RESEARCH ARTICLE

Association between Pesticide Use and Cholangiocarcinoma

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Abstract

Background: Thailand remains a primarily agricultural country and Thai farmers are heavy users of pesticides. Coincidentally the incidence of cholangio carcinoma (CCA) is high in parts of the country, but no previous study has examined any association between the two. Materials and Methods: The present matched, case-control study covered patients admitted to Srinagarind Hospital, Khon Kaen University, Thailand. The case group comprised 210 cases diagnosed with CCA and the control group 840 diagnosed with other diseases. Cases and controls were matched for sex, age within five years, and date of admission within three months. Multiple conditional logistic regression was used for the analysis. Results: After adjusting for potential confounders, pesticide use as compared with never used pesticide was not associated with CCA (ORadj=1.11, 95% CI: 0.77, 1.60) and neither was there any significant relationship between CCA and duration of pesticide use, type or number of types pesticide use. Conclusions: The current study thus found no association between pesticide use and CCA.

Keywords: Pesticides use - cholangiocarcinoma - cancer - North-East Thailand

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Introduction

Cholangiocarcinoma (CCA) is a malignancy of the bile duct. It is classified into three types: intrahepatic, perihilar, and distal (American Cancer Society, 2014; Darwin et al., 2015). CCA occurs worldwide, but high incidence rates of over 6.0 per 100,000 are reported in Thailand, Korea, and China. In Thailand, the incidence rate varies by region, ranging from 5.4 to 85.0 per 100,000: the highest rate is in the northeastern region (Bragazzi et al., 2011). The respective mortality rate among Thai men and women is around 33 and 11 per 100,000 (International Agency for Research on Cancer, 2015).

Epidemiological studies reveal that the major causes of CCA in Thailand are chronic infection with the liver fluke, *Opisthorchis viverrini* (*O. viverrini*), and consumption of foods containing nitrosamines (Pairojkul et al., 1991; Sripa et al., 2007; Watanapa and Watanapa, 2002). There is no evidence confirming the apparent association between pesticide exposure and CCA. However, since a function of the liver is to metabolize substances and excrete the metabolites in the bile through the bile duct, it is possible that pesticide exposure may contribute to carcinogenesis of the liver and/or bile duct. In a recent, large-scale, case-control study in Spain, people living in areas of high pesticide use had increased odds of developing a wide range of different forms of cancer (Parron et al., 2014). In a previous review, an association between pesticide use and cancers was found (Alavanja and Bonner, 2012).

Thailand is an agriculture-based nation (Thai Organic Trade Association, 2011). The number of agricultural holdings is 5.9 million, accounting for about 25 percent of households nationwide. The number of holdings engaged in cultivating crops is 4.5 million. The northeastern region has the highest number of holdings (2.7 million) and 72.4% of the holdings are for cultivating crops (National Statistical Office, Ministry of Information and Communication Technology, 2014).

To increase agricultural production, farmer in Thailand use pesticides. Pesticides include insecticides, herbicides, rodenticides, fungicides, and various other substances used to control pests (Michigan Department of Community Health, 2004). Pesticide imports into Thailand have increased from 110,000 tons in 2007 to 172,000 tons in 2013. The top ranking imports are herbicides, insecticides, and fungicides (Panuwet et al., 2012; Tawatsin et al., 2015). The report showed that 42.4% of holdings in the whole country and 32.5% in the northeastern region reported using chemical pesticides (National Statistical Office, Ministry of Information and Communication

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Technology, 2014).

Pesticides are toxic chemicals and can be hazardous to humans exposed to them (Bridget Hicks National Association of Geoscience Teachers (NAGT) Agricultural Pesticides and Human Health, 2013). From the perspective of carcinogenesis, the use of pesticide was found to be associated with human gene mutations, so pesticide use is potentially relevant to carcinogenesis. Indeed, some kinds of pesticide (e.g., organochlorides) are known tumor promoters in animal models (Acquavella et al., 2003).

Although Thai farmers are particularly heavy users of pesticides and the incidence rate of CCA is high (particular in northeastern region), no previous study has tested the association between pesticide use and CCA. This is significant as pesticide has long been suspected to be associated with CCA, (Wiwanitkit, 2009) so assessing the association between pesticide use and CCA was our aim.

Materials and Methods

Study design

The study used a matched case-control study. The matched variables were age, sex, and date of admission.

Participants

The data set used for analysis was the same as that compiled for an earlier hospital-based, case-control study, which included 210 cases and 840 controls (Kamsa-Ard et al., 2015). All cases and controls met the following eligibility criteria.

Inclusion criteria

Cases: 1). New inpatients with CCA diagnosed by physicians at Srinagarind Hospital, Khon Kaen University, Thailand. 1.1). Diagnosis was confirmed by histology and made between September 1, 2012 and August, 31, 2014; and 1.2). CCA was the primary diagnosis; and 2). Underwent surgery related to CCA between September 1, 2012 and August 31, 2014 at Srinagarind Hospital, Khon Kaen University, Thailand.

Controls: The controls were selected from the database at Srinagarind Hospital. 1). Inpatients admitted to Srinagarind Hospital, Khon Kaen University, Thailand, by the Departments of Otolaryngology, Ophthalmology, Rehabilitation Medicine, or Orthopedics between September 1, 2012 and August 31, 2014; 1.1). Matched with cases by sex, age (± 5 years), and date of admission to Srinagarind Hospital (± 3 months); and, 1.2). No history of liver diseases, liver cancer, CCA, or any other cancer.

Exclusion criteria

Any potential case or control: 1) Unable to speak Thai or the local Thai dialect (E-san); 2) Having difficulty communicating because of impaired cognitive function or illness.

Variables and measurement

The participants were interviewed by trained staff for (a) demographic data (b) detailed history of *O. viverrini* infection and treatment with praziquantel (PZQ) (c) tobacco and alcohol use (d) a family history of cancer and (e) history of pesticide use. Questions about pesticide use included (i) type (herbicide, rodenticide, insecticide, and fungicide) (ii) brand name(s), (iii) frequency of use and (iv) duration of exposure. The interviewers were blinded to the case-versus-control status of their interviewees, and all interviews were conducted before surgery for both cases and controls.

Statistical methods

A conditional logistic regression was used to calculate the odds ratios for the association between pesticide use and CCA. The covariates were educational level, involvement in horticultural work, raw fish consumption, fermented fish consumption, PZQ use, diabetic mellitus (DM), and family history of cancer. Backward elimination was used to build the final model. The initial model included pesticide use, educational level, involvement in horticultural work, raw fish consumption, fermented fish consumption, *O. viverrini* infection, PZQ usage, smoking, alcohol drinking, DM, and family history of cancer. Confounders with a p-value <0.05 were retained in the final model; these included: educational level, involvement in horticultural work, raw fish consumption,

Table 1. Baseline Characteristics of Case and Control Groups (210 Cases, 840 Controls)

Characteristics	Cases n (%)	Controls n (%)	p-value	Characteristics	Cases n (%)	Controls n (%)	p-value
1. Educational level			0.001	6. PZQ usage			<0.001
Primary school	166 (79.1)	571 (68.0)		No	102 (48.6)	550 (65.5)	
Higher than primary school	44 (20.9)	269 (32.0)		Yes	108 (51.4)	290 (34.5)	
2. Cultivation work			<0.001	7. Smoking			0.045
No	20 (9.5)	176 (20.9)		No	95 (45.2)	421 (50.1)	
Yes	190 (90.5)	664 (79.1)		Yes	115 (54.8)	419 (49.9)	
3. Raw fish consumption			<0.001	8. Alcohol drinking			0.036
No	81 (38.6)	518 (61.7)		No	71 (33.8)	334 (39.8)	
Yes	129 (61.4)	322 (38.3)		Yes	139 (66.2)	506 (60.2)	
4. Fermented fish consumption			<0.001	9. DM			<0.001
Never / Cooked	124 (59.0)	634 (75.4)		No	185 (88.1)	616 (73.3)	
Half-cooked / Raw	86 (41.0)	206 (24.6)		Yes	25 (11.9)	224 (26.7)	
5. OV infection			0.067	10. Family history of cancer			<0.001
No	78 (37.1)	331 (39.4)		No	102 (49.3)	584 (70.6)	
Yes	59 (28.1)	151 (18.0)		Yes	105 (50.7)	243 (29.4)	
Unknown	73 (37.8)	358 (42.6)					

Table 2. Association between Pesticide use and CCA (210 Cases, 840 Controls)

Model	Pesticide	Case n (%)	Control n (%)	ORcrude	ORadj	95% CI	p-value
1	Pesticide use						0.68
	No	117 (55.7)	577 (68.7)	1.00	1.00		
	Yes	93 (44.3)	263 (31.3)	1.74	1.08	0.75-1.57	
2	Duration of pesticide use						0.85
	0 year	117 (55.7)	577 (68.7)	1.00	1.00		
	≤ 5 years	40 (19.1)	98 (11.6)	2.16	1.20	0.72-1.99	
	> 5 years	24 (11.4)	72 (8.6)	1.80	1.17	0.65-2.08	
	Unknown	29 (13.8)	93 (11.1)	1.52	0.94	0.56-1.58	
3	Herbicide						0.29
	No	167 (79.5)	689 (82.0)	1.00	1.00		
	Yes	43 (20.5)	151 (18.0)	1.20	0.78	0.49-1.23	
4	Rodenticide						0.59
	No	148 (70.5)	680 (80.9)	1.00	1.00		
	Yes	62 (29.5)	160 (19.1)	1.81	1.12	0.75-1.66	
5	Insecticide						0.11
	No	184 (87.6)	732 (87.1)	1.00	1.00		
	Yes	26 (12.4)	108 (12.9)	0.95	0.64	0.37-1.10	
6	Fungicide						0.89
	No	203 (96.7)	820 (97.6)	1.00	1.00		
	Yes	7 (3.3)	20 (2.4)	1.45	0.93	0.36-2.40	
7	Number of pesticide types						0.10
	No	117 (55.7)	577 (68.7)	1.00	1.00		
	1 type	61 (29.1)	139 (16.6)	2.16	1.33	0.88-2.01	
	≥ 2 types	32 (15.2)	124 (14.7)	1.31	0.74	0.43-1.25	

OR_{adj} adjusted for education level, horticultural work, raw fish consumption, fermented fish consumption, PZQ usage, DM, and family history of cancer

fermented fish consumption, PZQ usage, DM, and family history of cancer.

Ethics

This study was approved by the Khon Kaen University Ethics Committee for Human Research (Reference No. HE551032 and HE581010).

Results

Males represented 60% of participants in both the case and control groups. The respective mean (SD) age was 60.2 (8.4) and 59.8 (8.6) years for the cases and controls. The average difference in admission date between the two groups was 34.7 (27.0) days; with a median difference of 27 (min=0; max=106) days. There were large differences between cases and controls in educational level, involvement in horticulture, raw fish consumption, fermented fish consumption, *O. viverrini* infection, PZQ usage, DM, and family history of cancer, but there was no significant difference in the proportion of smokers or drinkers (Table 1).

After controlling for educational level, horticultural work, raw fish consumption, fermented fish consumption, PZQ usage, DM, and family history of cancer, no statistically significant association could be confirmed between CCA and pesticide use, duration of pesticide exposure, type of pesticide (herbicides, rodenticides, insecticides, and fungicides), or number of pesticide types (Table 2).

Discussion

Pesticide use in Thailand is common-sprayed or broadcast-and use of personal protective equipment is uncommon (Panuwet et al., 2012). Numerous studies have demonstrated pesticide residues in groundwater in Thailand (Kearns, 2008). This is consistent with the sheer numbers of people who work in agriculture (i.e., 80-90 % of our cases and controls; albeit pesticide exposure among our groups was only between 30 and 45 %). Notwithstanding use and exposure to pesticides, our statistical analysis did not confirm any statistically significant association between pesticide use and occurrence of CCA. This main outcome is consistent with that of the previous study which showed no statistically significant association between CCA and exposure to herbicides, rodenticides, insecticides, fungicides, and number of different types of pesticide (Schmeisser et al., 2010).

The strengths of the study were: (a) the diagnosis of CCA was confirmed by histology; (b) the control group was selected from among patients admitted to the same hospital as the cases; (c) the controls had no history of liver diseases; (d) the potential confounders (age and sex) were controlled at the design stage as matching variables; (Kamsa-Ard et al., 2015) (e) any other confounding variables (i.e., education level, cultivation work, raw fish consumption, fermented fish consumption, PZQ usage, DM, and family history of cancer) were adjusted for in the conditional logistic regression; and, (f) the research assistants were blinded to the case/control status of the patients.

A limitation of the current study is that it relied upon data collected for another study for which the required sample size estimation was based on different objective (Kamsa-Ard et al., 2015). Thus, the current study had a calculated power of only 40%. Moreover, the collection of data regarding pesticides uses is difficult because there are many kinds of pesticides used in Thailand and the intensity of pesticide exposure is somewhat subjective. All of these may have contributed to the apparent non-association between pesticide use and CCA. We believe, however, that there may be some association between pesticide use and development of cholangiocarcinoma, so to clarify this issue, further study designed for this objective should be carried.

In conclusion, this study could not confirm any statistically significant association between pesticide use and development of CCA.

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