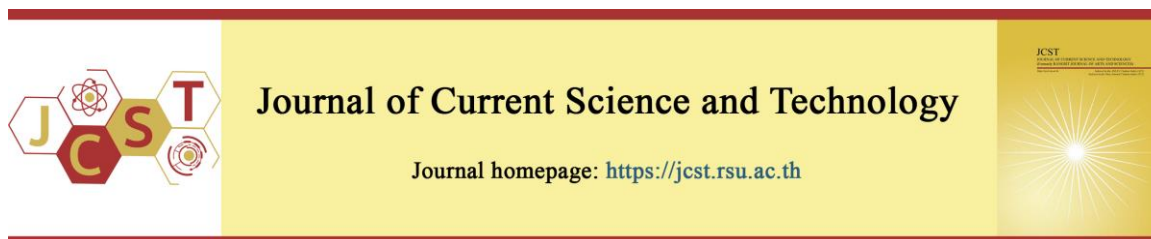


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Association between Praziquantel Treatment and Cholangiocarcinoma in Khon Kaen, Thailand: A Retrospective Cohort Study

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Abstract

Cholangiocarcinoma (CCA) is highly prevalent in northeastern Thailand, where *Opisthorchis viverrini* (OV) infection is endemic. Recurrent infection may lead to repeated praziquantel (PZQ) treatment, chronic biliary inflammation, and long-term carcinogenic processes. However, population-based longitudinal evidence regarding the association between PZQ use and CCA remains limited. Therefore, this study aimed to estimate the association between PZQ use and CCA incidence in a population-based cohort. This retrospective cohort study used data from the Khon Kaen Cohort Study conducted between 1990 and 2001. A total of 17,941 participants aged 30–70 years were followed through linkage with the Khon Kaen Population-Based Cancer Registry until 2022. Participants were classified by baseline history of PZQ use, obtained from structured interviews. Missing values for PZQ use and OV infection were addressed using logistic regression-based imputation models. Generalized linear models were used to estimate relative risks (RRs) and 95% confidence intervals (CIs). As a secondary exploratory analysis, the CCA Risk Lifestyle Index (CRLI), constructed from four factors, namely PZQ use, OV infection, alcohol consumption, and smoking (range 0–4), was evaluated separately. Among participants, 3,769 (21.01%) reported PZQ use. CCA occurred in 156 PZQ users (4.14%) and 456 non-users (3.22%). PZQ use was associated with a modestly increased risk of CCA (adjusted RR = 1.22, 95% CI: 1.01–1.48). Higher CRLI scores were associated with increased CCA risk (RR = 1.23, 95% CI: 1.13–1.33). PZQ use was associated with higher CCA incidence in this cohort. However, the findings should be interpreted cautiously because residual confounding related to recurrent OV infection may remain. Further longitudinal studies are needed to clarify this relationship.

Keywords: cholangiocarcinoma, *Opisthorchis viverrini*, praziquantel

1. Introduction

Cholangiocarcinoma (CCA) arises from the epithelial cells of the biliary tract, including internal and external biliary ducts (Khuntikao, 2005). According to 2022 estimates from the WHO Global Cancer Observatory, liver and bile duct cancer ranked seventh in incidence worldwide (age-standardized rate [ASR] = 9.5) (Ervik et al., 2024). In Thailand, national cancer registry data for 2016–2018 reported ASRs for liver and bile duct cancer of 33.2 per 100,000 in males and 12.2 per 100,000 in females, with a particularly high burden observed in northeastern Thailand (Rojanamatin et al., 2021).

Several factors have been found to influence the incidence of bile duct cancer, including sex, age, family history of cancer, residence near a water reservoir, consumption of undercooked food, and a history of bile duct disease (primary sclerosing cholangitis). However, the principal risk factor remains liver fluke infection (*Opisthorchis viverrini*) (Watanapa, 2002).

Liver fluke (*Opisthorchis viverrini*, OV) infection is highly prevalent in northeastern Thailand, and it is estimated that approximately 10% of the Thai population, or nearly 6 million people, are infected (Bureau of Epidemiology, 2010). Infection is primarily associated with the consumption of raw or partially cooked cyprinid fish containing infective metacercariae (Sripa et al., 2005; Watanapa, 2002). After ingestion, the parasites migrate to the biliary tract, where chronic irritation and obstruction of the bile ducts may contribute to cholangiocarcinoma development.

Repeated OV infection is common in northeastern Thailand because of persistent consumption of raw or undercooked freshwater fish (Sripa et al., 2005; Watanapa, 2002). Recurrent infection may lead to repeated praziquantel treatment, chronic biliary inflammation, oxidative stress, and repeated tissue injury, which are considered potential mechanisms contributing to cholangiocarcinoma development (Pinlaor et al., 2004; Dechakhamphu et al., 2008; Pinlaor et al., 2008; Songserm et al., 2020). Therefore, in epidemiological studies, Praziquantel (PZQ) use may function not only as a pharmacological exposure but also as a surrogate marker of cumulative OV infection burden and reinfection frequency, particularly in endemic areas where reinfection after treatment is common (Saengsawang et al., 2016).

PZQ is the standard drug for treating OV infections. When liver fluke infection is detected, it is treated with a single dose (Watanapa, 2002). PZQ exerts its antiparasitic effects by disrupting the

parasite's tegument and inducing sustained muscle contraction, leading to parasite death and expulsion. Experimental studies have suggested that praziquantel treatment may be associated with increased intracellular reactive oxygen species (ROS), oxidative stress, and elevated malondialdehyde (MDA), which have been proposed as potential mechanisms related to carcinogenesis (Dechakhamphu et al., 2008; Giri & Roy, 2016; Pinlaor et al., 2008; Wirasorn et al., 2014). Previous studies have reported that repeated praziquantel treatment for more than two OV infections was associated with an increased risk of cholangiocarcinoma (OR = 2.30, 95% CI: 1.20–4.40), intrahepatic CCA (OR = 4.21, 95% CI: 1.61–11.05), and extrahepatic CCA (OR = 1.38, 95% CI: 0.56–3.41) compared with individuals who had never received praziquantel treatment (Kamsa-ard et al., 2015; Luvira et al., 2018).

Most previous studies investigating the association between praziquantel use and cholangiocarcinoma have been hospital-based matched case-control studies (Kamsa-ard et al., 2015; Luvira et al., 2018), while population-based evidence remains limited (Thinkhamrop et al., 2021). In addition, several previous studies were conducted in selected clinical populations or specific geographic settings, which may limit generalizability to the broader at-risk population (Kamsa-ard et al., 2015; Steele et al., 2018). Therefore, the present study used data from the Khon Kaen Cohort Study (KKCS), a large population-based retrospective cohort established between 1990 and 2001 with follow-up through 2022. Participants were linked to the Khon Kaen Population-Based Cancer Registry to identify subsequent cholangiocarcinoma incidence according to praziquantel exposure history.

This study used robust population-based data to generate more generalizable and evidence-based findings regarding the association between praziquantel use and cholangiocarcinoma in Khon Kaen Province. The findings may help inform public health strategies regarding *Opisthorchis viverrini* control and praziquantel use in endemic areas.

2. Objectives

The objective of this study was to estimate the association between praziquantel use and cholangiocarcinoma incidence in Khon Kaen, Thailand. A secondary exploratory analysis was conducted to evaluate the Cholangiocarcinoma Risk Lifestyle Index (CRLI).

3. Materials and Methods

3.1 Study Design and Cancer Follow-up

This retrospective cohort study used data from the Khon Kaen Cohort Study (KKCS). The KKCS included residents of Khon Kaen Province aged 30–70 years who were enrolled between 1990 and 2001 and had no previous diagnosis of cancer ($n = 19,861$). All cohort subjects were followed up for 22 years until December 31, 2022, by linking data with a citizen identification number and name to detect Cholangiocarcinoma (CCA) with the population cancer registry database of Khon Kaen Province (population-based cancer registry in Khon Kaen: KKCR). CCA diagnoses were obtained using the International Classification of Diseases for Oncology, 3rd edition (ICD-O-3), and only cases coded C22.1, C24.0, C24.8, and C24.9 were included.

3.2 Sample Size

This study included the entire cohort of 17,941 eligible participants without conducting an a priori sample size calculation. A post hoc power analysis was performed using the sample size formula for cohort studies with binary outcomes proposed by Rosner (2000). Based on the observed parameters, the study's statistical power was estimated to exceed 99%. The large sample size provided substantial statistical precision for detecting associations.

Participants were eligible for inclusion if they were between 30 and 70 years of age at enrollment, had official residency in Khon Kaen Province, and had no prior diagnosis of any type of cancer. Participants were excluded if they lacked essential follow-up information, such as a valid national identification number. In addition, participants who developed cancers other than cholangiocarcinoma during follow-up were excluded from the present analysis.

3.3 Data Quality

Data quality from the population-based cancer registry was assessed using two key indicators. The percentage of microscopically verified cases (MV%) was 12.25%, which is consistent with previous reports for liver and bile duct cancers in Thailand.

The percentage of cases identified through death certificates only (DCO%) was 3.27%, which falls well below the international standard threshold of 10% as recommended by IARC (Curado et al., 2007). These indicators confirm that the cancer registry data were sufficiently complete for epidemiological analysis.

3.4 Exposure Assessment

All participants in the Khon Kaen Cohort Study (KKCS) were interviewed by trained nurses using structured questionnaires at baseline enrollment. Praziquantel (PZQ) exposure was defined as a binary variable based on self-reported history of ever-versus-never use for the treatment of OV infection. Information regarding treatment frequency or cumulative number of treatment courses was not available in the original cohort database.

3.5 Statistical Analysis

Missing data for the two primary variables, praziquantel treatment and OV infection, were addressed using logistic regression-based imputation models. Candidate variables for the initial imputation models included age, sex, smoking history, alcohol consumption, history of OV infection, and family history of cancer. Final imputation models were selected based on overall model performance assessed using the Hosmer–Lemeshow goodness-of-fit test and receiver operating characteristic (ROC) curve analysis. Predicted probabilities from the final models were converted into binary exposure classifications using ROC-derived cut-off values, and these classifications were used to replace missing exposure values.

The Cholangiocarcinoma Risk Lifestyle Index (CRLI) was constructed by summing four risk exposures: praziquantel use, OV infection, alcohol consumption, and smoking. Each exposure was assigned one point, yielding a total score ranging from 0 to 4. The CRLI was evaluated separately from the individual risk-factor analyses and was not included in the same regression models as its component variables to avoid multicollinearity issues.

To examine the association between praziquantel treatment and CCA development, generalized linear models with a binomial family and log link function were used to estimate relative risks (RRs), adjusted relative risks (Adj.RRs), corresponding 95% confidence intervals (CIs), and p-values. This approach was selected because the primary objective was to estimate the association between baseline praziquantel exposure and cumulative incidence of cholangiocarcinoma during follow-up, rather than to model time to diagnosis. Age and sex were included a priori as standard confounding variables because both are well-established determinants of cholangiocarcinoma incidence. Other variables, including smoking history, alcohol consumption, OV infection, and family history of cancer, were each evaluated individually with adjustment for age and sex.

As a sensitivity analysis, a fully adjusted generalized linear model was fitted to complete-case data, including praziquantel use, age, sex, alcohol consumption, smoking, family history of cancer, and OV infection. This analysis was conducted to evaluate the robustness of the primary findings after additional adjustment for established or plausible risk factors. Because alcohol consumption, smoking, and family history of cancer contained substantial missing data in the historical cohort database, the sensitivity analysis

was restricted to participants with complete data for all covariates.

All statistical analyses were performed using Stata software version 18 (licensed to Khon Kaen University). All tests were two-sided, and a p-value of less than 0.05 was considered statistically significant.

3.6 Ethical considerations

This research was approved by The Ethical Committee for Human Research of Khon Kaen University, Thailand (HE-671265).

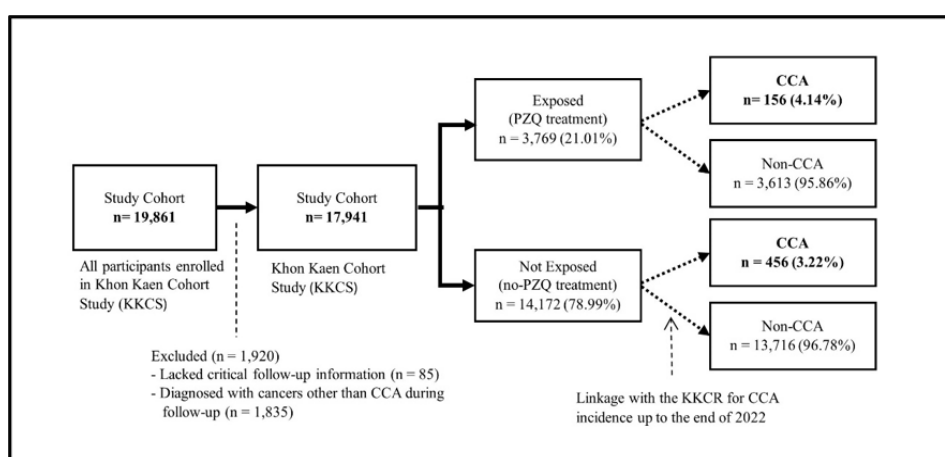


Figure 1 Flow Diagram of the Retrospective Cohort Study and CCA Outcome

Table 1 Demographic characteristics of Khon Kaen Cohort

Characteristics	total (n= 17,941)	non-PZQ (n = 14,172)	PZQ (n = 3,769)	p-value
Cholangiocarcinoma				0.006 ^a
No	17,329 (96.59)	13,716 (96.78)	3,613 (95.86)	
Yes	612 (3.41)	456 (3.22)	156 (4.14)	
Sex				< 0.001 ^a
Female	12,269 (68.39)	10,657 (75.20)	1,612 (42.77)	
Male	5,672 (31.61)	3,515 (24.80)	2,157 (57.23)	
Age at interview				< 0.001 ^b
Mean ± sd	50.13 ± 8.66	51.30 ± 8.07	45.77 ± 9.38	
Median (min, max)	50 (30,69)	51 (30,69)	44 (30,69)	
OV infection*				< 0.001 ^a
No	8,113 (48.67)	7,195 (52.45)	918 (31.08)	
Yes	8,558 (51.33)	6,522 (47.55)	2,036 (68.92)	
Alcohol*				<0.001 ^a
No	4,891 (58.22)	3,763 (60.99)	1,128 (50.56)	
Yes	3,510 (41.78)	2,407 (39.01)	1,103 (49.44)	
Smoking*				< 0.001 ^a
No	12,227 (74.45)	10,584 (78.52)	1,643 (55.85)	
Yes	4,195 (25.55)	2,896 (21.48)	1,299 (44.15)	
Family history of cancer*				< 0.001 ^a
No	12,303 (74.25)	10,643 (78.11)	1,660 (56.39)	
Yes	4,266 (25.75)	2,982 (21.89)	1,284 (43.61)	

PZQ, praziquantel treatment; a, chi-square test; b, Mann–Whitney test; n, number. *Numbers may not sum to the total because of missing data

4. Results

4.1 Demographics and Cohort Development

A total of 17,941 Khon Kaen Cohort Study participants met the inclusion and exclusion criteria. Of these, 3,769 individuals (21.01%) reported a history of praziquantel use, while 14,172 (78.99%) did not. Participants were followed until December 31, 2022, by linking with the Khon Kaen Population-Based Cancer Registry (KKPCR). Among praziquantel users, 156 individuals (4.14%) were diagnosed with cholangiocarcinoma, compared with 456 cases (3.22%) in the non-user group (Figure 1).

Baseline characteristics differed significantly between the two groups. Compared with non-users, praziquantel users had higher proportions of males (57.23% vs. 24.80%), history of OV infection (68.92% vs. 47.55%), alcohol consumption (49.44% vs. 39.01%), smoking (44.15% vs. 21.48%), and family history of cancer (43.61% vs. 21.89%). The only exception was mean age, which was higher in the non-user group (51.30 ± 8.07 years) compared with the user group (45.77 ± 9.38 years) (Table 1).

4.2 Model Development

The final model for imputing praziquantel use included age and sex, with acceptable model fit (Hosmer–Lemeshow $p = 0.6427$) and modest discriminatory performance (ROC = 0.6143). Before imputation, data on praziquantel exposure were missing in 27.59% of participants. After imputation, the proportion of participants classified as having a history of praziquantel use increased from 5.37% to 21.01%, while non-users increased from 67.05% to 78.98%.

For OV infection, the imputation model included family history of cancer and sex, with acceptable model fit (Hosmer–Lemeshow $p = 0.9133$) and limited discriminatory performance (ROC = 0.5291). Before imputation, OV infection data were missing in 63.16% of participants. After imputation, the proportion of participants with a history of OV infection increased from 19.47% to 47.70%, while those without a history of infection increased from 17.37% to 45.22%, with 7.08% of OV infection data remaining missing.

The increase in the estimated prevalence of PZQ use and OV infection after imputation reflects the high proportion of missing exposure data and the distribution of observed covariates used in the imputation models. Given the modest discriminatory performance of the imputation models, these imputed

exposure classifications should be interpreted cautiously.

4.3 Generalized Linear Models

Praziquantel use was significantly associated with an increased risk of cholangiocarcinoma (RR = 1.29, 95% CI: 1.08–1.54; $p = 0.006$). Other significant predictors included male sex (RR = 2.19, 95% CI: 1.88–2.56; $p < 0.001$), older age (RR = 1.05 per year, 95% CI: 1.04–1.06; $p < 0.001$), alcohol consumption (RR = 1.27, 95% CI: 1.03–1.56; $p = 0.027$), smoking (RR = 2.03, 95% CI: 1.72–2.40; $p < 0.001$), OV infection (RR = 1.52, 95% CI: 1.28–1.80; $p < 0.001$), and a family history of cancer (RR = 1.21, 95% CI: 1.01–1.45; $p = 0.004$) (Table 2).

In the multivariate analysis, sex and age were included as covariates. After adjustment, praziquantel use remained significantly associated with cholangiocarcinoma (Adj.RR = 1.22, 95% CI: 1.01–1.48). OV infection was also independently associated with increased risk (Adj.RR = 1.21, 95% CI: 1.00–1.45), as was having a first-degree relative with cancer (Adj.RR = 1.33, 95% CI: 1.11–1.60) (Table 2).

4.4 Sensitivity Analysis

As a sensitivity analysis, a fully adjusted generalized linear model was fitted to complete-case data ($n = 8,250$) and included praziquantel use, age, sex, alcohol consumption, smoking, family history of cancer, and OV infection. In this model, the association between praziquantel use and cholangiocarcinoma was attenuated and no longer statistically significant (Adj. RR = 0.94, 95% CI: 0.72–1.22; $p = 0.636$). Because the analysis was restricted to complete cases owing to substantial missing data for several covariates, these findings should be interpreted with caution and are presented as a sensitivity analysis rather than the primary analysis.

4.5 CCA Risk Lifestyle Index (CRLI)

Generalized linear models showed that each one-point increase in CRLI was associated with a 1.23-fold higher risk of CCA (RR = 1.23, 95% CI: 1.13–1.33; $p < 0.001$). Compared to those with a score of 0, participants with scores of 3 and 4 had elevated risks (RR = 1.63, 95% CI: 1.19–2.23 and RR = 2.16, 95% CI: 1.54–3.06, respectively). A binary classification (score > 2 vs. ≤ 2) was associated with nearly double the risk (RR = 1.98, 95% CI: 1.60–2.46; $p < 0.001$) (Table 2).

Table 2 Bivariate and Multivariate Analysis of the Factors Associated with CCA using Generalized Linear Models

Risk Factors	CCA (n = 612)		Crude RR	95 % CI, <i>p</i> -value	Adj. RR	95 % CI, <i>p</i> -value
	n	%				
Gender						
Female	304	2.48	1	-		
Male	308	5.43	2.19	(1.88 - 2.56), < 0.001	-	-
Age at interview						
1 year increase	612	-	1.05	(1.04 - 1.06), < 0.001	-	-
PZQ treatment						
No	456	3.22	1	-	1	
Yes	156	4.14	1.29	(1.08 - 1.54), 0.006	1.22	(1.01 - 1.48), 0.039
Alcohol						
No	176	3.60	1	-	1	
Yes	160	4.56	1.27	(1.03 - 1.56), 0.027	1.01	(0.8 - 1.27), 0.957
Smoking						
No	326	2.67	1	-	1	
Yes	227	5.41	2.03	(1.72 - 2.4), < 0.001	1.25	(0.93 - 1.69), 0.137
OV infection						
No	212	2.61	1	-	1	
Yes	340	3.97	1.52	(1.28 - 1.8), < 0.001	1.21	(1 - 1.45), 0.048
Family history of cancer						
No	387	3.15	1	-	1	
Yes	162	3.80	1.21	(1.01 - 1.45), 0.004	1.33	(1.11 - 1.6), 0.002
CRLI (continuous)						
1 score increase	612	-	1.23	(1.13 - 1.33), < 0.001	-	-
CRLI (binary)						
Score > 2 vs. ≤ 2	126	6.33	1.98	(1.60 - 2.46), < 0.001	-	-

CCA, cholangiocarcinoma; n, number; RR, relative risk; Adj.RR, adjusted relative risk; CI, confidence interval; CRLI, CCA Risk Lifestyle Index. Adj.RRs were estimated from separate multivariable generalized linear models, each adjusted for age and sex. All generalized linear models were fitted using the imputed analytic dataset

5. Discussion

5.1 Association between Praziquantel use and Cholangiocarcinoma

Praziquantel use was significantly associated with increased CCA incidence in the crude analysis. Participants with a history of praziquantel use had a 1.29-fold higher risk of developing CCA compared with non-users (RR = 1.29, 95% CI: 1.08–1.54).

These findings are consistent with two previous systematic reviews and meta-analyses. The first, combining four case-control studies (n = 1,921), reported a pooled OR of 2.02 (95% CI: 1.59–2.57), while the second, based on three studies (n = 360), reported an OR of 1.93 (95% CI: 1.2–3.1) (Kamsa-ard et al., 2018; Steele et al., 2018).

Similar findings were observed in a hospital-based matched case-control study conducted at Srinagarind Hospital (n = 1,050), where praziquantel use was associated with a 1.93-fold higher odds of CCA (OR = 1.93, 95% CI: 1.35–2.77) (Kamsa-ard et al., 2015), and in a large cross-sectional study from the Cholangiocarcinoma Screening and Care Program

(CASCAP) involving 264,720 participants in northeastern Thailand, which reported an OR of 1.51 (95% CI: 1.21–1.87) (Thinkhamrop et al., 2021).

In the present study, multivariable analysis adjusting for age and sex confirmed the association between praziquantel use and CCA (adjusted RR = 1.22, 95% CI: 1.01–1.48; *p* = 0.039). This finding is consistent with another matched case-control study that adjusted for raw fish consumption, family history of cancer, and education level (adjusted OR = 1.49, 95% CI: 1.02–2.20; *p* = 0.025) (Kamsa-ard et al., 2015).

In the sensitivity analysis using a fully adjusted complete-case model, the association between praziquantel use and cholangiocarcinoma was attenuated and was no longer statistically significant. This attenuation may reflect additional adjustment for OV infection and other measured risk factors, but it may also reflect the substantial reduction in sample size resulting from missing covariate data. Because the complete-case analysis included fewer than half of the original cohort, the findings may be affected by

selection bias and reduced statistical precision. Therefore, the primary analysis based on the larger cohort remains the principal analysis of this study, whereas the fully adjusted model should be interpreted as a sensitivity analysis.

Most of these findings were derived from observational epidemiological studies, because interventional studies in humans are not ethically feasible.

However, the observed association between praziquantel use and CCA should be interpreted cautiously. Because praziquantel is prescribed primarily for the treatment of OV infection, PZQ exposure in this study may partly represent a surrogate marker for cumulative OV infection burden, reinfection frequency, or other underlying risk factors rather than an isolated pharmacological exposure.

An important interpretive issue is the apparent protective-versus-harmful paradox of praziquantel treatment. Praziquantel is the standard treatment for OV infection and may reduce parasite-related biliary injury by eliminating the infection. Therefore, a positive association between PZQ use and CCA should not be interpreted as evidence that PZQ itself causes CCA. Rather, this association may reflect confounding by indication, whereby individuals receiving PZQ were more likely to have had OV infection, recurrent infection, or higher cumulative exposure to the liver fluke transmission cycle and related risk factors. This interpretation is also consistent with experimental and clinical evidence suggesting that praziquantel treatment may reduce certain markers of DNA damage among liver fluke-infected individuals (Dechakhamphu et al., 2008). Thus, the observed association in this study should be viewed as an epidemiological association requiring cautious interpretation rather than as evidence of a direct harmful pharmacological effect of PZQ.

5.2 Association of Other Risk Factors with Cholangiocarcinoma

Male sex was associated with a higher CCA risk (RR = 2.19, 95% CI: 1.88–2.56), consistent with pooled ORs from cross-sectional (3.00, 95% CI: 0.80–11.25) and case-control studies (2.33, 95% CI: 1.03–5.27) (Kamsa-ard et al., 2018), and with Korean cohort data (HR = 1.96, 95% CI: 1.64–2.35) (Cho et al., 2022). A Thai study, however, reported a lower risk in males (OR = 0.81, 95% CI: 0.67–0.99) (Thinkhamrop et al., 2021).

Age was associated with a 5% increase in risk per year (RR = 1.05, 95% CI: 1.04–1.06), aligning

with Korean cohort HRs ranging from 2.33 to 3.03 (Cho et al., 2022), and age-group analyses (>50: OR = 6.58 (Kamsa-ard et al., 2018); >60: OR = 4.68 (Thinkhamrop et al., 2021); 65–74 vs. >75: OR = 1.60 (Peng et al., 2018)).

Alcohol use was associated with an increased risk (RR = 1.27, 95% CI: 1.03–1.56), although this association was attenuated after adjustment. Meta-analyses have reported ORs of 3.01 (Kamsa-ard et al., 2018) and 2.61 (Steele et al., 2018). Smoking was also associated with an increased risk (RR = 2.03), although this association was not statistically significant in adjusted models. Meta-analyses: OR = 1.46 (Kamsa-ard et al., 2018), 1.33 (Steele et al., 2018). Other studies: UK (OR = 1.46–1.77) (Petrick et al., 2017, 2018), China (OR = 2.76) (Xiong et al., 2018), Thailand (OR = 2.69) (Thinkhamrop et al., 2021).

OV infection was associated with CCA (RR = 1.52; adjusted RR = 1.21, $p = 0.048$), supported by meta-analyses (OR = 4.8–6.35) (Kamsa-ard et al., 2018; Shin et al., 2010; Steele et al., 2018), and case-control studies in Korea (OR = 3.49) (Lee et al., 2015), Russia (OR = 2.9) (Fedorova et al., 2023), and Thailand (OR = 1.63) (Thinkhamrop et al., 2021).

A family history of cancer was associated with an increased risk (RR = 1.21; adjusted RR = 1.33), consistent with pooled OR = 2.48 (Kamsa-ard et al., 2018) and 3.00 (Steele et al., 2018).

5.3 The CCA Risk Lifestyle Index (CRLI)

In this secondary exploratory analysis, higher CRLI scores were associated with increased CCA risk. Each additional risk factor was associated with a higher risk of CCA (RR = 1.23, 95% CI: 1.13–1.33), and risk increased progressively across CRLI score categories. No previous studies have evaluated CRLI in relation to CCA incidence, although one study reported an association between CRLI and CCA mortality (Pupacdi et al., 2024).

This exploratory analysis suggests that cumulative lifestyle-related exposures may contribute to increased CCA risk. Although the CCA Risk Lifestyle Index (CRLI) combines medical treatment, infection status, and behavioral risk factors, it was developed to summarize cumulative exposures associated with cholangiocarcinoma in this endemic setting. Similar approaches that combine multiple risk factors into a single composite score have been used to evaluate the cumulative effects of multiple exposures on cancer outcomes, although the component variables differ across studies (Zhang et

al., 2020). Therefore, the CRLI should be interpreted as an exploratory composite index.

5.4 Study Limitations

This study had several limitations. Because praziquantel exposure was assessed using self-reported history without verification from medical records, exposure misclassification and recall bias may have occurred. In addition, information regarding treatment frequency or cumulative number of praziquantel treatment courses was not available in the original cohort database, limiting the ability to distinguish repeated OV infection from repeated treatment exposure. The imputation models demonstrated only modest discriminatory performance, which may have affected the precision of exposure classification. Data on risk factor exposure were collected only once, at cohort enrollment, with no subsequent follow-up or verification from other sources.

The high proportion of missing data for OV infection and the modest discriminatory performance of the imputation models may have led to misclassification of exposure. In addition, the large shift in exposure prevalence after imputation indicates that the imputed classifications were sensitive to assumptions about the missing-data mechanism.

The fully adjusted sensitivity analysis was restricted to complete cases because several covariates contained substantial missing data, which may have introduced selection bias and reduced statistical precision.

Furthermore, retrospective verification of key exposures was not feasible because cholangiocarcinoma is associated with poor survival and most surviving cohort participants are now elderly, which makes direct follow-up difficult.

In addition, although the relatively low proportion of microscopically verified cases (MV = 12.25%) is consistent with previous reports on liver and bile duct cancers in Thailand, diagnoses based primarily on characteristic CT findings rather than on histopathological confirmation may have introduced some diagnostic uncertainty.

6. Conclusion

Praziquantel use was associated with higher cholangiocarcinoma incidence in this population-based cohort. However, the findings should be interpreted cautiously because residual confounding related to recurrent OV infection and other underlying risk factors may remain. Given the observational design of the study and the limitations of the exposure

assessment, the present findings should not be interpreted as evidence of a direct causal effect of praziquantel treatment on cholangiocarcinoma development.

In this secondary exploratory analysis, higher CCA Risk Lifestyle Index (CRLI) scores were associated with increased CCA risk, suggesting that cumulative lifestyle-related exposures may contribute to cholangiocarcinoma development. Further longitudinal studies with more detailed exposure assessment are needed to clarify these relationships.

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8. Conflict of Interest

The authors declare that they have no conflicts of interest.

9. Abbreviations

Abbreviations	Full Term
Adj.RR	Adjusted Relative Risk
ASR	Age-Standardized Rate
CASCAP	Cholangiocarcinoma Screening and Care Program
CCA	Cholangiocarcinoma
CI	Confidence Interval
CRLI	CCA Risk Lifestyle Index
CT	Computed Tomography
DCO	Death Certificate Only
IARC	International Agency for Research on Cancer
ICD-O	International Classification of Diseases for Oncology
KKCR	Khon Kaen Population-based cancer registry
KKCS	Khon Kaen Cohort Study
MDA	Malondialdehyde
MV	Microscopically Verified

Abbreviations Full Term

OR	Odds Ratio
OV	<i>Opisthorchis viverrini</i>
PZQ	Praziquantel
ROC	Receiver Operating Characteristic
ROS	Reactive Oxygen Species
RR	Relative Risk

10. CRediT Statement

Chalongpon Santong: Conceptualization, Methodology, Software, Programming, Formal Analysis, Investigation, Resources, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Supervision, Project Administration

Pattapong Kessomboon: Conceptualization, Methodology, Investigation, Writing – Review & Editing, Supervision, Funding Acquisition

Nusaraporn Kessomboon: Conceptualization, Methodology, Writing – Original Draft, Writing – Review & Editing

Banchob Sripa: Validation, Writing – Original Draft, Writing – Review & Editing

Patravoot Vatanasapt: Validation, Writing – Original Draft, Writing – Review & Editing

Ricardo Angeles: Validation, Writing – Original Draft, Writing – Review & Editing

Gina Agarwal: Validation, Writing – Original Draft, Writing – Review & Editing, Funding Acquisition

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