TITLE PAGE

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

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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 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.

INTRODUCTION

Cholangiocarcinoma (CCA) and hepatocellular carcinoma (HCC), are ranked the most prevalent cancers in Southeast Asia.[1-3] The early-stages of CCA can manifest through obstructive jaundice, which is found in 30% of patients who are diagnosed with primary sclerosing cholangitis.[4] Other liver disorders: fatty liver disease, cirrhosis, and liver mass are likewise recognized risk factors for both CCA and HCC.[5-10] 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.[11 12] A previous study demonstrated that US screening is highly sensitive in identifying CCA through confirmed incidences of BDD.[13] 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.[14] 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 (BilIN), and intraductal papillary neoplasm of the bile duct (IPNB).[15 16] 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*.[17-21] 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.[22]

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.[8] As a result of this relationship, US detection has been utilized to identify people with PDF as a risk group for CCA development.[8 20 23 24] 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.[8] 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[8 17], 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-

up approach.[25] 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.[26] 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.[8] 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.[19]

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.

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

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>

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DISCUSSION

Liver cancer is one of the leading causes of death throughout the world.[27] CCA accounts for more than 60% of these liver cancer cases with Northeast Thailand reporting the highest incidence in the world.[28 29] PDF and BDD have been recognized as the key risk factors of CCA development.[8 17 21] 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.[17] In addition, studies conducted in Thailand report only PDF as a major risk factor of CCA development.[8 21 30]

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,[17-21] 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.[31] A study from Canada in 2014 found that older age was associated with bile duct diameters which increases with age.[32] 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.[33] 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.[34] HBV infection was also related to a 3.4-fold risk of ICC in China.[35] 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).[36]

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.[37] 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.[38 39] 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.[40] 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

All authors declare no conflict of interest.

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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.

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

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

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

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

REFERENCES

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

of the International Association for the Study of the Liver 2010;30(2):215-21. doi: 10.1111/j.1478-3231.2009.02149.x

7. Shaib YH, El-Serag HB, Nooka AK, et al. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma: a hospital-based case-control study. *The American journal of gastroenterology* 2007;102(5):1016-21. doi: 10.1111/j.1572-0241.2007.01104.x
8. Chamadol N, Pairojkul C, Khuntikeo N, et al. Histological confirmation of periductal fibrosis from ultrasound diagnosis in cholangiocarcinoma patients. *Journal of hepato-biliary-pancreatic sciences* 2014;21(5):316-22. doi: 10.1002/jhbp.64
9. Welzel TM, Graubard BI, El-Serag HB, et al. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma in the United States: a population-based case-control study. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* 2007;5(10):1221-8. doi: 10.1016/j.cgh.2007.05.020
10. Shaib YH, El-Serag HB, Davila JA, et al. Risk factors of intrahepatic cholangiocarcinoma in the United States: a case-control study. *Gastroenterology* 2005;128(3):620-6.
11. Hamilton SR, Aaltonen LA. Pathology and genetics of tumours of the digestive system. 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 Institue. Guidelines for screening, diagnosis and treatment of liver cancer and cholangiocarcinoma. Bankok: 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

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.

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

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.

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.

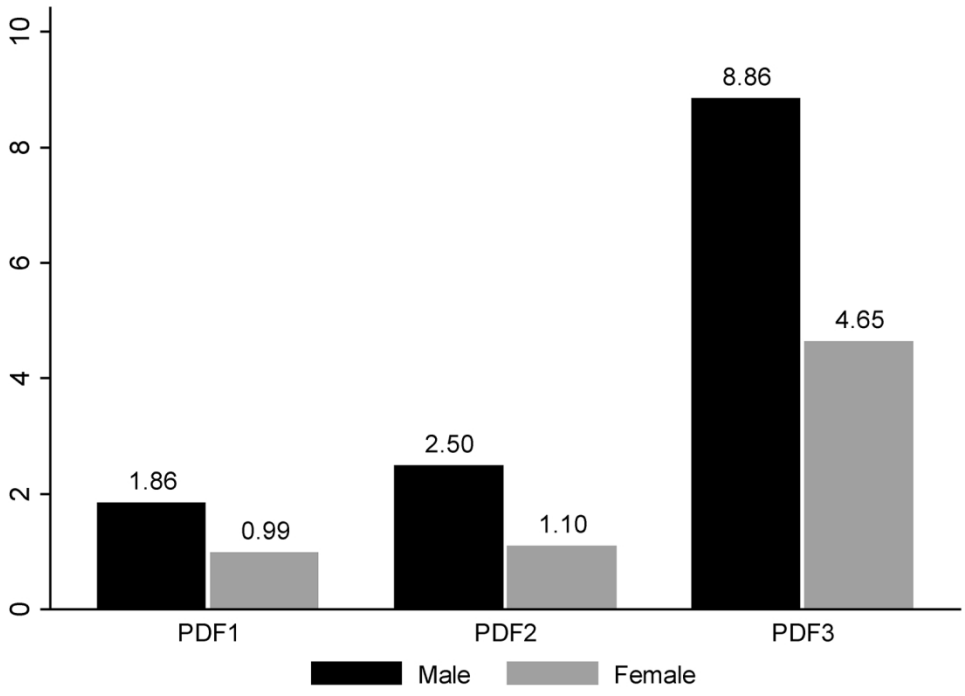

Figure 1 Percentage of BDD between male and female according to PDF1, 2, and 3.

143x104mm (300 x 300 DPI)

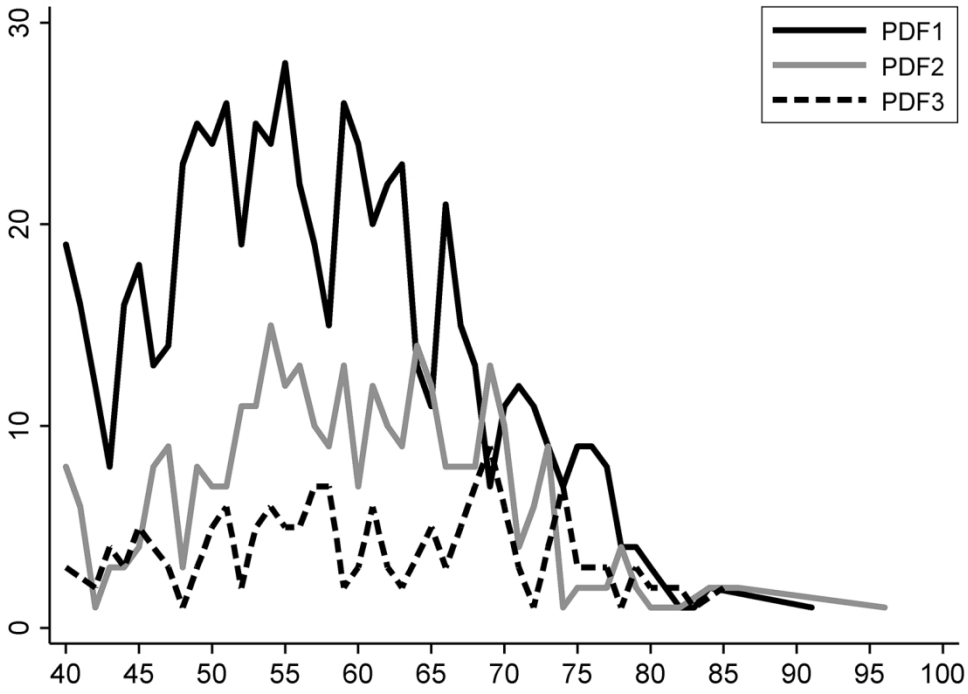

Figure 2 Number of BDD in PDF subjects by age range.

141x103mm (300 x 300 DPI)

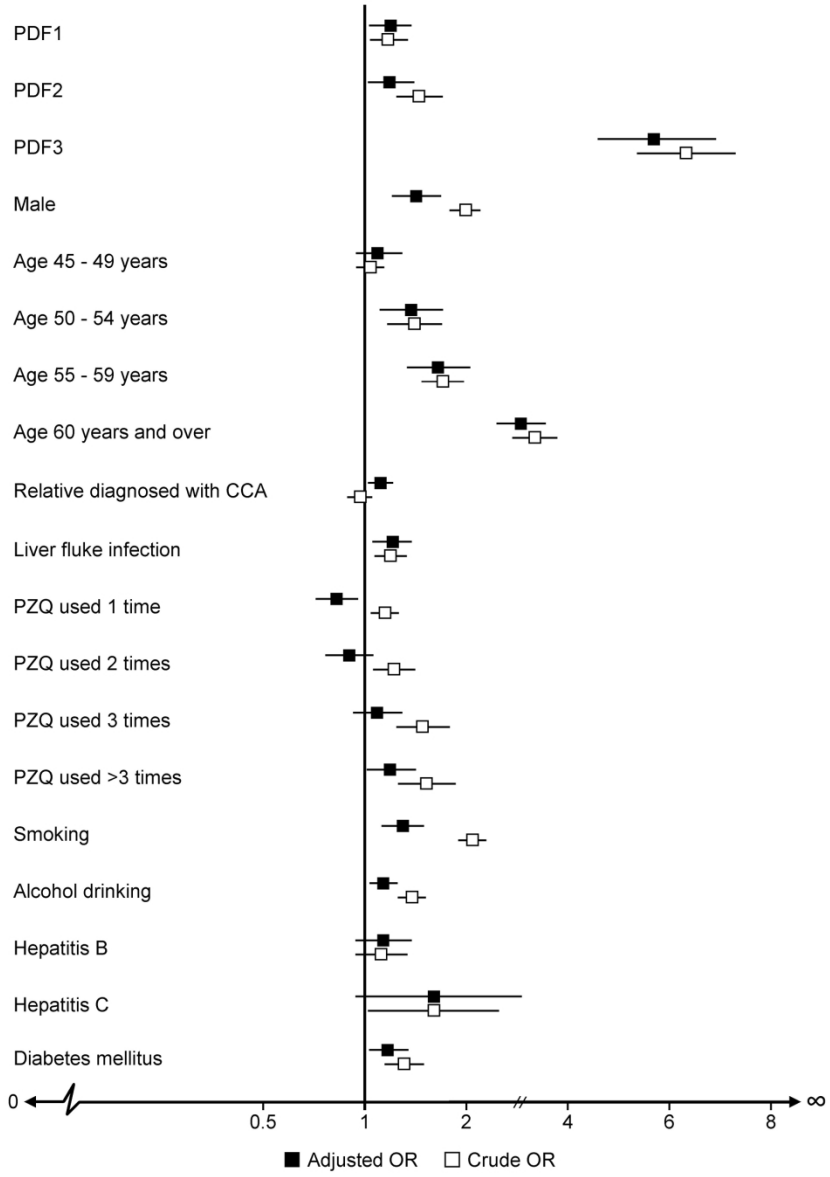

Figure 3 The adjusted OR and crude OR of the associated factors of BDD.

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

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) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Pgs9-10	*Cohort study*—Report numbers of outcome events or summary measures over time
		N/A	*Case-control study*—Report numbers in each exposure category, or summary measures of exposure
		Pg25	*Cross-sectional study*—Report numbers of outcome events or summary measures
Main results	16	Pgs9-10	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		N/a	(*b*) Report category boundaries when continuous variables were categorized
		N/a	(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	N/A	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
		Discussion	
Key results	18	Pg11-12	Summarise key results with reference to study objectives
Limitations	19	Pg4	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Pg14	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Pg14	Discuss the generalisability (external validity) of the study results
		Other information	
Funding	22	Pg15	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

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

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

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

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TITLE PAGE

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

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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.

INTRODUCTION

Cholangiocarcinoma (CCA) and hepatocellular carcinoma (HCC), are ranked the most prevalent cancers in Southeast Asia.[1-3] The early-stages of CCA can manifest through obstructive jaundice, which is found in 30% of patients who are diagnosed with primary sclerosing cholangitis.[4] Other liver disorders: fatty liver disease, cirrhosis, and liver mass are likewise recognized risk factors for both CCA and HCC.[5-10] 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.[11 12] A previous study demonstrated that US screening is highly sensitive in identifying CCA through confirmed incidences of BDD.[13] 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.[14] 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 (BilIN), and intraductal papillary neoplasm of the bile duct (IPNB).[15 16] 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*.[17-21] 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.[22]

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.[8] As a result of this relationship, US detection has been utilized to identify people with PDF as a risk group for CCA development.[8 20 23 24] 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.[8] 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[8 17], 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-

up approach.[25] 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.[26] 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.[8] 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.[19]

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.

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

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>

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DISCUSSION

Liver cancer is one of the leading causes of death throughout the world.[27] CCA accounts for more than 60% of these liver cancer cases with Northeast Thailand reporting the highest incidence in the world.[28 29] PDF and BDD have been recognized as the key risk factors of CCA development.[8 17 21] 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.[17] In addition, studies conducted in Thailand report only PDF as a major risk factor of CCA development.[8 21 30]

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,[17-21] 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.[31] A study from Canada in 2014 found that older age was associated with bile duct diameters which increases with age.[32] 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.[33] 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.[34] HBV infection was also related to a 3.4-fold risk of ICC in China.[35] 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).[36]

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.[37] 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.[38 39] 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.[40] 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.

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

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.

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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.

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

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

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

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

REFERENCES

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

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

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.

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

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.

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

559

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.

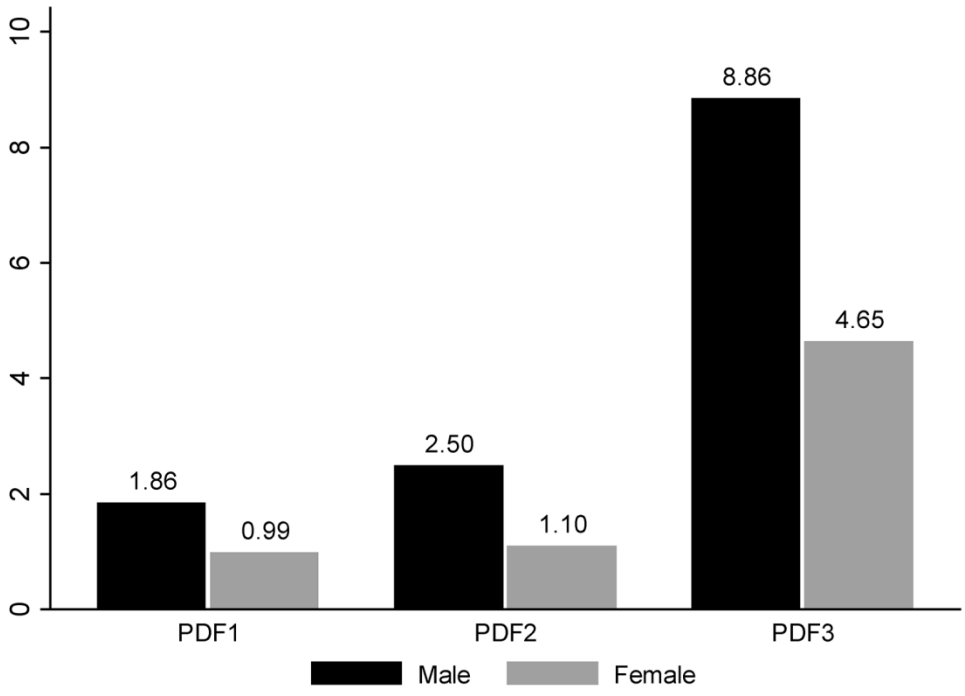

Figure 1 Percentage of BDD between male and female according to PDF1, 2, and 3.

143x104mm (300 x 300 DPI)

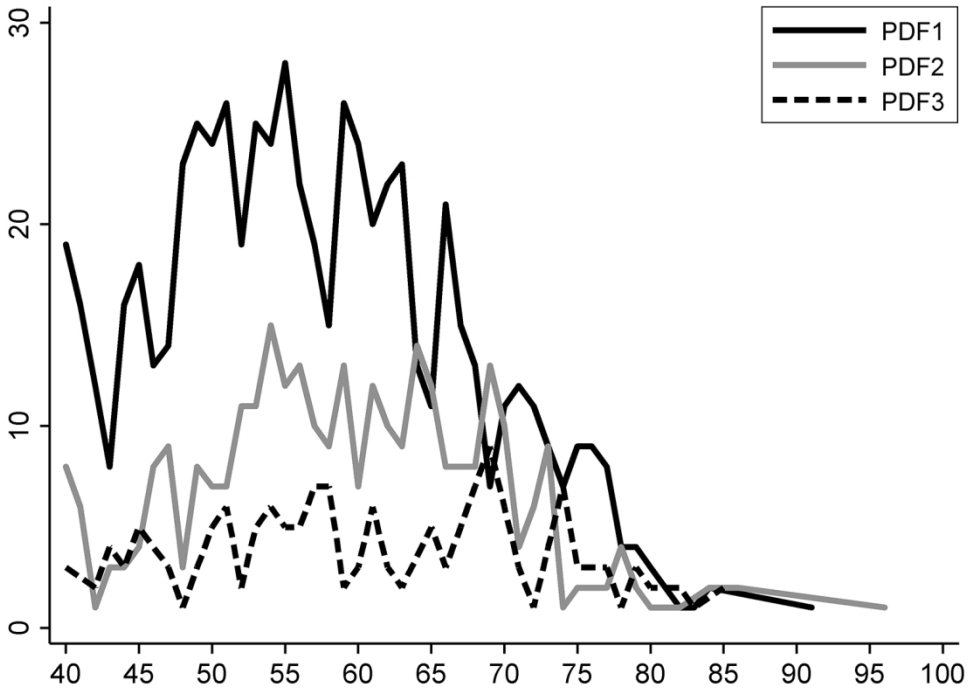

Figure 2 Number of BDD in PDF subjects by age range.

141x103mm (300 x 300 DPI)

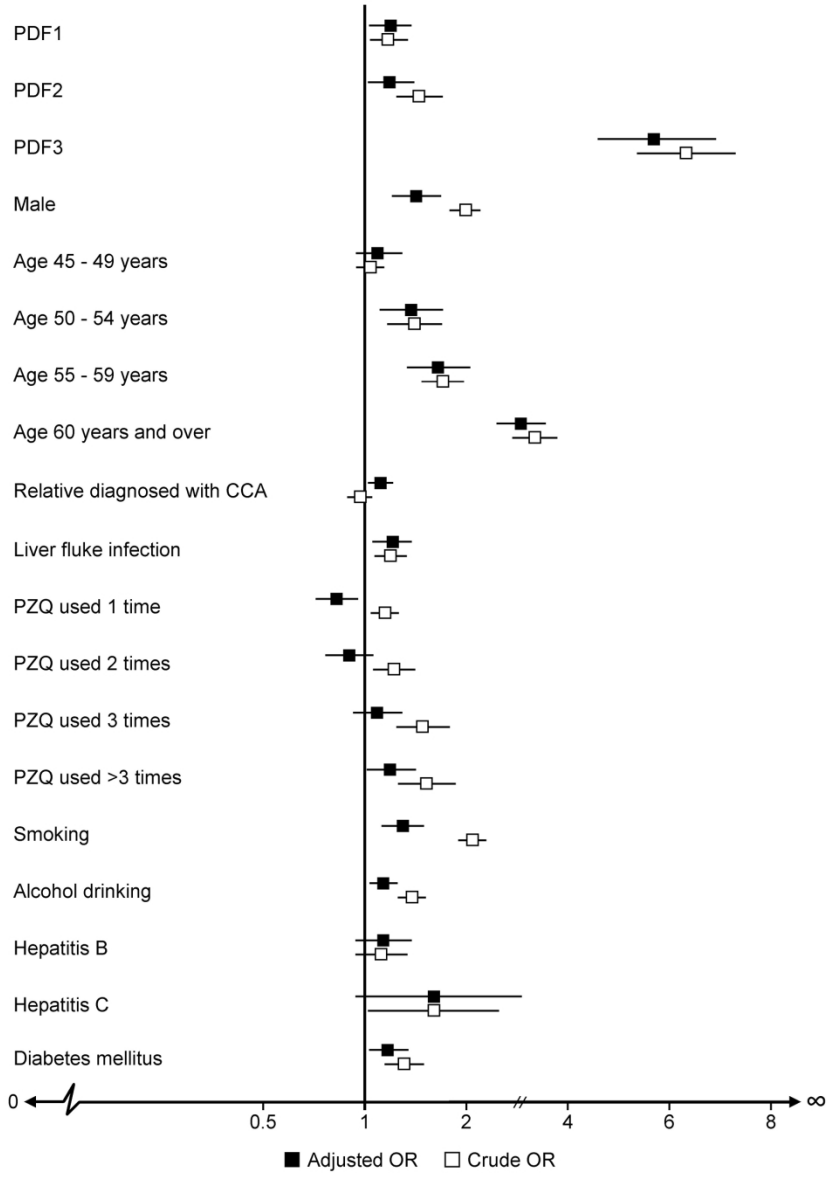

Figure 3 The adjusted OR and crude OR of the associated factors of BDD.

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

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) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Pgs9-10	*Cohort study*—Report numbers of outcome events or summary measures over time
		N/A	*Case-control study*—Report numbers in each exposure category, or summary measures of exposure
		Pg25	*Cross-sectional study*—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.