Journal of Public Health and Development
Vol.22 No.1 January-April 2024

ORIGINAL ARTICLE

The combination between praziquantel treatment and fatty liver disease in relation to diabetes mellitus in Northeast of Thailand

Dutsadee Khaweewong[1], Kavin Thinkhamrop[2,3,4*], Apiporn T. Suwannatrai[2,5], Attapol Titapun[2,3,6], Watcharin Loilome[2,3,7], and Matthew Kelly[8]

[1]Doctor of Public Health Program, Faculty of Public Health, Khon Kaen University, Khon Kaen, Thailand
[2]Cholangiocarcinoma Research Institute (CARI), Khon Kaen, Thailand
[3]Cholangiocarcinoma Screening and Care Program (CASCAP), Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
[4]Health and Epidemiology Geoinformatics Research (HEGER), Faculty of Public Health, Khon Kaen University, Khon Kaen, Thailand
[5]Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
[6]Department of Surgery, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
[7]Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
[8]Department of Applied Epidemiology, National Centre for Epidemiology and Population Health, Australian National University, Canberra, Australia

Corresponding Author: Kavin Thinkhamrop **Email:** kavith@kku.ac.th

Received: 7 September 2023 **Revised:** 19 November 2023 **Accepted:** 25 November 2023 **Available online:** January 2024
DOI: 10.55131/jphd/2024/220109

ABSTRACT

Diabetes mellitus (DM) is a growing global public health concern, including Thailand. Prior research has established an association between fatty liver disease (FLD) and DM. Moreover, the distribution of DM was also found in liver fluke endemic areas where praziquantel (PZQ) is used extensively. However, limited information exists regarding factors associated with DM, specifically in FLD patients undergoing PZQ treatment. Our study aimed to evaluate the association between the combination of PZQ treatment and FLD diagnosis, and DM in Northeast Thailand. We conducted the study among participants in northeastern Thailand who self-reported DM diagnosis and PZQ treatment history using health questionnaires. Additionally, FLD diagnosis was confirmed through ultrasonography from the Cholangiocarcinoma Screening Program data collected between 2013-2022. We employed multilevel mixed-effect logistic regression to assess the association between the combination of PZQ treatments and FLD diagnosis, and the occurrence of DM, represented as adjusted odds ratios (AOR) and their 95% confidence intervals (CI). Of 756,431 participants, the overall DM prevalence was 7%. Notably, 10.45% of individuals with FLD+ and 8.23% with PZQ+ were found to have DM. Specifically, the prevalence of DM in individuals with PZQ+ and FLD-, PZQ- and FLD+, and PZQ+ and FLD+ combinations were 7.14%, 10%, and 11.81%, respectively. In comparison to PZQ- and FLD-, participants with PZQ+ and FLD-, PZQ- and FLD+, and PZQ+ and FLD+ were associated with DM (AOR = 1.27; 95% CI: 1.24-1.30, AOR = 1.8; 95% CI: 1.76-1.85, and AOR = 2.19; 95% CI: 2.12-2.27, respectively). In conclusion, the combination of PZQ and FLD plays a crucial role in the association with DM, particularly in individuals with PZQ+ and FLD+. Our findings emphasize the importance of DM screening, with special attention to individuals with a combination of PZQ treatment and FLD diagnosis, in order to promote early detection and management of DM in Northeast Thailand.

Key words:
diabetes mellitus; praziquantel; fatty liver; combination; Northeast Thailand

Citation:
Dutsadee Khaweewong, Kavin Thinkhamrop, Apiporn T. Suwannatrai, Attapol Titapun, Watcharin Loilome, and Matthew Kelly. The combination between praziquantel treatment and fatty liver disease in relation to diabetes mellitus in Northeast of Thailand. J Public Hlth Dev. 2024;22(1):110-125 (https://doi.org/10.55131/jphd/2024/220109)

INTRODUCTION

Diabetes Mellitus (DM) is a chronic non-communicable disease that poses a significant global public health challenge[1]. Particularly, type 2 DM (T2DM), a widespread metabolic disorder on a global scale, has two primary etiological factors: impaired insulin secretion by pancreatic β-cells and inadequate responsiveness of insulin-sensitive tissues to insulin[2]. A previous study indicated that advancing age, higher body mass index (BMI), smoking, alcohol consumption, elevated uric acid levels, and low high-density lipoprotein (HDL) were identified as risk factors for T2DM[3, 4]. The global prevalence of DM in 2019 was estimated at 9.3%, and is projected to rise to 10.2% by 2030 and 10.9% by 2045[5]. In Thailand, DM prevalence of 9.9% in the adult population was found in 2018[6]. DM stands as a leading cause of death in Thailand, accounting for approximately 15% of the 200,000 chronic non-communicable disease related deaths in 2016[7]. Various factors have been associated with DM risk, including sex, advancing age, high body mass index, stress level, smoking cigarettes, alcohol consumption, and fatty liver disease (FLD)[8-12].

FLD, or hepatic steatosis, is a reversible condition marked by the buildup of sizable vacuoles containing triglyceride fat within liver cells[13]. Particularly nonalcoholic fatty liver disease (NAFLD), is recognized as a significant global health concern. As well as its connection with DM risk, the condition is also associated with cirrhosis, colorectal cancer, liver cancer, bile duct cancer, and DM[14-17]. NAFLD, a subtype of FLD, along with alcoholic fatty liver disease (AFLD)[18, 19], is common in the Thai population, particularly in the Northeast Region[20]. A study conducted in 2015 in Northeast Thailand revealed a notable prevalence of FLD in people with DM at 36.3%[21], indicating a relationship between the two conditions[22]. Furthermore, a study in 2017 reported the prevalence of FLD among DM patients in the same region as that of the 2015 study conducted in Thailand[20]. Another factor that may be associated with DM is praziquantel (PZQ) treatment. Past studies have reported links between PZQ and DM, with research indicating a significant increase in blood glucose levels in response to PZQ administration[23, 24].

PZQ is commonly used to eliminate *Opisthorchis viverrini*, a parasitic worm prevalent in endemic areas, particularly in the Northeast of Thailand[25, 26]. A study conducted in Thailand in 2021 reported that areas with a high prevalence of *O. viverrini* infection were also associated with a higher prevalence of DM than the areas with low prevalence of *O. viverrini* infection[27]. In contrast, a previous study from Lao PDR in 2018 examined the association between helminth infections and DM in adults. The study found that *Taenia* sp. infection was linked to elevated levels of HbA1c and an increased risk of DM. However, no association was observed between *O. viverrini* infection and DM in the study population[28]. Furthermore, a study from Thailand in 2020 demonstrated that individuals infected with *O. viverrini* had lower HbA1c levels compared to those not infected. Interestingly, the levels of HbA1c increased after PZQ treatment[24]. These findings suggest complex interactions between parasitic infections, PZQ treatment, and DM. The relationship between *O. viverrini* infection, PZQ treatment, and DM warrants further investigation to better understand the underlying mechanisms and potential implications for disease management and prevention.

These studies have demonstrated the link between FLD and DM, as well as the association between PZQ use and DM, particularly in populations living in areas at risk of liver fluke infection. These findings shed light on the longstanding health challenges faced in Thailand, especially in the northeastern region. Despite existing research indicating connections between FLD, PZQ treatment, and DM, there remain limitations regarding the risk of developing DM, especially in individuals using PZQ and diagnosed with FLD. Therefore, the primary aim of our study was to evaluate the association between the combination of PZQ treatments and FLD diagnosis and DM in Northeast Thailand. We aim to improve understanding of the interplay between PZQ treatment, FLD, and DM and to identify potential strategies for reducing risk of DM. Our research endeavors to contribute to efforts aimed at minimizing the distribution of PZQ use, FLD, and DM, with the ultimate goal of mitigating the risk of DM as much as possible.

METHODS

Study design

This cross-sectional study utilized data obtained from the Cholangiocarcinoma Screening and Care Program (CASCAP) conducted in the Northeast of Thailand. CASCAP is a pioneering initiative aimed at Cholangiocarcinoma (CCA) screening among high-risk populations, employing a community-based bottom-up approach with the goal of encompassing all residents in northeastern Thailand[29]. The study areas covered 2678 sub-districts across 20 provinces in Northeast Thailand. During the screening process, participants undergo an initial verbal assessment to identify potential risks of CCA, utilizing a CASCAP health questionnaire that covers various aspects such as socio-demographic information, history of PZQ treatments, and DM diagnosis. The ultrasound screening process aims to identify hepatobiliary abnormalities associated with CCA risk and includes FLD. The study area, as depicted in Figure 1, encompasses the entire northeastern region of Thailand, comprising a total of 20 provinces.

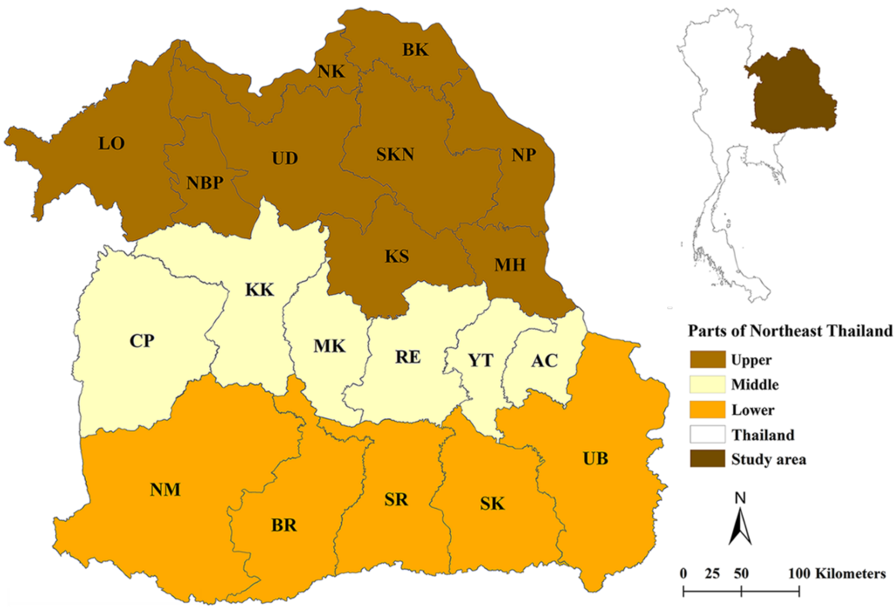

Figure 1. Map of study area. Provinces: AC Amnat Charoen, BK Bueng Kan, BR Buriram, CP Chaiyaphum, KS Kalasin, KK Khon Kaen, LO Loei, MH Mukdahan, MK Maha Sarakham, NP Nakhon Phanom, NR Nakhon Ratchasima, NBP Nong Bua Lamphu, NK Nong Khai, RE Roi Et, SKN Sakon Nakhon, SK Sisaket, SR Surin, UB Ubon Ratchathani, UD Udon Thani, YT Yasothon

Participants

This study encompasses all participants who actively participated in CASCAP across 20 provinces in the northeastern region of Thailand. The inclusion criteria comprised individuals aged 40 years and above, residing in the northeastern region, and duly enrolled in the CASCAP database during the period from 2013 to 2022. For our analysis, we considered those participants who provided information regarding their history of PZQ treatments, FLD diagnosis, and DM diagnosis, resulting in a total of 756,431 participants being included in our study. This extensive sample size serves as a reliable representation of the population, ensuring the accuracy of our study results and, in turn, contributing to the soundness of our study's conclusions.

Variables measurements

The primary outcome of this study was the history of DM diagnosis, which was collected by CASCAP's research assistant, a qualified medical staff member at the hospital. The DM data were categorized into two groups: non-DM (individuals without DM) and DM (individuals with DM) groups based on the available medical records.

The primary factors of interest in this study were initially divided into two categories: history of PZQ treatments and FLD diagnosis. Subsequently, these two factors were combined and categorized into four groups for analysis, namely: never used PZQ and FLD negative (PZQ- and FLD-), PZQ used and FLD negative (PZQ+ and FLD-), never used PZQ and FLD positive (PZQ- and FLD+), and PZQ used and FLD positive (PZQ+ and FLD+). Additionally, several covariates were considered in the analysis, including gender, age at enrollment, educational levels, main occupation, and history of alcohol consumption. These covariates were examined at both the individual and province levels to assess their potential influence on the association between the combined factors (PZQ treatments and FLD diagnosis) and the outcome variable (DM diagnosis).

Statistical analyses

Categorical demographic characteristics were summarized using frequencies and percentages, including information on gender, age groups, education levels, occupation, and history of alcohol consumption. On the other hand, continuous data were presented as mean, standard deviation (SD), median, and minimum and maximum values. These summarizations were performed separately based on FLD diagnosis and PZQ treatments to gain insights into potential differences and patterns within each group.

The rate of DM was calculated using a normal approximation to a binomial distribution. To account for the hierarchical structure of the participants, where individuals (level 1) were nested within provinces (level 2), a multilevel mixed-effects logistic regression model was applied. This model considered the association between the combination of PZQ and FLD, while adjusting for all other co-variates such as gender, age at enrollment, educational levels, occupation, and alcohol consumption (fixed effects) in relation to DM. The results were presented as adjusted odds ratios (AOR) with their 95% confidence intervals (CI). The model was further adjusted to account for the variation in individual-level and province-level effects (random effects). The model's fitness was assessed by estimating the highest value of maximum likelihood. All test statistics were two-tailed, and a p-value of less than 0.05 was considered statistically significant. For data analysis, we utilized the STATA version 18 statistical package (Stata, College Station, Texas, USA).

This analysis explores the spatial distribution of DM, FLD, and PZQ prevalence. The sub-districts' administrative borders were established using polygon shapefiles obtained from the DIVA-GIS website (www.diva-gis.org). Utilizing ArcGIS 10.5.1 (ESRI Inc. Redlands, CA, USA), the spatial datasets, which incorporated the overall prevalence rates of DM, PZQ, and FLD, were imported and subjected to analysis.

Ethics approval

The research protocol was approved by Khon Kaen University Ethics Committee for Human Research, with reference number HE661301. The data were provided by the Cholangiocarcinoma Screening and Care Program (CASCAP). The CASCAP data collection was conducted according to the principles of Good Clinical Practice, the Declaration of Helsinki, and national laws and regulations about clinical studies. It was approved by the Khon Kaen University Ethics Committee for Human Research under the reference number HE551404. All patients gave written informed consent for the study.

RESULTS

Baseline characteristics of the participants

Our study included 756,431 participants with a history of PZQ treatment, FLD, and DM diagnosis. Among them, around two-thirds (63.13%) were female, with a mean age of 55.4 ± 9.05 years. The majority had completed secondary and primary education (92.71%), worked in the agricultural sector (77.87%), and were aged 50 years and above (70.74%). Among the study participants, the overall prevalence of PZQ use was found to be 23.40% (177,010/756,431) and the overall prevalence of FLD was 21.90% (165,674/756,431). The prevalence of different combinations of PZQ treatments and FLD diagnosis was as follows: approximately two-thirds (60.17%) had PZQ- and FLD-, followed by 17.93% had PZQ+ and FLD-, 16.43% who had PZQ- and FLD+, and 5.47% who had both PZQ+ and FLD+ (Table 1).

Figure 2 illustrates the prevalence of PZQ treatment and FLD diagnosis. The prevalence of PZQ use was widely distributed across the upper and middle parts of the region, with a noticeable clustering in the lower part near the border with Cambodia. On the other hand, the prevalence of FLD exhibited a more evenly distributed pattern across both the upper and lower parts of the region.

Table 1. Baseline demographic characteristics of participants according to combinations of history of praziquantel treatment and fatty liver diagnosis

Characteristics	**Total (n = 756,431)**	**Fatty liver negative**		**Fatty liver positive**	
		Never use PZQ (n = 455,149)	**Used PZQ (n = 135,608)**	**Never use PZQ (n = 124,272)**	**Used PZQ (n = 41,402)**
	Number (%)	**Number (%)**	**Number (%)**	**Number (%)**	**Number (%)**
Gender					
Male	278,666 (36.85)	164,593 (36.17)	60,424 (44.57)	38,177 (30.73)	15,472 (37.38)
Female	477,623 (63.15)	290,493 (63.83)	75,149 (55.43)	86,060 (69.27)	25,921 (62.62)

Characteristics	Total (n = 756,431)	Fatty liver negative		Fatty liver positive	
		Never use PZQ (n = 455,149)	Used PZQ (n = 135,608)	Never use PZQ (n = 124,272)	Used PZQ (n = 41,402)
	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)
Age groups (years)					
< 50	213,952 (29.26)	134,365 (30.74)	33,972 (25.62)	35,724 (29.59)	9,891 (24.29)
50 – 60	310,378 (42.45)	178,544 (40.84)	56,992 (42.99)	55,039 (45.59)	19,803 (48.63)
> 60	206,848 (28.29)	124,230 (28.42)	41,615 (31.39)	29,973 (24.83)	11,030 (27.08)
Mean ± SD	55.4 ± 9.05	55.32 ± 9.31	56.21 (9.02)	54.76 ± 8.37	55.61 (8.06)
Median (Min : Max)	55 (40 : 110)	54 (40 : 110)	56 (40 : 100)	54 (40 : 110)	55 (40 : 110)
Educational levels					
Certificate and higher	39,619 (5.24)	24,711 (5.43)	5,107 (3.77)	7,905 (6.36)	1,896 (4.58)
Secondary and primary	701,288 (92.71)	419,254 (92.11)	128,703 (94.91)	114,237 (91.92)	39,094 (94.43)
Lower than primary	15,524 (2.05)	11,184 (2.46)	1,798 (1.33)	2,130 (1.71)	412 (1)
Occupation					
Agricultural	589,017 (77.87)	349,538 (76.8)	114,072 (84.12)	91,513 (73.64)	33,894 (81.87)
Others	167,414 (22.13)	105,611 (23.2)	21,536 (15.88)	32,759 (26.36)	7,508 (18.13)
Alcohol consumption					
No	431,871 (57.09)	269,204 (59.15)	66,985 (49.4)	74,264 (59.76)	21,418 (51.73)
Yes	324,560 (42.91)	185,945 (40.85)	68,623 (50.6)	50,008 (40.24)	19,984 (48.27)

Max=Maximum; Min=Minimum; PZQ=Praziquantel; SD=Standard deviation;

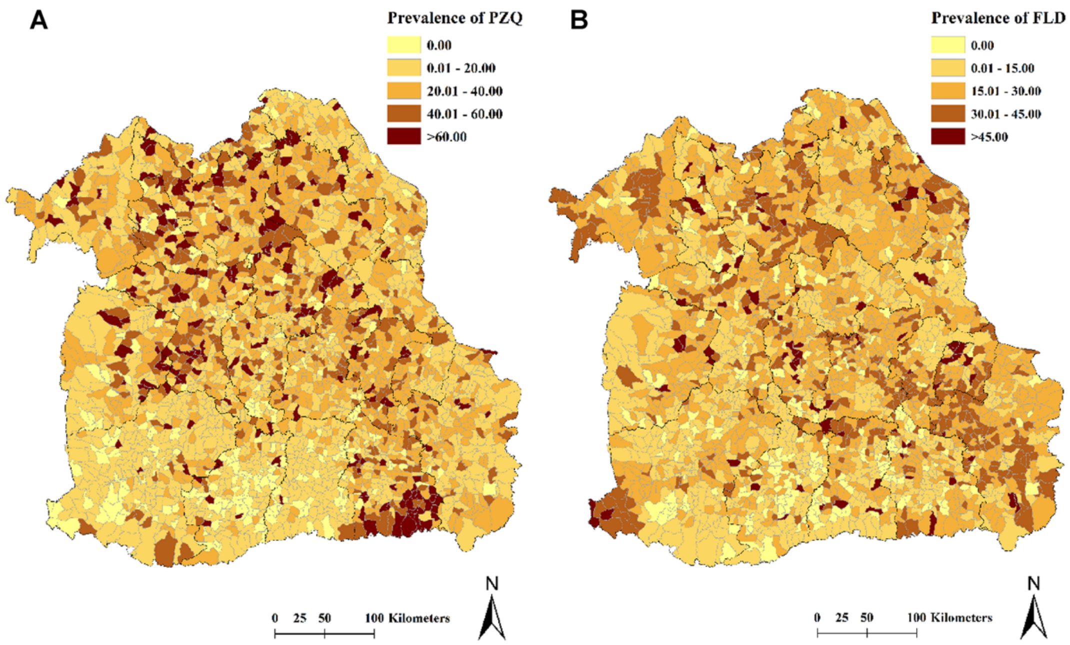

Figure 2. The prevalence of praziquantel used (A) and diagnosed with fatty liver (B)

Prevalence of diabetes mellitus and factors associated with diabetes mellitus

Out of the 756,431 participants, the overall prevalence of DM was found to be 7%. Specifically, the prevalence of DM was 8.23% among those who had used PZQ and 10.45% in the FLD positive group. The prevalence of DM was higher in females (7.77%) than in males (5.68%), and the highest prevalence was observed in participants aged over 60 years (10.78%) (Table 2). Figure 3 presents the prevalence of DM, PZQ with DM, FLD with DM, and PZQ with FLD and DM. The prevalence of DM alone was observed across all parts of the region (upper, middle, and lower). PZQ with DM prevalence was distributed in the upper and middle parts, while FLD with DM was distributed in all parts of the region. Moreover, the prevalence of PZQ with FLD and DM was predominantly common in the upper part of the region.

The results from both simple and multivariable analyses indicated that all factors examined were significantly associated with DM. These factors included PZQ use, FLD positivity, being female, age 50 years and above, completion of secondary education or higher, working in the agricultural sector, and history of alcohol consumption. In the multivariable model, after controlling for the effects of all these factors, our findings revealed that participants who used PZQ were more likely to have DM (AOR 1.25; 95% CI: 1.23-1.28) compared to those who never used PZQ. Similarly, participants with FLD positive status were also more likely to have DM (AOR 1.78; 95% CI: 1.75-1.82) compared to those with FLD negative state.

Table 2. Association of praziquantel use, fatty liver diagnosis, and other factors with diabetes mellitus using multilevel mixed-effects logistic regression

Factors	**Number of participants**	**Diabetes**	**Crude analysis**			**Adjusted analysis**		
		Cases (%)	**COR**	**95% CI**	**p-value**	**AOR**	**95% CI**	**p-value**
Overall	756,431	52,927 (7)	NA	NA	NA	NA	NA	NA
Praziquantel treatment					< 0.001			< 0.001
No	579,421	38,356 (6.62)	1			1		
Yes	177,010	14,571 (8.23)	1.28	1.25-1.30		1.25	1.23-1.28	
Fatty liver disease					< 0.001			< 0.001
No	590,757	35,615 (6.03)	1			1		
Yes	165,674	17,312 (10.45)	1.83	1.79-1.86		1.78	1.75-1.82	
Gender					< 0.001			< 0.001
Male	278,666	15,818 (5.68)	1			1		
Female	477,623	37,101 (7.77)	1.40	1.37-1.42		1.46	1.43-1.50	
Age groups (years)					< 0.001			< 0.001
< 50	213,952	7,341 (3.43)	1			1		
50 – 60	310,378	22,635 (7.29)	2.21	2.15-2.27		2.21	2.15-2.27	
> 60	206,848	22,289 (10.78)	3.39	3.30-3.49		3.53	3.43-3.63	
Educational levels					< 0.001			< 0.001
Certificate and higher	39,619	2,300 (5.81)	1			1		
Secondary and primary	701,288	49,379 (7.04)	1.25	1.19-1.30		1.14	1.09-1.20	
Lower than primary	15,524	1,248 (8.04)	1.51	1.40-1.62		1.09	1.01-1.18	

Factors	Number of participants	Diabetes	Crude analysis			Adjusted analysis		
		Cases (%)	COR	95% CI	p-value	AOR	95% CI	p-value
Occupation					< 0.001			< 0.001
Agricultural	589,017	38,894 (6.6)	1			1		
Others	167,414	14,033 (8.38)	1.27	1.25-1.30		1.33	1.30-1.36	
Alcohol consumption					< 0.001			< 0.001
No	431,871	33,173 (7.68)	1			1		
Yes	324,560	19,754 (6.09)	0.77	0.76-0.79		0.95	0.93-0.97	

AOR=Adjusted odds ratios; CI=Confidence interval; COR=Crude odds ratios; NA=Not available

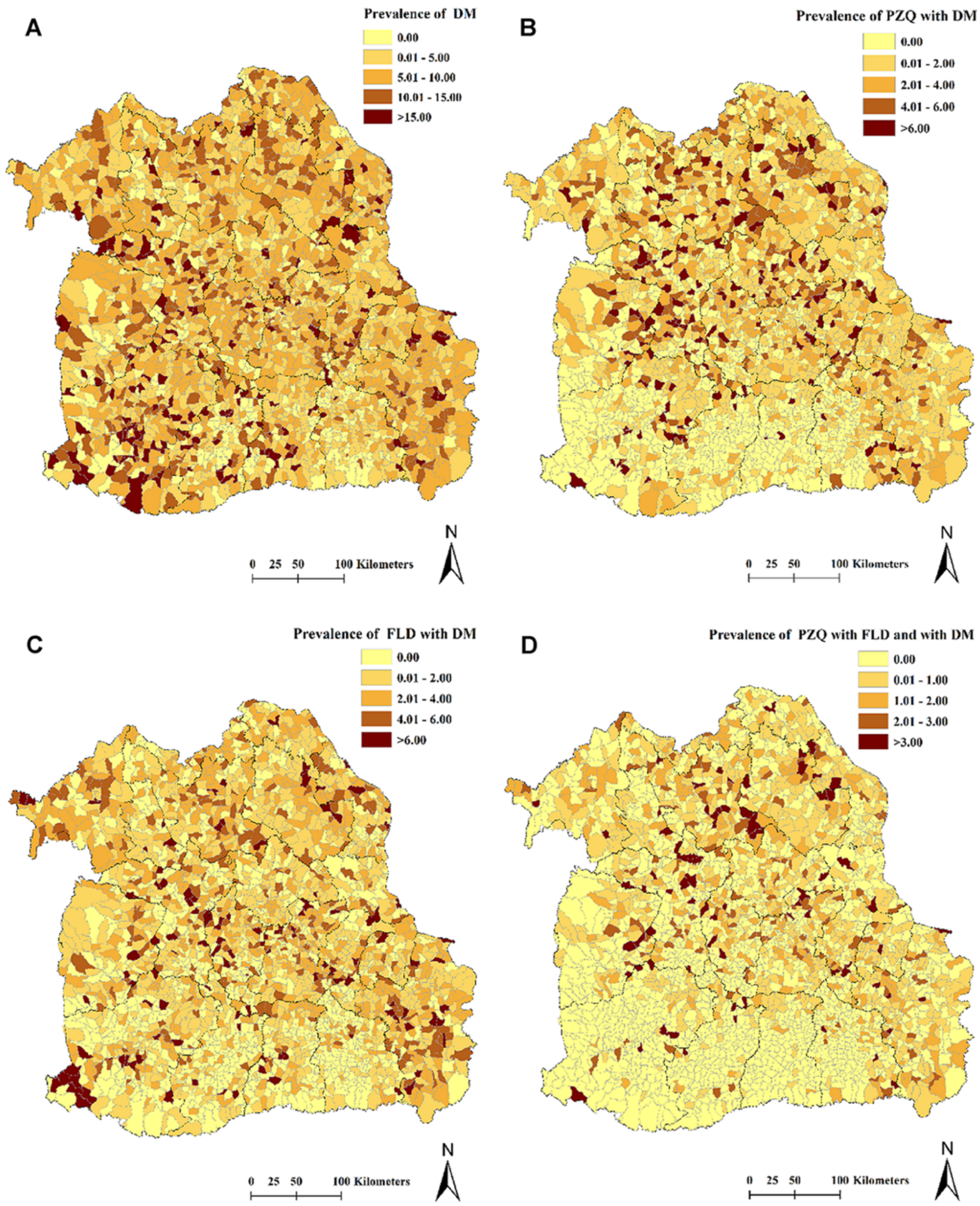

Figure 3. The prevalence of DM alone (A), PZQ with DM (B), FLD with DM (C), and PZQ with FLD and DM (D)

Association between the combination of praziquantel use and fatty liver, and diabetes mellitus

The combination of PZQ treatments and FLD diagnosis showed that the highest prevalence of DM was found in participants who had used PZQ and had a positive FLD diagnosis (11.81%), followed by those who had never used PZQ but had a positive FLD diagnosis (10%).

The association of combinations of PZQ and FLD with DM was assessed using a multilevel mixed-effects logistic-regression model, comparing each group to the reference group (PZQ- and FLD-). In the crude analysis, participants with PZQ+ and FLD-, PZQ- and FLD+, and PZQ+ and FLD+ showed a statistically significant association with DM. Similarly, in the multivariable analysis, after controlling for co-variates such as gender, age at enrollment, educational levels, occupation, and alcohol consumption (fixed effects), as well as the variation at the province level (random effects), participants with PZQ+ and FLD-, PZQ- and FLD+, and PZQ+ and FLD+ were more likely to have DM positive status (AOR 1.27; 95% CI: 1.24-1.30, AOR: 1.8; 95% CI: 1.76-1.85, and AOR 2.19; 95% CI: 2.12-2.27, respectively) with a p-value < 0.001 (Table 3). Figure 4 displays a comparison between the COR and the AOR for the association of the combination of PZQ and FLD with DM. The results demonstrate a consistent and significant association in both the crude and adjusted analyses, indicating a strong relationship between PZQ use, FLD diagnosis, and the likelihood of developing DM in the study population.

Table 3. Association of combinations of praziquantel use and fatty liver with diabetes mellitus using multilevel mixed-effects logistic regression

Factors	Number of participants	Diabetes	Crude analysis			Adjusted analysis		
		Cases (%)	COR	95% CI	p-value	AOR	95% CI	p-value
Combination of praziquantel and fatty liver								
PZQ- and FLD-	455,149	25,935 (5.7)	1		< 0.001	1		< 0.001
PZQ+ and FLD-	135,608	9,680 (7.14)	1.28	1.25-1.32		1.27	1.24-1.30	
PZQ- and FLD+	124,272	12,421 (10)	1.85	1.80-1.89		1.80	1.76-1.85	
PZQ+ and FLD+	41,402	4,891 (11.81)	2.27	2.20-2.35		2.19	2.12-2.27	

AOR=Adjusted odds ratios; CI=Confidence interval; COR=Crude odds ratios; FLD=Fatty liver disease; PZQ=Praziquantel; PZQ- and FLD-=Never used PZQ and FLD negative; PZQ+ and FLD-=PZQ used and FLD negative; PZQ- and FLD+= Never used PZQ and FLD positive; PZQ+ and FLD+=PZQ used and FLD positive

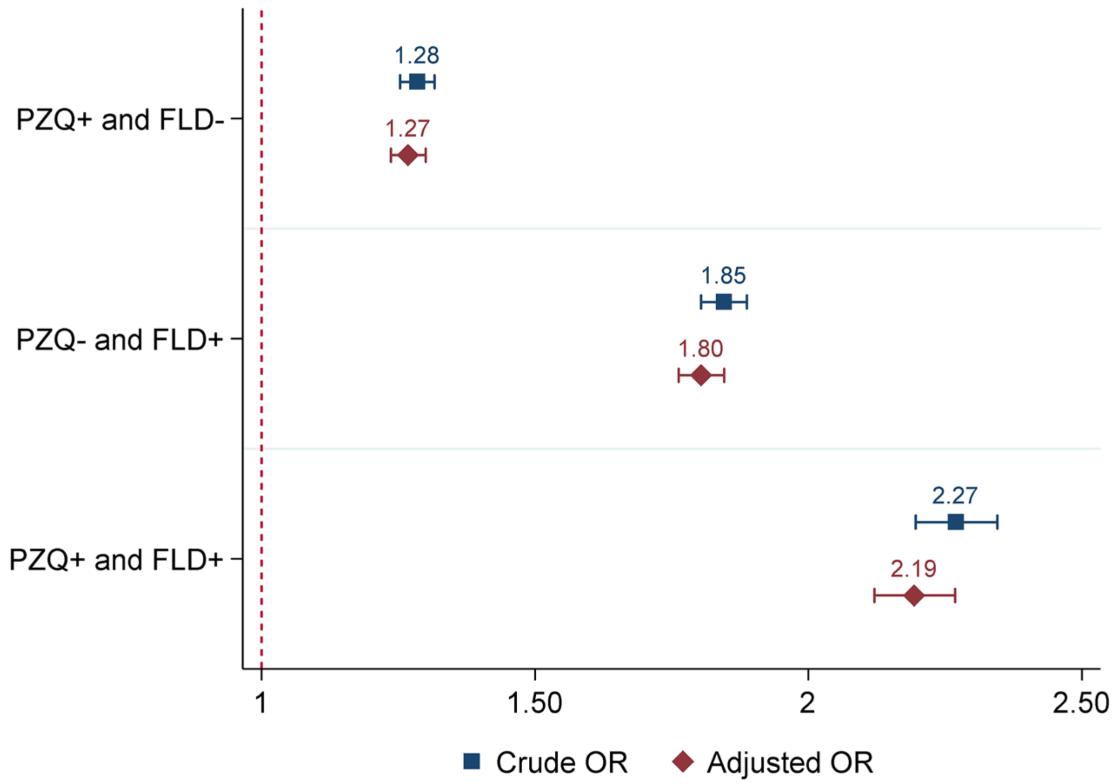

Figure 4. Crude and adjusted odds ratios of the association between the combination of PZQ and FLD, and DM

DISCUSSION

In our investigation, we explored the association between combinations of PZQ treatments and FLD diagnosis and DM in Northeast Thailand. The results indicated that the combination of PZQ and FLD showed a stronger association with DM compared to either PZQ alone or FLD alone. This finding is of significant epidemiological importance, as it was observed in a large population in northeastern Thailand, and addressed one of the most significant chronic disease burdens in the community. The insights gained from this research contribute to our understanding of the interplay between these factors and their potential implications for public health in the region.

Our study revealed a prevalence of PZQ use and FLD in approximately one-quarter of the participants (23.4% and 21.9%, respectively). The prevalence of PZQ use in our study was lower than that reported in previous studies from Thailand in 2019 (42.2%)[26], but was similar to findings from a study conducted in 2022 (27%)[30]. The high prevalence of PZQ use observed in our study can be attributed to the Northeast region, which is known to have the highest prevalence of liver fluke infection[31, 32]. Comparing the prevalence of FLD in our study, previous studies from Spain in 2023 reported a prevalence of nonalcoholic FLD (NAFLD) of 22.3%[33], which aligns with our findings from northeastern Thailand in 2017 (21.9%)[20]. However, a study conducted in Thailand in 2021 reported a higher prevalence of NAFLD (32.17%) in the Thai population[34], which may be attributed to differences in the study populations and regions examined. It is important to note that our study focused specifically on the northeastern region of Thailand, which could account for variations in the prevalence rates compared to nationwide studies. Overall, our findings contribute valuable insights into the prevalence of PZQ use and FLD in the northeastern region of Thailand, where liver fluke infection is more prevalent.

The overall prevalence of DM in our study was 7%, which is consistent with global DM prevalence reported in 2019 (9.3%)[5] and the prevalence found in the Thai population in 2018 (9.9%)[6]. Our study was specifically conducted in the northeastern region of Thailand, and the DM prevalence of 7% aligns with a previous study conducted in the same region in 2021, which reported a DM prevalence of 8.22%[27]. Among our study participants, those who had used PZQ for treating liver fluke infection and those diagnosed with FLD had DM prevalence of 8.23% and 10.45%, respectively. These results are in line with previous studies from Thailand, which reported a significant increase in blood glucose levels in individuals who received PZQ treatment after *O. viverrini* infection[24]. Additionally, previous studies from Thailand have shown that areas with a high prevalence of *O. viverrini* infection also have a higher prevalence of DM[27], likely due to the corresponding prevalence of PZQ use to eliminate *O. viverrini*[21, 22]. Regarding the prevalence of DM in FLD patients, our study findings are consistent with previous studies from Thailand in 2015, which found a common occurrence of FLD in people with DM[21], and a study from 2017, which reported a high FLD prevalence in DM patients[20]. When considering the combination of PZQ use and FLD diagnosis, our study found the highest DM prevalence in participants who had used PZQ and were diagnosed with FLD (11.81%). These findings are in line with evidence from previous studies, indicating a higher prevalence of DM among individuals with liver fluke infection who received PZQ treatment and those with concomitant FLD. This suggests a potential link between PZQ, FLD, and the prevalence of DM in this population.

Our findings regarding connections between FLD, PZQ and DM are consistent with a longitudinal study conducted in Thailand in 2023, which revealed that FLD was associated with the development of DM[35]. Additionally, a study from Korea in 2023 reported a higher risk of type 2 DM in women with NAFLD than in men[36]. Moreover, a study in 2020, which is similar to our findings, reported that people infected with *O. viverrini* had lower HbA1c levels than those not infected, but their HbA1c levels increased after being treated with PZQ[24]. Although there have been no specific studies examining the combination of PZQ and FLD and their association with DM, previous research has shown associations between PZQ treatments, FLD, and DM, which supports our findings regarding the increased risk of DM with the combination of PZQ treatments and FLD diagnosis.

Our study has several limitations that should be considered when interpreting the results. Firstly, the data on history of PZQ treatments were obtained from self-reports provided by the participants, which may introduce information bias. Although efforts were made to ensure accurate reporting, there is a possibility of misclassification or underreporting. Secondly, our study reports cross-sectional findings only. We cannot provide evidence of causation between our variables. Finally, our study was conducted only in the Northeast region of Thailand, which may not fully represent the entire country's population. Given the potential regional variations in the prevalence of FLD throughout Thailand, extrapolating our findings to the entire country should be done with caution. However, it is important to note that our study had a very large sample size, including participants from 20 provinces in the Northeast region. Despite the regional focus, the extensive representation of participants allows for more robust statistical analyses and increases the generalizability of our findings within the studied region. Additionally, the use of multilevel mixed-effects logistic regression helped to account for the hierarchical structure of the data, which considered individual-level and province-level effects, improving the accuracy of our estimates.

CONCLUSION

In conclusion, our study highlights the significant association between the combination of PZQ and FLD with DM. Particularly, individuals who have undergone PZQ treatments and have been diagnosed with FLD were found to be more likely to have DM. These findings emphasize the importance of focusing DM screening efforts on individuals with this combination of risk factors. To address the increased risk observed in individuals with PZQ and FLD, targeted educational initiatives should be implemented. Increasing awareness regarding the potential development of DM in areas with high prevalence of PZQ use, FLD, and DM is crucial. These educational efforts could play a vital role in preventing and managing DM, thereby improving public health outcomes in these regions. Furthermore, ongoing monitoring and evaluation of DM prevalence in areas with a high burden of PZQ use and FLD is essential to ensure early detection and timely interventions. Collaborative efforts between healthcare providers, public health officials, and community leaders can strengthen preventive measures and support effective healthcare planning. Overall, our study contributes valuable insights into the relationship between PZQ, FLD, and DM. The identification of individuals at higher risk for DM allows for targeted interventions and resource allocation, ultimately leading to improved health outcomes in the Northeast of Thailand.

RECOMMENDATION

We strongly recommend conducting a prospective follow-up study on liver fluke-infected individuals who have been treated with PZQ and diagnosed with FLD. This type of study would allow a better understanding of the long-term outcomes and potential complications associated with this combination of risk factors, particularly in relation to the development and progression of DM. To ensure the robustness and generalizability of the findings, it is essential to include a sample group that is representative of all areas of the country. Consequently, individuals with a history of PZQ treatments and FLD were utilized as criteria for evaluating the factors associated with DM, in conjunction with residents inhabiting regions susceptible to *O. viverrini* infection and CCA issues. This specific target demographic should be the focus of interventions aimed at instigating health behavior modifications and enhancing knowledge to mitigate the risk of DM.

ACKNOWLEDGEMENTS

The authors are truly thankful for all members of CASCAP, particularly the cohort members and staff from all participating institutions including the Ministry of Public Health, Ministry of Interior, and Ministry of Education of Thailand. This research was supported by NSRF under the Basic Research Fund of Khon Kaen University through Cholangiocarcinoma Research Institute.

REFERENCES

1. Al-Lawati JA. Diabetes Mellitus: A Local and Global Public Health Emergency! Oman Med J. 2017;32(3):177-9. doi: 10.5001/omj. 2017.34.
2. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of Type 2 Diabetes Mellitus. Int J Mol Sci. 2020;21(17). doi: 10.3390/ijms 21176275.
3. Papier K, Jordan S, D'Este C, Bain C, Peungson J, Banwell C, et al. Incidence and risk factors for type 2 diabetes mellitus in transitional Thailand: results from the Thai cohort study. BMJ Open. 2016;6(12):e014102. doi: 10.1136/ bmjopen-2016-014102.
4. Li J, Ye Q, Jiao H, Wang W, Zhang K, Chen C, et al. An early prediction model for type 2 diabetes mellitus based on genetic variants and nongenetic risk factors in a Han Chinese cohort. Front Endocrinol (Lausanne). 2023;14: 1279450. doi: 10.3389/fendo.2023. 1279450.
5. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9(th) edition. Diabetes Res Clin Pract. 2019;157:107843. doi: 10.1016/ j.diabres.2019.107843.
6. Aekplakorn W, Chariyalertsak S, Kessomboon P, Assanangkornchai S, Taneepanichskul S, Putwatana P. Prevalence of Diabetes and Relationship with Socioeconomic Status in the Thai Population: National Health Examination Survey, 2004-2014. J Diabetes Res. 2018;2018: 1654530. doi: 10.1155/2018/1654530.
7. Reutrakul S, Deerochanawong C. Diabetes in Thailand: Status and Policy. Curr Diab Rep. 2016;16(3):28. doi: 10.1007/s11892-016-0725-7.
8. Hanprathet N, Lertmaharit S, Lohsoonthorn V, Rattananupong T, Ammaranond P, Jiamjarasrangsi W. Increased Risk Of Type 2 Diabetes And Abnormal FPG Due To Shift Work Differs According To Gender: A Retrospective Cohort Study Among Thai Workers In Bangkok, Thailand. Diabetes Metab Syndr Obes. 2019; 12:2341-54. doi: 10.2147/DMSO. S219524.
9. Sae-Wong J, Chaopathomkul B, Phewplung T, Chaijitraruch N, Sahakitrungruang T. The Prevalence of Nonalcoholic Fatty Liver Disease and Its Risk Factors in Children and Young Adults with Type 1 Diabetes Mellitus. J Pediatr. 2021;230:32-7.e1. doi: 10.1016/j.jpeds.2020.10.043.
10. Dejkhamron P, Santiprabhob J, Likitmaskul S, Deerochanawong C, Rawdaree P, Tharavanij T, et al. Young-onset diabetes patients in Thailand: Data from Thai Type 1 Diabetes and Diabetes diagnosed Age before 30 years Registry, Care and Network (T1DDAR CN). J Diabetes Investig. 2022;13(5):796-809. doi: 10.1111/jdi.13732.
11. Nooseisai M, Viwattanakulvanid P, Kumar R, Viriyautsahakul N, Muhammad Baloch G, Somrongthong R. Effects of diabetes self-management education program on lowering blood glucose level, stress, and quality of life among females with type 2 diabetes mellitus in Thailand. Prim Health Care Res Dev. 2021;22:e46. doi: 10.1017/ S1463423621000505.
12. Golabi P, Paik JM, Kumar A, Al Shabeeb R, Eberly KE, Cusi K, et al. Nonalcoholic fatty liver disease (NAFLD) and associated mortality in individuals with type 2 diabetes, pre-diabetes, metabolically unhealthy, and

metabolically healthy individuals in the United States. Metabolism. 2023; 146:155642. doi: 10.1016/j.metabol.2023.155642.

13. Chen YY, Yeh MM. Non-alcoholic fatty liver disease: A review with clinical and pathological correlation. J Formos Med Assoc. 2021;120(1 Pt 1):68-77. doi: 10.1016/j.jfma.2020.07.006.
14. Miller MJ, Harding-Theobald E, DiBattista JV, Zhao Z, Wijarnpreecha K, Lok AS, et al. Progression to cirrhosis is similar among all ages in nonalcoholic fatty liver disease, but liver-related events increase with age. Hepatol Commun. 2023;7(6). doi: 10.1097/HC9.0000000000000148.
15. Wu PH, Chung CH, Wang YH, Hu JM, Chien WC, Cheng YC. Association between nonalcoholic fatty liver disease and colorectal cancer: A population-based study. Medicine (Baltimore). 2023;102(21):e33867. doi: 10.1097/MD.0000000000033867.
16. Younossi ZM, Otgonsuren M, Henry L, Venkatesan C, Mishra A, Erario M, et al. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. Hepatology. 2015;62(6):1723-30. doi: 10.1002/hep.28123.
17. Wongjarupong N, Assavapongpaiboon B, Susantitaphong P, Cheungpasitporn W, Treeprasertsuk S, Rerknimitr R, et al. Non-alcoholic fatty liver disease as a risk factor for cholangiocarcinoma: a systematic review and meta-analysis. BMC Gastroenterol. 2017;17(1):149. doi: 10.1186/s12876-017-0696-4.
18. Toshikuni N, Tsutsumi M, Arisawa T. Clinical differences between alcoholic liver disease and nonalcoholic fatty liver disease. World J Gastroenterol. 2014;20(26):8393-406. doi: 10.3748/wjg.v20.i26.8393.
19. Haonon O, Liu Z, Dangtakot R, Pinlaor P, Puapairoj A, Cha'on U, et al. *Opisthorchis viverrini* infection induces metabolic disturbances in hamsters fed with high fat/high fructose diets: Implications for liver and kidney pathologies. J Nutr Biochem. 2022;107: 109053. doi: 10.1016/j.jnutbio.2022.109053.
20. Summart U, Thinkhamrop B, Chamadol N, Khuntikeo N, Songthamwat M, Kim CS. Gender differences in the prevalence of nonalcoholic fatty liver disease in the Northeast of Thailand: A population-based cross-sectional study. F1000Res. 2017;6:1630. doi: 10.12688/f1000research.12417.2.
21. Thinkhamrop K, Khuntikeo N, Phonjitt P, Chamadol N, Thinkhamrop B, Moore MA, et al. Association between Diabetes Mellitus and Fatty Liver Based on Ultrasonography Screening in the World's Highest Cholangiocarcinoma Incidence Region, Northeast Thailand. Asian Pac J Cancer Prev. 2015;16(9):3931-6. doi: 10.7314/apjcp.2015.16.9.3931.
22. Tanase DM, Gosav EM, Costea CF, Ciocoiu M, Lacatusu CM, Maranduca MA, et al. The Intricate Relationship between Type 2 Diabetes Mellitus (T2DM), Insulin Resistance (IR), and Nonalcoholic Fatty Liver Disease (NAFLD). J Diabetes Res. 2020;2020: 3920196. doi: 10.1155/2020/3920196.
23. El-Kashef HA, Salem HA, Said SA, Elmazar MM. Effect of praziquantel on serum glucose and insulin levels in normal and hyperglycemic rats. Arzneimittelforschung. 1996;46(4): 433-5.
24. Muthukumar R, Suttiprapa S, Mairiang E, Kessomboon P, Laha T, Smith JF, et al. Effects of *Opisthorchis viverrini* infection on glucose and lipid profiles in human hosts: A cross-sectional and

prospective follow-up study from Thailand. Parasitol Int. 2020;75: 102000. doi: 10.1016/j.parint.2019. 102000.
25. Saengsawang P, Promthet S, Bradshaw P. Infection with *Opisthorchis viverrini* and use of praziquantel among a working-age population in northeast Thailand. Asian Pac J Cancer Prev. 2013;14(5):2963-6. doi: 10.7314/apjcp. 2013.14.5.2963.
26. Thinkhamrop K, Khuntikeo N, Sithithaworn P, Thinkhamrop W, Wangdi K, Kelly MJ, et al. Repeated praziquantel treatment and *Opisthorchis viverrini* infection: a population-based cross-sectional study in northeast Thailand. Infect Dis Poverty. 2019;8(1):18. doi: 10.1186/ s40249-019-0529-5.
27. Thinkhamrop K, Khuntikeo N, Laohasiriwong W, Chupanit P, Kelly M, Suwannatrai AT. Association of comorbidity between *Opisthorchis viverrini* infection and diabetes mellitus in the development of cholangiocarcinoma among a high-risk population, northeastern Thailand. PLOS NEGL TROP DIS. 2021;15(9): e0009741. doi: 10.1371/journal.pntd. 0009741.
28. Htun NSN, Odermatt P, Paboriboune P, Sayasone S, Vongsakid M, Phimolsarn-Nusith V, et al. Association between helminth infections and diabetes mellitus in adults from the Lao People's Democratic Republic: a cross-sectional study. Infect Dis Poverty. 2018;7(1): 105. doi: 10.1186/s40249-018-0488-2.
29. Khuntikeo N, Chamadol N, Yongvanit P, Loilome W, Namwat N, Sithithaworn P, et al. Cohort profile: cholangiocarcinoma screening and care program (CASCAP). BMC Cancer. 2015;15:459. doi: 10.1186/s12885-015-1475-7.
30. Prathumkam P, Thinkhamrop K, Khuntikeo N, Chamadol N, Thuanman J, Kelly M, et al. Association between the Number of Repeated Praziquantel Treatments and Kidney Parenchymal Change in Northeast Thailand. Asian Pac J Cancer Prev. 2022;23(7):2397-405. doi: 10.31557/APJCP.2022.23. 7.2397.
31. Sripa B, Suwannatrai AT, Sayasone S, Do DT, Khieu V, Yang Y. Current status of human liver fluke infections in the Greater Mekong Subregion. Acta Trop. 2021;224:106133. doi: 10.1016/ j.actatropica.2021.106133.
32. Martviset P, Phadungsil W, Na-Bangchang K, Sungkhabut W, Panupornpong T, Prathaphan P, et al. Current prevalence and geographic distribution of helminth infections in the parasitic endemic areas of rural Northeastern Thailand. BMC Public Health. 2023;23(1):448. doi: 10.1186/ s12889-023-15378-4.
33. Cusacovich I, Sánchez-Lite I, Toribio B, González JM, Pérez-Rubio A, Andaluz-Ojeda D. Prevalence of nonalcoholic fatty liver disease in a Spanish town: a population-based study. Rev Clin Esp (Barc). 2023; 223(7):396-404. doi: 10.1016/j.rceng. 2023.04.012.
34. Phisalprapa P, Prasitwarachot R, Kositamongkol C, Hengswat P, Srivanichakorn W, Washirasaksiri C, et al. Economic burden of non-alcoholic steatohepatitis with significant fibrosis in Thailand. BMC Gastroenterol. 2021; 21(1):135. doi: 10.1186/s12876-021-01720-w.
35. Han WM, Apornpong T, Lwin HMS, Thammapiwan S, Boonrungsirisap J, Gatechompol S, et al. Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis With Liver Fibrosis as Predictors of New-Onset Diabetes Mellitus in People With HIV: A Longitudinal Cohort Study. Clin Infect Dis. 2023;77(12):1687-95. doi: 10.1093/cid/ciad433.

36. Cho Y, Chang Y, Ryu S, Wild SH, Byrne CD. Nonalcoholic fatty liver disease without overlapping metabolic-associated fatty liver disease and the risk of incident type 2 diabetes. Liver Int. 2023;43(11):2445-54. doi:10.1111/liv.15661.