

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	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
AUTHORS	Chamadol, Nittaya; Khuntikeo, Narong; Thinkhamrop, Bandit; Thinkhamrop, Kavin; Suwannatrai, Apiporn; Kelly, Matthew; Promthet, Supanee

VERSION 1 - REVIEW

REVIEWER	Yasunori Sato Keio University School of Medicine, Japan
REVIEW RETURNED	14-May-2018

GENERAL COMMENTS	<p>The study by Chamadol et al. is of interest considering the association between periductal fibrosis and bile duct dilatation in ultrasonography screening. The present paper is well written and the study design is appropriate to assess association between PDF and BDD. The conclusions are sound and based on the results that were obtained from the study. I would have no strong reservations about the publication of the paper. However, the following point needs clarification:</p> <p>PDF was categorized into three groups by ultrasound echo. It is not clear the classification where, how, and by whom. Please explain and discuss the internal validity and external validity of this classification.</p>
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REVIEWER	Masayuki Nakano Shounann Fujisawa Tokushuukai Hospital, Diagnostic Pathology Japan
REVIEW RETURNED	15-Aug-2018

GENERAL COMMENTS	<p>A lot of cases are considered, but it's consideration only of an image. If there is no consideration with some pathological finding under an image, we can't judge whether your interpretation of an image is right. So, you need an information about histopathology that you study cases.</p>
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REVIEWER	Peter Odermatt Swiss Tropical and Public Health Institute Switzerland I am associated with the CASCAP Team in Project in Lao PDR
REVIEW RETURNED	22-Aug-2018

GENERAL COMMENTS	<p>In Northeast Thailand cholangiocarcinoma (CCA) has the highest incidence worldwide which is around 50 times higher than in any other country. CCA is linked with the frequent consumption of raw fish of large part of the population which results in high infection rates with the Asian liver fluke <i>Opisthorchis viverrini</i> and severe hepato-biliary morbidity.</p> <p>Since more than thirty years, the Thai health services are combatting the infection and the resulting high CCA incidence. In recent years the Cholangiocarcinoma Screening and Care Program (CASCAP) was launched with the aim to early identify persons at risk and include them into early care programs (follow-up and early surgery if necessary). The current manuscript reports on data from CASCAP which includes almost 400'000 enrolled participants and addresses the question of how periductal fibrosis (PDF) is associated with bile duct dilatations (BDD) in view of defining (additional) early markers for CCA. The study concludes that PDF and BDD are strongly associated. Hence, PDF can also be used as CCA risk marker. The authors report on further interesting findings, namely on gender, age and smoking history which were also associated with BDD.</p> <p>This is a most interesting report based an enormous amount of data collected in CASCAP. It will enrich the discussion on prevention and early detection of CCA in Thailand and the neighbouring countries in Southeast Asia.</p> <p>There are a few important points to address:</p> <ol style="list-style-type: none"> 1. CASCAP is a screening program, and it is the basis of the current study population. Detailed including and excluding criteriae need to be presented in the report in order that study population is well characterised. 2. Following 1, it is recommended in this report not to use the terms "cross-sectional study" or "cohort study" as this is somehow misleading. Alternatively, the authors can use the term "CASCAP has a cross-sectional design". 3. PDF assessment: Given the key importance of this indicator the authors should explain in detail how in CASCAP the PDF's are staged into PDF 1, 2 and 3 (what are the critariae). The reference given refers to a document on schistosomiasis. Therefore, detailed information is required. 4. Raw and insufficiently cooked fish consumption is a key risk factor for liver fluke infections and therefore, also for CCA development. In the result and discussion section it should be mentioned. 5. Repeated praziquantel (PZQ) treatment: PZQ treatment is the key control measure for liver fluke infection and related morbidity promoted by WHO. The authors found an association between the
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	<p>number PZQ treatments and BDD in the bivariate analysis but not in the multivariable analysis. Given the public health importance of PZQ treatment, it would most important to discuss this issue in the discussion section.</p> <p>6. Finally, can the authors provide their thoughts on the limitations of the study. E.g., given that the study population (CASCAP) is not randomly selected, in how far does this influence the results?</p>
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VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name: Yasunori Sato

Institution and Country: Keio University School of Medicine, Japan

Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below

The study by Chamadol et al. is of interest considering the association between periductal fibrosis and bile duct dilatation in ultrasonography screening. The present paper is well written and the study design is appropriate to assess association between PDF and BDD. The conclusions are sound and based on the results that were obtained from the study. I would have no strong reservations about the publication of the paper. However, the following point needs clarification:

PDF was categorized into three groups by ultrasound echo. It is not clear the classification where, how, and by whom. Please explain and discuss the internal validity and external validity of this classification.

We have added the following text to the Methods section to clarify how PDF was categorised and by whom.

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.²³ 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.

Reviewer: 2

Reviewer Name: Masayuki Nakano

Institution and Country: Shounann Fujisawa Tokushuukai Hospital, Diagnostic Pathology Japan

Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below A lot of cases are considered, but it's consideration only of an image. If there is no consideration with some pathological finding under an image, we can't judge whether your interpretation of an image is right. So, you need an information about histopathology that you study cases.

We agree that histopathological confirmation of the ultrasound derived diagnoses in our study is important. In the CASCAP project which this report is part of, there is a stepped process of diagnoses to treatment in the development of CCA. The first stage as reported here is the identification of participants with potential hepatobiliary abnormalities through ultrasound. In cases where the ultrasound identifies serious problems the patients will then be offered the opportunity for histopathological confirmation of their diagnosis. As this is a large ongoing study future analyses and publications will address the next aspect of the project.

Ultrasound however has been found to be reliable in predicting histological confirmation. We have added the following text in the introduction to clarify:

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

Reviewer: 3

Reviewer Name: Peter Odermatt

Institution and Country: Swiss Tropical and Public Health Institute
Switzerland

Please state any competing interests or state 'None declared': I am associated with the CASCAP Team in Project in Lao PDR

Please leave your comments for the authors below

In Northeast Thailand cholangiocarcinoma (CCA) has the highest incidence worldwide which is around 50 times higher than in any other country. CCA is linked with the frequent consumption of raw fish of large part of the population which results in high infection rates with the Asian liver fluke *Opisthorchis viverrini* and severe hepato-biliary morbidity.

Since more than thirty years, the Thai health services are combatting the infection and the resulting high CCA incidence. In recent years the Cholangiocarcinoma Screening and Care Program (CASCAP) was launched with the aim to early identify persons at risk and include them into early care programs (follow-up and early surgery if necessary). The current manuscript reports on data from CASCAP which includes almost 400'000 enrolled participants and addresses the question of how periductal fibrosis (PDF) is associated with bile duct dilatations (BDD) in view of defining (additional) early markers for CCA. The study concludes that PDF and BDD are strongly associated. Hence, PDF can also be used as CCA risk marker. The authors report on further interesting findings, namely on gender, age and smoking history which were also associated with BDD.

This is a most interesting report based on an enormous amount of data collected in CASCAP. It will enrich the discussion on prevention and early detection of CCA in Thailand and the neighbouring countries in Southeast Asia.

There are a few important points to address:

1. CASCAP is a screening program, and it is the basis of the current study population. Detailed including and excluding criteria need to be presented in the report in order that study population is well characterised.

Thanks for your comment, and it is true that the study population should be clearly defined. For this study however, there are no strict inclusion or exclusion criteria given the aim of recruiting all Northeast Thai residents aged 40 years or over. We have added the following sentence to the Methods, Study design section to make that clear:

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.

2. Following 1, it is recommended in this report not to use the terms “cross-sectional study” or “cohort study” as this is somehow misleading. Alternatively, the authors can use the term “CASCAP has a cross-sectional design”.

The CASCAP project is strictly speaking a cohort study in that it has recruited a pool of participants and will follow them up over time with periodic screening of participants and treatment where symptoms have been identified. The analysis presented in this paper however are cross sectional results from the first baseline data of participants. We have more carefully considered our description of the study and clarified where needed.

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.²⁶ 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.

3. PDF assessment: Given the key importance of this indicator the authors should explain in detail how in CASCAP the PDF's are staged into PDF 1, 2 and 3 (what are the criteria). The reference given refers to a document on schistosomiasis. Therefore, detailed information is required.

We have added the following text to the Methods section to give more detail on this method:

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.²³ 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.

4. Raw and insufficiently cooked fish consumption is a key risk factor for liver fluke infections and therefore, also for CCA development. In the result and discussion section it should be mentioned.

We agree that this fish consumption behaviour is one of the main risk factors for CCA development in Thailand. We have added the following text in the introduction:

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.

And in the discussion.

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.

5. Repeated praziquantel (PZQ) treatment: PZQ treatment is the key control measure for liver fluke infection and related morbidity promoted by WHO. The authors found an association between the number PZQ treatments and BDD in the bivariate analysis but not in the multivariable analysis. Given the public health importance of PZQ treatment, it would most important to discuss this issue in the discussion section.

The association between history of PZQ treatment and current liver fluke infection and hepatobiliary abnormalities is the focus of other analyses for the CASCAP project. Given that the relationship is not significant here we prefer not to distract from the main issues discussed in this paper by going further into that relationship.

6. Finally, can the authors provide their thoughts on the limitations of the study. E.g., given that the study population (CASCAP) is not randomly selected, in how far does this influence the results?

Thanks for the suggestion we have added the following to the discussion:

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 behaviours in terms of smoking and alcohol consumption were self-reported leading to some potential bias in their measurements.

VERSION 2 – REVIEW

REVIEWER	Masayuki Nakano Division of Surgical Pathology, Shounann Fujsawa Tokushuukai Hospital. Kanagawa prefecture, Japan.
REVIEW RETURNED	22-Oct-2018

GENERAL COMMENTS	I under stand your opinion that early detections of CCA symptom is essential and very important especially in the prevalent country. And PDF 3 with BBD are corelation symptom maybe correct as you consider, however it requires these symptom come from CCA scientifically. Authors may have a plan to do so as next study, but it is inverse order.
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REVIEWER	Peter Odermatt Swiss Tropical and Public Health Institute KKU and Swiss TPH are collaborating on liver fluke infection / morbidity Projects in Lao PDR.
REVIEW RETURNED	02-Nov-2018

GENERAL COMMENTS	With the Revision the authors have addressed the points raised.
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