RESEARCH ARTICLE

Risk Factors for Cholangiocarcinoma in the Lower Part of Northeast Thailand: a Hospital-based Case-control Study

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Abstract

Background: Cholangiocarcinoma (CCA) is the most common cancer in Northeast Thailand. It is also a crucial health problem for Thai people. Various risk factors for CCA have been identified in the upper part of Northeast Thailand, but no similar studies of risk factors have been conducted in the lower parts of the region. This study aimed to investigate factors associated with CCA in the resident population. Materials and Methods: A hospital-based case-control study was conducted during 2009-2012 with the recruitment of 123 CCA cases and 123 non-CCA patient controls, matched for sex, age and residential area. Information was collected by interview with a structured questionnaire. Blood samples were collected for assays of anti-OV antibodies. Associations between various personal factors, dietary habits, family history, the presence of anti-OV antibodies and CCA were analyzed using multiple conditional logistic regression. Results: Patients who consumed raw meat (beef, pork) and alcoholic beverages ≥3 times per week had a higher risk of CCA than non-consumers (OR_{adj}=4.33; 95%CI=1.14-16.35 and OR_{adj}=2.13; 95%CI=1.00-4.55, respectively). Patients who had a family history of cancer had a higher risk than those who did not (OR_{adj}=4.34; 95%CI=1.80-10.43). Also, patients who had anti-OV antibodies (AU>23.337) had a higher risk than those whose anti-OV antibodies were below the cut-off (AU≤23.34) (OR_{adj}=3.09; 95%CI=1.04-9.16). Conclusions: As is the case in the upper part of Northeast Thailand, OV infection is a crucial risk factor for CCA in people who live in lower part of the region. Similarly, a family history of cancer and the consumption of alcohol are risk factors for CCA.

Keywords: Risk factors - cholangiocarcinoma - *Opisthorchis viverrini* - case-control study

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Introduction

Cholangiocarcinoma (CCA), the second most common histological type of primary liver cancer, is the most common cancer in the northeast region of Thailand and remains a significant public health problem for Thai people (Parkin et al., 1993; Sripa et al., 2012).

Based on data from the Cancer Registry of Ubon Ratchathani, the estimated age standardized incidence rates of CCA have been 74.9 and 34.7 per 100,000 for males and females, respectively (Khuhaprema et al., 2007). The role of the liver fluke, *Opisthorchis viverrini* (OV), has been recognized as a causal agent in CCA (IARC, 1994), and subsequent studies have confirmed this (Chernrungroj, 2000; Sriamporn et al., 2004; Honjo et al., 2005; Sripa et al., 2007; Poomphakwaen et al., 2009; Songserm et al., 2011). However, most of the studies of OV infection and other risk factors for CCA have been conducted in the upper part of Northeast Thailand. Although there is considerable interest in the incidence of CCA in the lower part of Northeast Thailand, few studies have been done to investigate the risk factors in this part of the region. We therefore conducted a hospital-based case-control study to investigate factors associated with CCA in a population of lower part of the northeast region.

Materials and Methods

This was a hospital-based, case-control study in which cases of CCA and matched controls were compared in terms of various potential risk factors for CCA.

Subjects

A total of 123 new cases of CCA were recruited from Sappasit Prasong Hospital and Ubon Ratchathani Cancer Center, Ubon Ratchathani Province, between June, 2009, and June, 2012. All the patients were from Ubon Ratchathani Province or a neighboring province, were histologically confirmed to have CCA, and were interviewed within two months of diagnosis. During the same period, a control subject matched for sex, age (±5 years) and residential area was recruited for each case.

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Subjects with gastrointestinal disease or any form of cancer were excluded. All gave informed consent for their participation in the study. Subjects refusing to complete the interview were excluded. A 10-ml blood sample was obtained from all cases and their matched controls, and the samples transferred to the laboratory for investigation of anti-OV antibodies.

Interview

All subjects were interviewed by nurses trained in the use of a structured questionnaire, which was in two sections.The first section consisted of items about socio-demographic status and various personal life-style and historical factors, such as smoking history, liver fluke (OV) infection, history of praziquantel use, betel nut chewing and a family history of cancer. The second section was concerned with dietary habits and contained items about the frequency (never or <once per month, 1-4 times per month, 3 times per week) with which alcohol and certain types of food items were consumed prior to becoming sick with their present illness (one year earlier). All subjects were reminded of this condition throughout this section of the interview.

Laboratory methods

Anti-OV antibody was extracted from the serum of cases and their matched controls in the Parasitological Laboratory at the Faculty of Medicine, Khon Kaen University, Thailand, using the indirect enzyme-linked immunosorbent assay (ELISA) technique previously described by Parkin et al. (1991) and Honjo et al. (2005). The ELISA results were expressed in terms of mean arbitrary units (AU) of two duplicate cells, and the cut-off value selected was >23.337 and ≤23.337 AU.

Table 1. Association of Factors with Cholangiocarcinoma (univariate analysis)

Factors		Cases (n=123)		Controls (n=123)		OR	95%CI	p value
		No.	%	No.	%			
Family history of cancer	No	85	69.1	107	87	1		
	Yes	38	30.9	16	13	3.6	1.70-7.45	0.001
Betel nut chewing	No	108	87.8	115	93.5	1		
	Yes	15	12.2	8	6.5	3	0.81-11.08	0.1
History of praziquantel use	Never used	89	72.4	84	68.3	1		
	Ever used	29	23.6	18	14.6	1.8	0.84-3.67	0.13
	May have used/unsure tablet name	5	4.1	15	12.2	0.3	0.11-1.04	0.06
Vegetables (grown in water)	No/<1 per month	30	24.4	30	24.4	1		
	1-4 times per month	26	21.2	42	34.2	0.57	0.28-1.19	0.213
	3 times per week	67	54.4	51	41.4	1.48	0.74-2.96	0.273
Vegetables (local)	No/<1 per month	30	24.4	28	22.8	1		
	1-4 times per month	28	22.8	39	31.7	0.85	0.28-1.33	0.729
	3 times per week	65	52.8	56	45.5	1.36	0.61-3.02	0.456
Raw freshwater cyprinoid fish	No/<1 per month	37	30.1	49	39.8	1		
	1-4 times per month	23	18.7	16	13	3.08	1.06-8.95	0.038
	3 times per week	63	51.2	58	47.2	3.4	1.05-11.01	0.041
Sour fish, jom	No/<1 per month	34	27.6	43	35	1		
	1-4 times per month	29	23.6	21	17	1.77	0.81-3.85	0.15
	3 times per week	60	48.8	59	48	1.6	0.57-4.51	0.376
Raw shellfish	No/<1 per month	40	32.5	42	34.1	1		
	1-4 times per month	26	21.2	21	17	1.3	0.55-3.07	0.556
	3 times per week	57	46.3	60	48.8	0.85	0.27-2.74	0.789
Sour shrimp, jom	No/<1 per month	42	34.1	49	39.8	1		
	1-4 times per month	22	17.9	15	12.2	2.2	0.83-5.83	0.114
	3 times per week	59	48	59	48	1.93	0.47-7.93	0.36
Raw meat (beef, pork)	No/<1 per month	30	24.4	34	27.6	1		
	1-4 times per month	32	26	45	36.6	1.1	0.53-2.32	0.792
	3 times per week	61	49.6	44	35.8	3.38	1.25-9.12	0.016
Sour meat (beef, pork)	No/<1 per month	32	26	33	26.8	1		
	1-4 times per month	35	28.5	38	30.9	1.01	0.46-2.19	0.99
	3 times per week	56	45.5	52	42.3	1.37	0.51-3.65	0.53
Raw sausages, mum (beef, pork)	No/<1 per month	34	27.6	33	26.8	1		
	1-4 times per month	37	30.1	42	34.2	0.89	0.44-1.79	0.749
	3 times per week	52	42.3	48	39	1.19	0.48-2.97	0.703
Cooked fermented fish (plara)	No/<1 per month	1	0.8	3	2.4	1		
	1-4 times per month	33	26.8	31	25.2	3	0.31-28.84	0.341
	3 times per week	89	72.4	88	71.5	-	-	0.99
Uncooked fermented fish (plara)	No/<1 per month	37	30.1	47	38.2	1		
	1-4 times per month	37	30.1	32	26	1.54	0.81-2.91	0.188
	3 times per week	49	39.8	44	35.8	1.75	0.74-4.19	0.205
Seasonal fruit	No/<1 per month	25	20.3	34	27.6	1		
	1-4 times per month	25	20.3	27	21.9	1.24	0.55-2.77	0.6
	3 times per week	73	59.3	62	50.4	1.72	0.85-3.47	0.131
Alcoholic beverages	No/<1 per month	38	30.9	53	43.1	1		
	1-4 times per month	16	13	23	18.7	1.07	0.47-2.35	0.868
	3 times per week	69	56.1	47	38.2	2.27	1.23-4.17	0.008
Anti-OV	AU ≤23.337	13	10.6	23	18.7	1		
	AU>23.337	110	89.4	99	80.5	2.29	0.94-5.56	0.07

Statistical analysis

The associations between CCA and potential risk factors were evaluated using odds ratios (ORs) and 95% confidence intervals (95%CIs) derived from a conditional logistic regression analysis. Crude and adjusted odds ratios were estimated for each independent variable. Factors included in the multivariate analysis were those found to be strongly associated with CCA in a univariate analysis (p value<0.10).Those variables, which were not found to be strongly associated with CCA in the univariate analysis, but which are reported in the literature as having an important role as risk factors for CCA, were also included. Crude ORs were adjusted for age, sex and residential area. Statistical analyses were performed using STATA version 11. Statistical significance was set at p≤0.05.

This study was approved by the Research Ethics Committee, Ubon Ratchathani University, reference no. 1/2009, on 26 February 2009.

Results

The distributions of general characteristics in cases and controls were similar because this was a matched case-control study; the distributions of sex, age and province of residence were the same in both groups. Most subjects were laborers engaged in agricultural work and were not educated beyond a lower primary school level (Prathom 1-4). The total family average annual income for both cases and controls was similar (at around 30,000 baht per year).

In the univariate analysis (Table 1), subjects with a family history of cancer had a statistically significantly higher risk of CCA than those who had not (OR=3.6; 95%CI=1.70-7.45). Although none of the other demographic personal factors were significantly associated with CCA, betel nut chewing emerged as a possible risk factor (OR=3.0; 95%CI=0.81-11.08). For the univariate analyses of dietary intake, the lowest level of consumption (never or <once per month) as was used to define the reference group. Statistically significant associations with CCA were found for the consumption of raw fresh-water cyprinoid fish 1-4 times month and three or more times per week (OR=3.08; 95%CI=1.06-8.95 and 3.40; 95%CI=1.05-11.01, respectively) and with the consumption of raw meat (beef or pork) or use of alcohol three or more times per week (OR=3.38; 95%CI=1.25-9.12 and OR=2.27; 95%CI=1.23-4.17, respectively).

In addition to those found to have an association with CCA in the univariate analysis, the following previously reported factors were included in the multivariate analysis: history of praziquantel use, vegetables (grown in water), vegetables (local), raw shrimp (som jom), uncooked fermented fish (plara), betel nut chewing, and anti-OV antibody.

Table 2 shows the adjusted ORs and 95%CIs from the multivariate analysis. The risk factors which remained statistically significant were family history of cancer (OR=4.34; 95%CI=1.80-10.43), consumption of raw meat (beef or pork) at least three times week (OR=4.33; 95%CI=1.14-16.35), drinking an alcoholic beverage at least three times a week (OR=2.13; 95%CI=1.00-4.55), and presence of anti-OV antibodies (AU>23.337) (OR=3.09: 95%CI=1.04-9.16).

Table 2. Association of Factors and Cholangiocarcinoma (multivariate analysis)

Variables		Crude OR	Adj. OR+	95%CI	p value
Family history of cancer	No	1	1		
	Yes	3.6	4.34	1.80-10.43	0.001
Betel nut chewing	No	1	1		
	Yes	3	1.85	0.26-12.94	0.54
History of praziquantel use					
Never used		1	1		
Ever used		1.76	1.97	0.55-7.12	0.3
May have used/unsure tablet name		0.34	0.39	0.06-2.48	0.32
Vegetables	No/<1 per month	1	1		
(grown in water)	1-4 times per month	0.57	0.58	0.23-1.49	0.261
	3 times per week	1.48	0.9	0.35-2.34	0.836
Vegetables	No/<1 per month	1	1		
(local)	1-4 times per month	0.61	0.7	0.26-1.86	0.473
	3 times per week	1.35	1.67	0.54-5.12	0.37
Sour shrimp, jom	No/<1 per month	1	1		
	1-4 times per month	2.2	1.67	0.52-5.32	0.387
	3 times per week	1.93	0.28	0.04-1.92	0.195
Raw meat	No/<1 per month	1	1		
(beef, pork)	1-4 times per month	1.1	1.16	0.47-2.86	0.751
	3 times per week	3.38	4.33	1.14-16.35	0.031
Alcoholic beverages	No/<1 per month	1	1		
	1-4 times per month	1.07	1.01	0.38-2.71	0.978
	3 times per week	2.27	2.13	1.00-4.55	0.05
Uncooked fermented fish (plara)					
	No/<1 per month	1	1		
	1-4 times per month	1.54	0.92	0.33-2.58	0.87
	3 times per week	1.75	0.72	0.18-2.91	0.65
Anti-OV	AU≤23.337	1	1		
	AU>23.337	2.29	3.09	1.04-9.16	0.042

Discussion

In the present study, the authors recruited CCA cases from Sappasit Prasong Hospital and Ubon Ratchathani Cancer Center, a tertiary hospital which specializes in cancer treatment. We were able to recruit a sufficient number of cases for the study because a great number of cancer patients come to these two hospitals for treatment.

The significant risk factors for CCA found in the present study were a family history of cancer, a high consumption raw meat (beef or pork), the consumption of alcoholic beverages, and anti-OV antibodies. The strongest risk factor was family history of cancer, and this is similar to a finding of a nested case-control study within cohort study in Khon Kaen Province, Thailand (Poomphakwaen et al., 2009).

A high level of consumption of raw meat (beef, pork) was associated with a significantly increased in the risk of CCA in this study, but no such association was found in a previous study of CCA in Khon Kaen (Poomphakwaen et al., 2009). Songserm et al. (2012) reported an association with the consumption of beef, but not with pork.

There was no association between smoking and risk of CCA in our study which is consistent with Parkin et al. (1991), but not with Haswell-Elkins et al. (1994).

The similar lack of a statistically significant association with smoking has been found in more recent studies by Honjo et al. (2005), Poomphakwaen et al. (2009), and Songserm et al. (2012).

We found that people who drink alcoholic beverages three times per week were significantly at risk for

CCA. This finding is supported by Honjo et al. (2005), who reported that both ex-drinkers and current regular consumers of alcohol were at increased risk of CCA. The finding is also consistent with the results of the study by Songserm et al. (2012), who reported a strong positive relationship between the risk of CCA and the monthly number of units of alcohol consumed. The results of the study by Poomphakwaen et al. (2009) are less clear, but the consumption of more than 0.5 units per day was a risk factor in their univariate analysis.

In this present study we found that those who have an OV antibody titre >23.337 compared with ≤23.337 have a higher risk for CCA (OR=3.09; 95%CI: 1.05-9.16). This positive finding is similar to those reported in other studies (Parkin et al., 1991; Honjo et al., 2005).

In the present study, no association was found between vegetable consumption and the development of CCA. However, vitamin C from vegetable or fruit consumption has been shown to inhibit cancer development (Srivatanakul et al., 1991; Lampe, 1999). Parkin et al. (1991) included various vegetables and fruits as items in their food intake questionnaire. Only fresh fruit had a significant protective association with CCA in their univariate analysis, and none of the vegetables or fruits emerged as protective factors in the multivariate analysis. In the study by Poomphakwaen et al. (2009), fruit consumption emerged from their multiple logistc regression analysis as a significant protective factor; vegetables were not a significant factor, even in their univariate analaysis. On the other hand, Songserm et al. (2012) found that both vegetables and fruit were protective factors.

Raw fish consumption was risk factor for CCA in our univariate analysis, but not in the final model. However, in other Northeast Thailand studies, strong associations have been found between the consumption of raw fish and CCA (Honjo et al., 2005; Songserm et al., 2012). Both these studies were conducted in the more northern parts of the region.

Perhaps the most important limitation of this study was a possible memory bias in the recall of dietary intake and various other potentially risky habits one year before becoming ill with a current disease. While the research interviewer repeatedly emphasised the timeframe, this may not have overcome all the distortions in recall which can arise when people, especially very sick people, are asked to remember details of habits so long ago in the past. It also the case that people are often reluctant to provide veridical feedback on socially undesirable behaviours, such as drinking and smoking.

In conclusion, as is the case in the upper part of Northeast Thailand, OV infection is a crucial risk factor for CCA in people, who live in lower part of the region. Similarly, a family history of cancer and the consumption of alcohol are also risk factors for CCA. Resemblances between the lower and upper parts of the region in terms of the role of various dietary habits are less clear.

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