RESEARCH COMMUNICATION

Risk Factors for Cholangiocarcinoma in Khon Kaen, Thailand : A Nested Case-Control Study

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Abstract

The present nested case-control study within the Khon Kaen cohort study was conducted to assess risk factors for cholangiocarcinoma (CCA) development. Cases were 108 subjects with proven CCA by ultrasound at least and controls also numbered 108, matched by sex, age (not more than 3 years difference) and period of recruitment to the cohort (not more than 3 months difference). A questionnaire was constructed based on that employed for the Khon Kaen cohort study recruitment. McNemar's chi-square test and conditional logistic regression were used for crude analysis and multivariables analysis. Results revealed a sex ratio of 2:1 for males:females. The current study found a statistically significant association when adjusted for other potential covariate factors between cholangiocarcinoma and the consumption of total fruits 1.0-2.08 times per day (OR 0.32; 95%CI: 0.12-0.88) and history of *Opisthorchis viverrini* (OV) eggs in stools at recruitment plus consumption of meat < 0.45 times per day (OR 2.99; 95%CI: 1.04-8.62). The findings suggest that OV infestation is the strongest risk factor for development of cholangiocarcinoma and also suggests decrease in risk among individuals who consume more fruit.

Key Words: Cholangiocarcinoma - nested case-control study - dietary factors - *Opisthorchis viverrini* - Thailand

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Introduction

Liver cancer is an important health problem worldwide (Uttaravichien et al., 1996). In 2002, the age-standardized incidence rates around the world were 15.7 per 100,000 population in males and 5.8 in females. The incidence is generally low in developed countries but high in developing countries (Ferlay et al., 2004). The incidence of liver cancer in Thailand is particularly high, with estimated rates for the whole country of 38.6 in males and 17.2 in females (Sriplung et al., 2003). Within the country itself, there is variation in the incidence among the regions and Khon Kaen Province, which is in the northeast of Thailand, has the highest incidence 94.8-97.4 in males and 37.2-39.4 in females (Parkin et al., 1992; Parkin et al., 1997). Most of the liver cancer patients in Khon Kaen have cholangiocarcinomas (CCA) (82-89%) (Vatanasapt et al., 1993; 1995; Deerasamee et al., 1999; Sriplung et al., 2003; Khuhaprema et al., 2007).

CCA is the cancer of epithelial cells of the biliary tract which include the bile duct in the liver (intrahepatic cholangiocarcinoma) and outside of the liver (extrahepatic cholangiocarcinoma) but not the gall bladder and ampulla of Vater (Uttaravichien et al., 1996). It is reported that, in each year, worldwide there are 315,000 new cases of cholangiocarcinoma (Parkin et al., 1997).

In Thailand, CCA is one of the leading causes of death and a major problem in the northeast of Thailand (Vatanasapt et al., 1990). There is evidence that high incidences are found in regions or countries with high prevalence of liver flukes such as the northeast of Thailand, Laos and south of Vietnam. Thus an association exists between CCA and infestation of species *Opisthorchis viverrini* and *Clonorchis sinensis* (Haswell-Elkins et al., 1994; Sriamporn et al., 2004).

Other possible factors include alcoholic beverages, smoking, food consumption, environmental agents and past illness (i.e. primary sclerosing cholangitis, ulcerative colitis, choledocal cysts, Caroli's disease, infection with hepatitis B and viruses). In Thailand, a number of studies of risk factors for cholangiocarcinoma have been performed, but most of them had a hospital-based case-control and descriptive study design, very few had a prospective cohort study design. Therefore in the current study, the authors aimed to conduct an analytic study to study the risk factors for cholangiocarcinoma using cohort study data, to evaluate personal behaviour, and the influence of health care system and also health education.

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Subjects and Methods

Subjects

There were 108 subjects from the cohort who developed primary site cholangiocarcinomas (proven by ultrasound at least). Subjects who do not develop to be cholangiocarcinoma were randomly selected as controls, totally 108 controls (case : control = 1:1). Cases and controls were matched by sex and age (± 3 years) and recruitment to the cohort within the same period (± 3 months).

The Khon Kaen Cohort study

The initiated recruitment was conducted at the Cancer Unit of the Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, Thailand during 1990-2001. The cohort study collected data from the population of Khon Kaen Province aged 35 year and above. The study team set up as a mobile cancer screening programme and moved to the villages, inviting the participants to join the project. The participants signed the consent form and were interviewed. Biological specimens were collected for further study. In total, there were about 25,000 participants joined the project. The cohort groups were followed-up by data linkage between the Khon Kaen cohort data and the population-based cancer registry to find if any of the participants developed a cancer (Sriamporn et al., 2005).

Data collection

A structured questionnaire was constructed based on the questionnaire used at the Khon Kaen cohort study recruitment. The variables of interest were: general information of the study subjects, information about smoking, alcoholic beverage consumption, food consumption habits and food items, history of *O. viverrini* infestation, number of eggs in stool and history of praziquantel used.

Statistical analysis

Univariate analysis was carried out to study the association between individual exposure and cholangiocarcinoma using McNemar's Chi-square test and the conditional logistic regression technique. Multivariables analysis was made controlling the potential effect of each variable using multiple conditional logistics regression.

For the analysis of smoking, there were categorized as smokers and nonsmokers. Smokers included those who smoked any type of cigarette. Average number of cigarettes per year were computed based on all smoking periods reported and dichotomized on the median of the controls. Average number of cigarette was calculated as annual cigarettes (filtered and unfiltered) plus 1.5 times annual Yamuan (home-made cheroot). The 1.5 correction factor was used to allow for the longer size of Yamuan compared with the regular cigarettes. The amount of cigarettes was categorized based on the 50th percentile of the controls and dichotomized into low and high levels. There were two categories for alcohol drinking: drinkers and nondrinkers. Drinkers, was defined as who have consumed at least one type of all alcoholic beverages (beer, Sato, white whisky and red whisky) and consumed within range of every day to once a month. The average amount of alcohol consumption were analyzed based on gram per day, that is unit of alcohol consumption measure and percentile of alcohol volume (alc. % vol.) of beer was defined as 5.0%, Sato was defined as 7.0%, white whisky was defined as 40% and red whisky was defined as 35%. Average amount of alcohol consumption were categorized into 3 categories including No, ≤ 20 gram per day and > 20 gram per day. Alcohol unit per day was categorized into 3 categories including No, ≤ 0.50 and > 0.50 alcohol unit per day.

For the measure of dietary intake within a previous year, there were categorized 3 levels as low, medium and high. History of OV eggs in stool at recruitment measured by Stoll's egg count technique, the unit being eggs/gram. Division was into 3 categories: negative, no eggs in stool; positive, eggs found at recruitment; and unknown.

The analysis of this study utilized conditional logistic regression model with backwards selection to choose a final model to explore the relationship between there factors and interesting factors in the hypothesis. Conditional logistic regression is useful in investigating the relationship between an outcome and a set of risk factors in a match case-control studies (Chernrungroj, 2000). For those exposure variables with statistically significant (p-value <0.05) association with CCA in a univariate analysis and those with no statistically significant association with CCA but found statistically significant from the reviewed literatures were also included in the initial model of multiple conditional logistic regression analysis, with the exception that did not allow the removal of main effect terms for CCA until all interaction terms had been removed. At each step, the term with highest p-value (provided it was greater than 0.05) was removed from the model and the model was refit with all remaining terms. This process was continued until no remaining terms could be removed.

Results

There were more male cases than females with a ratio

Table 1. Characteristics of Cases and Controls

Variables		Cases		Controls	
Sex	Male	71	65.7	71	65.7
	Female	37	34.3	37	34.3
Age at recruitment					
	≤50	27	25.0	27	25.0
	51 – 55	22	20.4	18	16.7
	56 – 60	20	18.5	24	22.2
	>60	39	36.1	39	36.1
	Mean (SD)	56.7 (9.4)		56.5 (9.6)	
	Median (Min: Max)	57 (31:79)		57 (30:79)	
Occupation					
	Farmer	88	81.5	94	87.0
	Non-farmer	20	18.5	14	13.0
Education level					
	Illiterate	4	3.7	5	4.6
	Primary	99	91.7	98	90.8
	Secondary	5	4.6	5	4.6

Data are numbers and percentages

of 2:1. The mean age at recruitment of cases and controls were 56.7 and 56.5 years respectively and mean age at diagnosis date of cases was 60.8. All cases had confirmed diagnosis by ultrasound, only 7.4% of cases had histological confirmed. Some 13.9% were at late stage (stage IV), 86.1% were unknown. The most common occupation was farmer with only a primary school education (Table 1). Most of the subjects were married.

Table 2. Crude Analysis of Potential Factors Associated with Cholangiocarcinoma

Variables	Cases		Controls		OR*	95% CI	p-value
Smoking (n= 216)							
No	48	44.4	50	46.3	1.00		
Yes	60	55.6	58	53.7	1.17	0.50-2.76	0.695
Filter cigarettes							
No	48	71.6	50	70.4	1.00		
Yes	19	28.4	21	29.6	1.33	0.23-9.10	0.999
Non-filter cigarettes							
No	48	84.2	50	86.2	1.00		
Yes	9	15.8	8	13.8	1.50	0.17-17.9	0.999
Home made cigarettes							
No	48	49.5	50	53.8	1.00		
Yes	49	50.5	43	46.2	1.25	0.44-3.64	0.815
Average number of cigarettes per year							
No	48	44.4	50	46.3	1.00		
Low	38	35.2	40	37.0	1.07	0.47-2.42	0.868
High	22	20.4	18	16.7	1.37	0.55-3.45	0.501
Ever drink alcohol (n= 216)							
No	41	38.0	52	48.1	1.00		
Yes	67	62.0	56	51.9	1.69	0.88-3.35	0.093
Beer							
No	41	56.2	52	67.5	1.00		
Yes	32	43.8	25	32.5	1.75	0.69-4.81	0.201
Sato							
No	41	78.8	52	91.2	1.00		
Yes	11	21.2	5	8.8	3.00	0.24-28.8	0.625
White whisky							
No	41	42.3	52	52.0	1.00		
Yes	56	57.7	48	48.0	2.00	0.93-4.57	0.055
Red Whisky							
No	41	64.1	52	80.0	1.00		
Yes	23	35.9	13	20.0	5.00	1.07-46.9	0.039
Frequency of alcohol consumption							
No	41	38.0	52	48.2	1.00		
< 1/month	17	15.7	17	15.7	1.49	0.61-3.65	0.385
Monthly	35	32.4	26	24.1	1.88	0.91-3.85	0.086
≥Weekly	15	13.9	13	12.0	1.53	0.65-3.59	0.326
Average amount of alcohol (grams per day)							
No	51	47.2	67	62.0	1.00		
≤ 20	45	41.7	32	29.6	2.02	1.07-3.08	0.030
> 20	12	11.1	9	8.4	1.94	0.73-5.14	0.185
Alcohol unit per day							
No	51	47.2	67	62.0	1.00		
≤ 0.50	29	26.9	22	20.4	1.86	0.92-3.79	0.085
> 0.50	28	25.9	19	17.6	2.18	1.02-4.66	0.045
OV eggs in stool (n=145)							
Negative	47	61.8	52	75.4	1.00		
Positive	29	38.2	17	24.6	1.77	0.87-3.59	0.115
Number of *O. viverrini* eggs (eggs per gram) (n=145)							
Negative	47	61.8	52	75.4	1.00		
1-1,000	13	17.1	11	15.9	1.17	0.48-2.80	0.731
>1,000	16	21.1	6	8.7	3.37	1.05-10.7	0.041
History of praziquantel used (n=120)							
No	22	20.4	25	23.2	1.00		
Yes	37	34.2	36	33.3	1.19	0.53-2.66	0.670
History of family cancer (n=216)							
No	83	76.8	99	91.7	1.00		
Yes	25	23.2	9	8.3	3.00	1.30-7.72	0.005

OR*= Odds ratio from matched case-control analyses

Table 2 presents number of cases and controls, crude OR and 95% Confidence Interval (95%CI) for each variable.

The results of crude analysis revealed that the odds ratios of drinking red whisky, amount of alcoholic beverage consumption of ≤20 grams per day, number of OV eggs in stool more than 1000 eggs per gram, consumption of nitrite-containing food, and history of having cancer in the family were more than 1 with statistical significant (OR=5.00, 95%CI: 1.07-46.9; OR=2.02, 95%CI: 1.07-3.08; OR=3.37, 95%CI:; 1.05-10.70; OR=4.91, 95%CI: 1.04-23.24; OR=3.00, 95%CI: 1.30-7.72). That suggested that these exposures were potential to be risk factors for CCA.

Total fruit consumption of 1.0-2.08 times per day was protective factor for cholangiocarcinoma with statistical significant (OR=0.40, 95%CI: 0.18-0.87).

There were no association of CCA and other variables such as smoking, frequency of alcohol consumption, history of praziquantel used, meat and pork consumption as shown in Tables 2 and 3. For those exposure variables with statistical significant association with CCA in a crude analysis and those with no statistical significant association but found significant from the reviewed literatures were included in the initial model of multivariables analysis by multiple conditional logistic regression.

The result of the final model when adjusted for potential confounder the variables that still appeared to

Table 3. Crude Analysis of Dietary Factors Associated with Cholangiocarcinoma

Variables	Cases		Controls		OR*	95% CI	p-value
Total vegetables (times per day) (n=190)							
<1.7	84	88.4	78	82.1	1.00		
1.7-2.8	6	6.3	12	12.6	0.37	0.11-1.25	0.109
>2.8	5	5.3	5	5.3	0.74	0.20-2.82	0.663
Total fruits (times per day) (n=156)							
<1.0	57	72.2	41	53.2	1.00		
1.0-2.08	14	17.7	26	33.8	0.40	0.18-0.87	0.021
>2.08)	8	10.1	10	13.0	0.65	0.25-1.70	0.382
Total fish: Fish and shellfish (times per day) (n=147)							
<0.4	23	31.6	27	36.5	1.00		
0.4-0.8	25	34.2	24	32.4	1.23	0.56-2.67	0.605
>0.8	25	34.2	23	31.1	1.31	0.56-3.04	0.528
Total meat: beef and pork (times per day) (n=216)							
<0.45	75	69.4	69	63.9	1.00		
0.45-1.0	27	25.0	27	25.0	0.87	0.46-1.66	0.682
>1.0	6	5.6	12	11.1	0.31	0.08-1.20	0.090
Poultry (times per day) (n=216)							
<0.2	47	43.5	49	45.4	1.00		
0.2-0.4	52	48.2	44	40.7	1.25	0.65-2.38	0.502
>0.4	9	8.3	15	13.9	0.62	0.23-1.70	0.354
Total nitrite-containing foods (n=216)							
Never	55	50.9	64	59.3	1.00		
Sometime	26	24.1	24	22.2	1.26	0.67-2.36	0.418
Often	27	25.0	20	18.5	4.91	1.04-23.24	0.045

OR*= Odds ratio from matched case-control analyses

Table 4. Odds Ratio for Cholangiocarcinoma Adjusted for Other Potential Factors by Multiple Conditional Logistic Regression.

Variables	Crude OR	95% CI	Adjusted OR	95% CI	p-value
History of family cancer					
No	1.00		1.00		
Yes	3.00	1.30-7.72	2.12	0.79-5.68	0.135
Smoking					
No	1.00		1.00		
Yes	1.17	0.50-2.76	0.87	0.32-2.33	0.780
Average amount of alcohol (gram per day)					
No	1.00		1.00		
≤ 20	2.02	1.07-3.08	2.25	0.99-5.09	0.052
> 20	1.94	0.73-5.14	2.39	0.62-9.26	0.206
Total vegetables (times per day)					
L (<1.7)	1.00		1.00		
M (1.7-2.8)	0.37	0.11-1.25	0.31	0.06-1.70	0.177
H (>2.8)	0.74	0.20-2.82	1.60	0.26-9.78	0.609
Total fruits (times per day)					
L (<1.0)	1.00		1.00		
M (1.0-2.08)	0.40	0.18-0.87	0.32	0.12-0.88	0.027
H (>2.08)	0.65	0.25-1.70	0.59	0.16-2.14	0.420
Poultry (times per day)					
L (<0.2)		1.00			1.00
M (0.2-0.4)	1.25	0.65-2.38	2.38	0.88-6.39	0.085
H (>0.4)	0.62	0.23-1.70	1.26	0.30-5.36	0.751

L, low; M, medium; H, high

confer an elevated risk for CCA were history of family cancers (adjusted OR=2.12, 95%CI:0.79-5.68) and average amount of alcohol ≤ 20 and > 20 gram per day when compared with non drinker being risk for CCA but not statistical significant. Also the medium and high level consumption of poultry were higher risk for CCA than those who consumed low level. While the variables seemed to be lower risk or protective factors for cholangiocarcinoma when adjusted for potential confounder were fruits consumption (adjusted OR=0.32, 95%CI: 0.12-0.88) as shown in Table 4.

There was interaction effect between OV eggs in stool at recruitment and meat (times per day) consumption, which including beef and pork, found the statistical significant of who had both positive of OV eggs and consumed meat at low level (< 0.45 times per day) had higher risk for CCA when adjusted for other potential factors (adjusted OR = 2.99, 95%CI: 1.04-8.62). Also, in those who had both positive of OV eggs and consumed meat at medium level and who had both negative of OV eggs and consumed meat in high level but not statistical significant. While group of who had both negative of OV eggs and consumed meat in medium level and group of who had both positive of OV eggs and consumed meat in high level consumption seemed to be protective for CCA but not statistical significant as shown in Table 5.

Table 5. Odds Ratio of the Association between Cholangiocarcinoma and OV Eggs in Stool at Recruitment with Reference to Meat Consumption

Meat	OV	*OR	95% CI	p-value
Low (< 0.45)	Negative	1.00		
	Positive	2.99	1.04-8.62	0.043
Medium (0.45-1.0)	Negative	0.95	0.25-3.60	0.943
	Positive	6.20	0.57-67.1	0.133
High (> 1.0)	Negative	1.97	0.18-21.8	0.581
	Positive	0.39	0.02-6.29	0.510

*OR= Adjusted odds ratio

Discussion

This study is a nested case-control study within the cohort study, which analyzed from the questionnaires which interviewed at recruitment to the cohort study and stool examination results for OV eggs were analyzed. The advantages and disadvantages of nested case-control within the cohort can be explained i.e. prospective study-minimal recall bias, and interviewer bias (Dos Santos, 1999). This is especially important when dealing with questionnaire data, for example on dietary factors and alcoholic beverage consumption. When cancer cases and non-cancer; controls are interviewed, reported the use of praziquantel may also be a problem in a conventional case-control study. Also, some important variables are altered when cancer is present (as in conventional case-control study), especially dietary. But, for CCA we have the additional problem that the cases have obstructive jaundice, and this may result in cessation of passage of OV eggs via the bile duct (even if the parasite is still present when the cancer has advanced). On the other hand, the Khon Kaen cohort study interviewed subjects only on entry. There were no repeat interviews. So, the time between recording exposure and diagnosis is quite variable.

This study is the first prospective study of risk factors for cholangiocarcinoma, the assumption of this study, cases had the exposure before developed to be CCA and the conditional logistic regression was used for match case-control in a part of analysis of this study similar to the study in Nakhon Phanom (Honjo et al., 2005). But in the previous case-control studies in Thailand unconditional logistic regression was applied (Parkin et al., 1991; Chernrungroj, 2000). Therefore, the finding of this study should be more reasonable than the others.

Cases of cholangiocarcinoma, the diagnosis were rarely based on the gold standard of histology. Any resulting misclassification (of some cases) would weaken any observed associations. The sex ratio between male and female was 2: 1 in the present study. The previous hospital-based case-control study reported the sex ratio of 2.2: 1 (Parkin et al., 1991). Similarly, the incidence of cholangiocarcinoma in male in the edemic areas of Thailand is about twice that in females (Vatanasapt et al., 1995).

The current study is one of the few case-control studies to measure the association between CCA and its suspect risk factors. Therefore, the result of this study will be considered within the relatively sparse literature concerning the epidemiology of CCA. Studies are fewer than on hepatocellular carcinoma, which have mainly been carried out in western countries and some other developed countries, where research collaboration with western countries is available. Thailand is one of those countries,

and the findings suggest that there was association of cholangiocarcinoma, OV and carcinogens from foods stuffs. High incidences of CCA appear to always occur where prevalence of liver fluke are very high (Haswell-Elkins et al., 1994; Parkin et al., 1991; Srivatanakul et al., 1991; Sriamporn et al., 2004).

The results of this study showed that; in a multivariables analysis, there was interaction effect of meat; beef and pork (times per day) and OV eggs in stool at recruitment. The result of current study identified statistically significant relationships following adjustment for other potential covariate factors, with adjustment the calculation odds ratio for cholangiocarcinoma and who had positive of OV in stool at recruitment plus meat consumption in low level had risk for CCA 2.99 times (adjusted OR = 2.99, 95%CI: 1.04-8.62) of who had negative of OV eggs in stool at recruitment include plus meat consumption in low level. In univariate analysis, a trend of increasing risk with higher OV egg count is found. This result is quite a low level of risk. It is difficult to compare with other studies using eggs count, since this is not a good measure of exposure in case-control study (except Haswell-Elkins'study, 1994), who identified asymptomatic, early cases of cholangiocarcinoma, with no obstruction, as case group. OR of 2.99 is not compatible with high risk of cholangiocarcinoma in Northeast of Thailand. But, it is many times higher than in USA, for example. Therefore, 2.99 is an underestimate. Why? Likely that many chronic infections not detectable: not shedding eggs, or prhaps because of recent treatment with praziquantel to kill the parasites.

Mechanisms by which OV causes CCA, were studied elsewhere. In case of liver fluke-induced is generally accepted to be the result of chronic inflammation (Sripa et al., 2000; Sripa et al., 2003). It is almost certain that a combination of pathologies-mechanical damage, parasite secretions, and immunopathology lead to CCA development after chronic infection with OV. The primary pathologic change such as epithelial desquamation may be due to mechanical irritation caused by the liver fluke and/or its metabolic products. However, immunopathologic processes may contribute to the long-standing hepatobiliary damage. During liver fluke infestation, inflammation, periductal fibrosis, and proliferative responses, including epithelial hyperplasia, goblet cell metaplasia and adenomatous hyperplasia, may represent predisposing lesions that enhance susceptibility of DNA to carcinogens (Kim, 1984). Moreover, there are some studies have been demonstrated about molecular pathogenesis. Most of them were interested regarding the nitrosation of proline and thioproline increased in infected patients (Satarug et al., 1996), suggest that nitric oxide (NO) activity may also contribute to cholangiocarcinogenesis. Recently, it has been demonstrated that inducible nitric oxide synthase was activated in response to inflammation reaction, resulting in increased production of NO, which could directly cause DNA damage (Pinlaor et al., 2004a; Pinlaor et al., 2004b).

The result of the current study found that there was no association between smoking and CCA, the adjusted odds ratio was 0.87 and did not achieve statistically significant. Similarly, the previous case-control study by Parkin et al and a case-control study by Chernrungroj, that reported no association between cholangiocarcinoma and tobacco smoking. But, opposite in Honjo et al study which found that smoking had slightly increase association with cholangiocarcinoma. Frequency of tobacco used was obviously different between male and female. Only 0.85% of female while 99.5% of male were considered as smokers (Parkin et al., 1991; Chernrungroj, 2000; Honjo et al., 2005). However, the findings suggest that those individual who had current smoking benefited from a reduction in the risk of cholangiocarcinoma. In order to calculate the amount of cigarette, pack-year should be estimated, but the duration of smoking was not available so pack-year was not available to calculate. However in current study found that average number of cigarette per year of low and high level relative to who never smoking seemed to be risk for cholangiocarcinoma but not statistically significant.

Alcoholic beverage consumption included beer, Sato (home made), white whisky and red whisky were calculated odds ratio to find the association with cholangiocacinoma. The result of this study, multivariate analysis when adjusted for other potential factors, found that average amount of alcohol consumption had risk for cholangiocarcinoma when compared those who had ever drunk alcoholic beverage ≤ 20 gram per day and > 20 gram per day with who never drunk but not statistically significant. Also, the result in the univariate anlysis found that who had drunk alcoholic beverage any type had risk for cholangiocarcinoma, especially in each type of alcoholic beverage found that higher risk for cholangiocarcinoma. Similar to those findings elsewhere such as; a case-control study by Parkin et al, in Northeast of Thailand reported that those who regularly drink alcoholic beverage had higher risk of cholangiocarcinoma. The findings are similar to the study of Honjo et al, in a population-base case-control that studied the environmental factors and cholangiocarcinoma in Nakhon Phanom Province, reported that alcohol consumption had higher risk for cholangiocarcinoma (Parkin et al., 1991; Honjo et al., 2005). In a population-based case-control in Denmark and a study by Shaib et al found the association of alcohol, liver disease and cholangiocarcinoma (Welzel et al., 2007; Shaib et al., 2007). A multi-center case-control study of primary sclerosing cholangitis in the US found that those who have regular alcohol consumption have higher risk and can develop to be cholangiocarcinoma (Chalasani et al., 2000). In a hospital-based case-control study in Khon Kaen found an association between cholangiocarcinoma and who ever-drinker and current drinker compared with non-drinker but not statistically significant also, found association between average number of drinks/week and cholangiocarcinoma, when compared 1-7 drink per week and >7 drink per week with never drink (Chernrungroj, 2000).

The result of this study suggests a higher in risk of cholangiocarcinoma among subject with history of cancer family. However after adjusted for other potential factors, history of family cancer did not remain a significant risk factor (adjusted OR = 2.12, 95%CI: 0.79-5.68). Similar

to a hospital-based case-control study in Khon Kaen by Chernrungroj (2000). The finding might reflect the role of genetic factors or common environmental factors among family members. A more definitive approach is needed to elucidate the role of genetic factors and their interaction with environmental factors in CCA.

Analysis of dietary factors showed a low level consumption of total fruits and vegetables consumption to be a risk factor, similar to a hospital-based case-control study of cholangiocarcinoma by Chernrungroj (2000), although a case-control study by Parkin et al (1991) did not find any protective effects.

There was no association between CCA and meat consumption, the result of this study in univariate analysis suggested a protective effect when compared medium and high level consumption with low level consumption, but in multivariate analysis when adjusted for other potential factors found that the medium level consumption of total meat plus OV negative in stool at recruitment also suggested a protective effect. The results of the current study did suggest an important relationship between the risk of cholangiocarcinoma and the consumption of nitrite-containing foods, which consist of preserved fresh water fish (Pla-som, Pla-chom), salted fresh water fish (Pla-kem), fermented fish (Pla-ra), preserved beef (Nam), preserved pork (Nam), sausage beef and sausage pork, are in line with earlier findings (Parkin et al., 1991; Chernrungroj, 2000; Honjo et al., 2005).

As with case-control studies in general, it is important to consider the possible avenues by which systematic errors in collecting information could have influence the study results. One problem is with disease misclassification, most cases being diagnosed by ultrasound, so that it is quite possible that other types of cancer might have been inadvertently included.

In the part of exposure misclassification, information about drinking and smoking were not from direct measurement, but directly provided by individuals themselves or their close relatives. Unless the amount of alcohol usually drunk is close to zero it is difficult to describe. For many people, the consumption of alcohol has emotional and moral connotations, respondents may underestimate the amount of drink from feeling of guilt. Results regarding smoking could also have been biased in the same way. The food frequency questionnaire used in this study might also produce some misclassification of an individual's true usual intake.

In conclusion, the results from this study as well as the previous studies, we can conclude that OV infestation is a strong association with the risk for development of cholangiocarcinoma. This study also suggests that there is an association of meat consumption plus the status of OV infestation and cholangiocarcinoma, the consumption of more fruit decreases risk of cholangiocarcinoma. Therefore, it is essential to take these findings into account of the policy on cancer prevention and also for further research.

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