RESEARCH ARTICLE

Factors Associated with Periductal Fibrosis Diagnosed by Ultrasonography Screening among a High Risk Population for Cholangiocarcinoma in Northeast Thailand

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Abstract

Background: The population in northeast Thailand continues to present with hepatobiliary abnormalities, particularly periductal fibrosis (PDF) which is the result of chronic infection with liver fluke (*Opisthorchis viverini*; OV) and may lead to the development of cholangiocarcinoma (CCA). Although the prevalence of OV infection has been decreased due to a liver fluke control program over decades, the prevalence of PDF remains high. This study aimed to investigate demographic factors associated with PDF risk based on ultrasonography (US) screening. Materials and Methods: This cross-sectional study is part of the Cholangiocarcinoma Screening and Care Program (CASCAP), a prospective cohort study. Multiple logistic regression was used for data analysis. Results: In 55,246 subjects, the overall prevalence of PDF was 33.0% (95%CI: 32.6 - 33.4). Males (33.9 %) were at higher risk for developing PDF than females (32.2 %) (ORcrude = 0.93; 95%CI: 0.89 - 0.96; p-value < 0.001). Factors associated with an increased PDF risk, in addition to OV infection, included old age (≥ 70 years) (ORadj = 1.28, 95% CI: 1.14 - 1.44, p < 0.001) and hepatitis B infection (ORadj = 1.31, 95% CI: 1.11 - 1.55, p = 0.001). In contrast, number of praziquantel treatments (> 2 times) (ORadj = 0.54, 95% CI: 0.47 - 0.63, p < 0.001) and diabetes mellitus (ORadj = 0.57, 95% CI: 0.49 - 0.65, p < 0.001) were significantly associated with a decreased PDF risk. Conclusions: Future US screening should closely examine older people and hepatitis B subjects for the purpose of PDF surveillance among high risk groups for CCA. However, the results of inverse associations require further investigation in order to confirm our findings.

Keywords: Periductal fibrosis - demographic factors - ultrasonography - cholangiocarcinoma

Asian Pac J Cancer Prev, **17 (8)**, 4131-4136

Introduction

Cholangiocarcinoma (CCA), also known as bile duct cancer arising along the intra- or extra-hepatic biliary tree. It accounts for approximately 10–25% of all hepatobiliary malignancies worldwide (Gatto et al., 2010). CCA remains an important public health problem in countries of mainland Southeast Asia, especially in northeast Thailand, which has been reported the world's highest incidence of CCA (Sripa and Pairojkul, 2008; Moore et al., 2010; Shin et al., 2010). The incidence is rather high accounting for 87.7 per 100,000 in males and 36.3 per 100,000 in females (Khuhaprema T et al., 2010). Since, Thailand is an endemic area of liver flukes (Opisthorchis viverrini; OV), a major etiology of CCA, that cause chronic inflammation and are considered to be carcinogens (Rizvi and Gores, 2013). Most subjects with CCA have a poor prognosis and typically present at advanced stages. This is likely to be a neglected tropical disease.

It has been already known that OV infection is associated with asymptomatic hepatobiliary abnormalities, particularly periductal fibrosis (PDF). Although, the prevalence of OV infection over a 14-year period has decreased due to a liver fluke control program by the Ministry of Public Health (Yeoh et al., 2015), the previous community-based ultrasound study revealed the prevalence of PDF in endemic area of northeast Thailand was 23.6% (Of 3,359 subjects) (Mairiang et al., 2012).

In addition, PDF is a prominent finding based on ultrasonography (US) screening conducted by the ongoing cohort study of Cholangiocarcinoma Screening and Care Program (CASCAP). CASCAP comprises two cohorts-the screening and the patient cohorts. This is the first project on CCA that involves screening population who

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resident in endemic areas of northeast Thailand at the community level (Khuntikeo et al., 2015). There might be other potential factors associated with PDF risk such as demographic factors, unhealthy behaviors and underlying diseases.

This study aimed to investigate factors associated with PDF based on US screening among population who were at high risk for CCA in endemic areas. A large sample size of this community-based study could represent the prevalence of PDF among population in northeast Thailand and confirm their associated factors.

Materials and Methods

Study design

This cross-sectional study is part of the ongoing cohort study of CASCAP. The present study emphasized on the screening cohort. The abdominal US screening will be conducted regularly at least annually to determine the abnormalities of current bile duct and/or liver among population at risk in northeast Thailand (Khuntikeo et al., 2015).

Study population

A total of 85,950 individuals enrolled in the screening cohort of CASCAP project from August 2013 to May 2015. This study recruited all 55,246 subjects, who underwent the US examination with their willingness (Figure 1). These subjects were recruited under these criteria; they have to be typical northeast Thai aged of 40 years and over with any one or more of the following; 1) ever been infected by liver fluke or 2) ever treated with praziquantel or 3) ever having consumed raw freshwater fish with scales.

Data collection

Information on demographic factors was obtained from questionnaires. The independent factors were gender, age, OV infection, number of praziquantel treatments, family history of CCA, smoking, alcohol drinking, hepatitis B, hepatitis C and diabetes mellitus (DM). The dependent variable was PDF based on US findings which conducted by well trained radiologists. A radiologist who performed US examination did not concern the history of OV infection among these subjects. The PDF was defined by an increased periportal echo (IPE) caused by thickening of the bile duct wall which runs parallel to the portal vein (Benedetti et al., 2008).

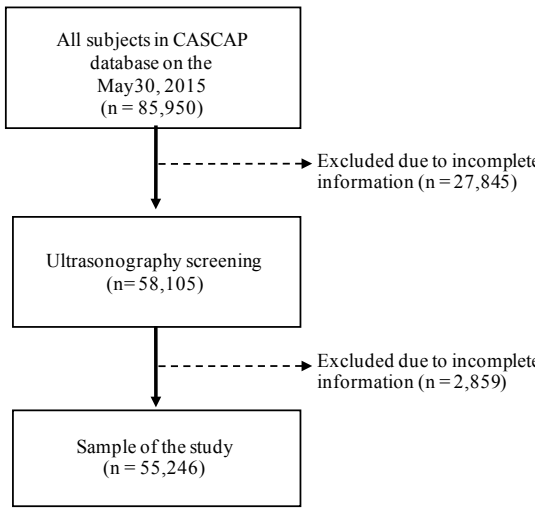

Figure 1. Algorithm of Sample Selection

Data analysis

Descriptive statistics were used to describe demographic characteristics. Frequency and percentage were used to present all categorical variables. In case of continuous variables, mean (± standard deviation) and median (minimum: maximum) were used. The bivariate analysis using simple logistic regression was carried out to explore the association between PDF and each factor, presented as crude odds ratio (ORcrude) and their 95% confident intervals (95% CI).

Factors associated with PDF in the bivariate analysis (p-value <0.25) and factors without association but shown to be potential risk factors for PDF based on literature reviews were recruited in the multivariate analysis. The adjusted odds ratio (OR_{adj}) and their 95% CI were computed by using multiple logistic regression. All test statistics were two-tailed and a p-value <0.05

Table 1. Demographic Characteristics of Subjects Undergoing Ultrasonography Screening

Characteristics	Number	Percentage
Gender (n = 48,117*)		
Male	20,155	41.9
Female	27,962	58.1
Age (years) (n = 54,108*)		
40 to 49	20,692	38.2
50 to 59	19,525	36.1
60 to 69	10,615	19.6
≥ 70	3,276	6.1
Mean (standard deviation)	53.7 (±9.2)	
Median (Min : Max)	52 (40 : 99)	
History of OV infection (n = 33,862*)		
No	17,759	52.5
Yes	16,103	47.5
No. of PZQ treatments (times) (n = 52,829*)		
0	29,574	56
1	17,645	33.4
2	3,524	6.6
> 2	2,086	4
Family history of CCA (n = 54,591*)		
No	35,713	65.4
Yes	18,878	34.6
Smoking (n = 54,259*)		
No	41,698	76.8
Yes, current or previous	12,561	23.2
Alcohol drinking (n = 54,612*)		
No	29,996	54.9
Yes, current or previous	24,616	45.1
Hepatitis B (n = 50,009*)		
No	48,855	97.7
Yes	1,154	2.3
Hepatitis C (n = 50,009*)		
No	49,920	99.8
Yes	89	0.2
Diabetes Mellitus (n = 50,009*)		
No	47,440	94.9
Yes	2,569	5.1

*The total number of subjects for each factor was different as a result of missing values

Table 2. Prevalence of Periductal Fibrosis and Bivariate Analysis of Associations between Potential Factors and PDF Risk

Factors	Number	% PDF	ORcrude	95% CI	p-value
Overall	55,246	33.0	NA**	NA**	NA**
Gender					<0.001
Male	20,155	33.9	1		
Female	27,962	32.2	0.93	0.89 - 0.96	
Age (years)					<0.001
40 to 49	20,692	33.5	1		
50 to 59	19,525	31.2	0.9	0.86 - 0.94	
60 to 69	10,615	32.9	0.98	0.93 - 1.03	
≥ 70	3,276	38.6	1.25	1.16 - 1.35	
History of OV infection					0.017
No	17,759	31.6	1		
Yes	16,103	32.8	1.06	1.01 - 1.11	
No. of PZQ treatments (times)					<0.001
0	29,574	34.6	1		
1	17,645	31.4	0.86	0.83 - 0.90	
2	3,524	29.8	0.8	0.74 - 0.87	
> 2	2,086	25.4	0.64	0.58 - 0.71	
Family history of CCA					0.004
No	35,713	32.6	1		
Yes	18,878	33.9	1.06	1.02 - 1.10	
Smoking					<0.001
No	41,698	32.6	1		
Yes, current or previous	12,561	34.3	1.08	1.03 - 1.12	
Alcohol drinking					0.063
No	29,996	32.7	1		
Yes, current or previous	24,616	33.5	1.03	0.99 - 1.07	
Hepatitis B					<0.001
No	48,855	32.4	1		
Yes	1,154	38.6	1.31	1.16 - 1.48	
Hepatitis C					0.004
No	49,920	32.5	1		
Yes	89	47.2	1.85	1.22 - 2.81	
Diabetes Mellitus					<0.001
No	47,440	33.2	1		
Yes	2,569	20.7	0.52	0.48 - 0.58	

**Not applicable

was considered significant. All statistical analyses were performed with STATA version 10 (Stata Corp, 2007).

Ethical consideration

Ethical approval for this study was obtained from the Khon Kaen University Ethics Committee for Human Research based on the Declaration of Helsinki and the ICH Good Clinical Practice Guidelines (Reference no: HE591048).

Results

Demographic characteristics

A total of 55,246 subjects who underwent the US screening, majority were females accounting for 58.1% and 41.9% were males. The mean age of all subjects was 53.7 (±9.2) and the age range of 40 to 99 years.

Nearly half of them had the history of OV infection accounting for 47.5 %. In addition, 34.6% of subjects had the family history of CCA (Table 1).

Prevalence of periductal fibrosis

The overall prevalence of PDF was 33.0 % (95%CI: 32.6 - 33.4) with significant gender differences (ORcrude = 0.93; 95%CI: 0.89 - 0.96; p-value < 0.001). Moreover, there were significant associations between each potential factor and PDF risk as assessed using bivariate analysis (Table 2).

Factors associated with periductal fibrosis based on ultrasonography screening

The data for associations between all potential factors and PDF risk using multiple logistic regression are shown in Table 3. Demographic factors which were significantly associated with an increased PDF risk, in addition to history of OV infection, included male gender, an age of 70 years and over and hepatitis B.

On the other hand, number of PZQ treatments and diabetes mellitus were significantly associated with a decreased PDF risk. However, there were no significant associations between family history of CCA, alcohol drinking and PDF risk in the present analysis. With regard to smoking, it was considered to be an inconclusive factor for PDF risk due to the observed trivial magnitude of effect (OR_{adj} = 1.09, 95% CI: 1.01 - 1.18, p = 0.04) in the present study.

Table 3. Multivariate Analysis of Associations between Potential Factors and PDF Risk, Presented as Adjusted Odds Ratio (OR_{adj}) and 95% Confidence Intervals (95% CI) Using Multiple Logistic Regression

Factors	Number	% PDF	ORcrude	ORadj	95% CI	p-value
Gender						0.032
Male	20,155	33.9	1	1		
Female	27,962	32.2	0.93	0.93	0.87 - 0.99	
Age (years)						<0.001
40 to 49	20,692	33.5	1	1		
50 to 59	19,525	31.2	0.9	0.96	0.90 - 1.02	
60 to 69	10,615	32.9	0.98	1.04	0.97 - 1.12	
≥ 70	3,276	38.6	1.25	1.28	1.14 - 1.44	
History of OV infection						<0.001
No	17,759	31.6	1	1		
Yes	16,103	32.8	1.06	1.37	1.28 - 1.47	
No. of PZQ treatments (times)						<0.001
0	29,574	34.6	1	1		
1	17,645	31.4	0.86	0.75	0.69 - 0.80	
2	3,524	29.8	0.8	0.7	0.63 - 0.78	
> 2	2,086	25.4	0.64	0.54	0.47 - 0.63	
Family history of CCA						0.963
No	35,713	32.6	1	1		
Yes	18,878	33.9	1.06	1.01	0.95 - 1.06	
Smoking						0.04
No	41,698	32.6	1	1		
Yes, current or previous	12,561	34.3	1.08	1.09	1.01 - 1.18	
Alcohol drinking						0.527
No	29,996	32.7	1	1		
Yes, current or previous	24,616	33.5	1.03	1.02	0.96 - 1.09	
Hepatitis B						0.001
No	48,855	32.4	1	1		
Yes	1,154	38.6	1.31	1.31	1.11 - 1.55	
Diabetes Mellitus						<0.001
No	47,440	33.2	1	1		
Yes	2,569	20.7	0.52	0.57	0.49 - 0.65	

Discussion

This study showed the overall prevalence of PDF was 33.0 % (95%CI: 32.6 - 33.4). We found significantly higher in males (33.9%) than in females (32.2%). Our findings were similar to the study by Mairiang and colleagues. They emphasized on the prevalence of advanced PDF which was 23.6% (95%CI: 22.2 - 25.0) which found in males (28.3%) more than in females (19.0%) with significant difference (Mairiang et al., 2012). Males are known to have higher risk for OV infection, a major cause of PDF, than female due to their eating raw or undercooked fresh water fish with scales coupled with alcohol consumption behaviors (Songserm et al., 2012; Thaewnongiew et al., 2014; Yeoh et al., 2015).

The older subjects, particularly the age of 70 and over, showed significantly higher risk for PDF than other age groups ($OR_{adj.}$ = 1.28, 95% CI: 1.14 - 1.44, p < 0.001). Since the incident of CCA mostly found in the increasing age groups, likewise, the progression of PDF which could develop for decades and usually be detected in the older person (Shaib and El-Serag, 2004; Chamadol et al., 2014). In contrast, another study found that younger people (less than 30 years old) had higher risk for PDF than the older people ($OR_{adj.}$ = 0.47, 95% CI: 0.33 - 0.68, p<0.001) (Mairiang et al., 2012).

Our results demonstrated that subjects with the history of OV infection had 37% more likely to have PDF than subjects without this history ($OR_{adj.}$ = 1.37, 95% CI: 1.28 - 1.47, p <0.001). In case of OV infection, it already established to be a major cause of chronic inflammation in the bile ducts which can lead to the progression of PDF (Intuyod K, 2012; Sripa et al., 2012a; Sripa et al., 2012b). The mechanism of PDF progression is likely to pass through the pro-inflammatory cytokine networks, particularly interleukin-6 (IL-6), which induces DNA damage then leading to the pathological changes of bile duct epithelium (Jaiswal et al., 2000; Watanapa and Watanapa, 2002; Chaiteerakij et al., 2015; Zhang et al., 2016). The elevated levels of IL-6 were the marker of chronic inflammation among OV infected subjects and significantly associated with the development of PDF (Sripa et al., 2009). In addition, elevated levels of IL-6 have been reported in almost every chronic inflammatory disease of the liver including hepatitis B virus (HBV) infection (Lan et al., 2015). The mechanism of IL-6 also supports the relationship between hepatitis B and PDF which is consistent with this study. Our data revealed that hepatitis B subjects were significantly associated with PDF risk (ORadj. = 1.31, 95% CI: 1.11 - 1.55, p = 0.001).

The association between smoking and CCA risk seems likely to be inconclusive issues both in Asian and Western studies (Shaib et al., 2004; Welzel et al., 2007; Poomphakwaen et al., 2009; Songserm et al., 2012; Chaiteerakij et al., 2013). In the present study, smoking was considered to be inconclusive factor for PDF risk

due to a trivial magnitude of effect (ORadj. = 1.09, 95% CI: 1.01 - 1.18, p = 0.040). Even though, it is already known that many chemical components in tobacco smoke act as carcinogens, these agents can cause DNA damage and induce hepatobiliary lesions both in vitro and in vivo (Watanapa and Watanapa, 2002). Further study should collect more details regarding quantity of cigarettes smoked daily and the age at which smoking started (Songserm et al., 2014) in order to elucidate this association.

Surprisingly, our findings showed the significant association between number of PZQ treatments (> 2 times) and a decreased PDF risk (ORadj = 0.54, 95% CI: 0.47 - 0.63, p < 0.001). PZQ has been established the antihelminthic drug of choice to treat OV infection due to chemotherapy effects, widely available and providing more than 90% of cure rate (Jongsuksuntigul and Imsomboon, 2003). There are many studies tried to investigate the relationship between number of repeated PZQ treatments and CCA risk both in animal and human models. However, the results in human subjects, based on systematic review and meta-analysis, remain inconclusive (Kamsa-ard S, 2013). Previous studies indicated that a history of PZQ uses at least once associated with OV re-infection and acute inflammation. Whereas, PDF induced by chronic infection usually remains long after OV eradication by PZQ (Pinlaor et al., 2009; Saengsawang et al., 2016). Thus, PZQ by itself might not be the direct risk factor for PDF and CCA development (Sripa et al., 2012a; Saengsawang et al., 2013; Sithithaworn et al., 2014). Additional studies demonstrated that PZQ has affected the degradation of PDF in OV-infected hamsters due to tissue resorption. This is the benefit of an early treatment. However, some fibroses are irreversible, especially in chronic infected subjects. Therefore, chronic treatment with PZQ may not be recommendable because it could induce the expression of fibrogenic factors (Pinlaor et al., 2009; Charoensuk et al., 2016). Nevertheless, the exact mechanisms in human have to be further elucidated.

Interestingly, we also found that DM was associated with a decreased PDF risk (OR_{adj} = 0.57, 95% CI: 0.49 - 0.65, p<0.001). This association might be due to a protective effect of treatment with metformin which is usually used as a first-line treatment when type 2 DM was diagnosed (He L, 2009; Chaiteerakij et al., 2013). Since the incidence of type 2 DM is common in Thailand (Deerochanawong and Ferrario, 2013; Thinkhamrop et al., 2015), it is possible that metformin has been widely used among these subjects. A recent study (Incio et al., 2015), have examined the effect of metformin on fibrosis and inflammation among pancreatic ductal adenocarcinoma (PDAC) patients with type 2 DM. They found that metformin alleviates desmoplasia, which is disproportionate formation of fibrous connective tissue. Moreover, they indicated that metformin also decreases tumor inflammation by reducing the expression of inflammatory cytokines both in vitro and in vivo experiments. Another study (Chaiteerakij et al., 2013) also showed a similar trend that metformin use was associated with a 60% reduction in intrahepatic cholangiocarcinoma (ICC) risk among diabetic patients (OR_{adj} = 0.4, 95% CI: 0.2 – 0.9, p = 0.04). However, the mechanisms of metformin on cancer have not been completely clarified. Further studies should to prove the cause and effect relationship between metformin use and PDF risk in order to validate our findings.

We did not find the associations between the family history of CCA, alcohol drinking and PDF. There might be due to the limitation such as information bias from self-report questionnaire at enrollment. However, the major strength of this study was the US screening on a very large sample size of high risk groups in the endemic areas which is the world's highest incident of CCA. Our findings could represent the prevalence of PDF among population in northeast Thailand and confirm their associated factors.

In conclusion, this study shows a high prevalence of PDF. Factors associated with an increased PDF risk, in addition to OV infection, included the old age (≥ 70 years) and hepatitis B. On the other hand, number of praziquantel treatments and diabetes mellitus were significantly associated with a decreased PDF risk. However, the results of inverse associations require further investigation in order to elucidate our findings. The next US screening should closely examine among older people and hepatitis B subjects for the purpose of PDF surveillance.

Acknowledgements

This work was supported by Khon Kaen University through the CASCAP project, the National Research Council of Thailand through the Medical Research Network of the Consortium of Thai Medical Schools. We are also grateful to all subjects for sacrificing their time and cooperating as well including to Data Management and Statistical Analysis Center (DAMASAC) team for their supports in data management and analysis.

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