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Review Article

Helicobacter pylori and Biliary Tract Cancers: A Meta-Analysis

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Background. *Helicobacter pylori* is detected in various extragastric diseases, including biliary tract cancers. Besides, gallbladder cancers, extracholangiocarcinomas, and intracholangiocarcinomas are highly lethal cancers with limited survival due to their late diagnosis. Epidemiological data on *Helicobacter pylori* infection and biliary tract cancer have been contradictory. *Aim*. The aim of this study is to explore and evaluate the association between the *Helicobacter pylori* infection and biliary tract cancer. *Materials and Methods*. Systematic literature research was carried out to identify all eligible articles. All relevant publications from 2000 to 2019 were retrieved using comprehensive combinations of keywords. We used a random effects model to calculate pooled prevalence estimates, and 95% confidence intervals (CIs) for odds ratio were also calculated. Quantitative assessment of heterogeneity was explored by the chi-square test and was measured using I^2. *Results*. Thirteen case-control studies published between 2001 and 2018 were included. The overall meta-analysis favoured a significant association between *Helicobacter pylori* infection and biliary tract cancer (OR, 2.57; 95% CI, 1.35–4.91; $I^2 = 58\%$). Geographic distribution-based subgroup analysis showed a higher prevalence of *H. pylori* in Asian and North American countries. Evidence supporting the higher presence of *Helicobacter pylori* in a cancer group was found by PCR. In another subgroup, the ORs were 4.18 (2.03, 8.58) in cholangiocarcinoma, 1.36 (0.34, 5.44) in gallbladder cancer, and 5.93 (1.89, 18.63) in other biliary tract cancers. *Conclusion*. This meta-analysis suggests that infection of the biliary tract with *Helicobacter pylor*i is related to an increased risk of biliary tract cancers.

1. Introduction

The estimated total of infection attributable to cancer in 2002 is more than one million case, and 5.5% are caused by *Helicobacter pylori (H. pylori)* [1]. In fact, this well-known pathogen is recognized as a type I carcinogen responsible for chronic gastritis, gastroduodenal ulcers, and gastric carcinoma. In recent studies, *H. pylori* was detected in extragastric cancers such as biliary tract cancer [2, 3].

Biliary tract carcinoma (BTC) is a group of rare incidence tumors that includes gallbladder cancers (GBCs), extra cholangiocarcinomas (ECCs), and intracholangiocarcinomas (ICCs) [4]. These malignancies are highly lethal, aggressive, and poorly understood of all cancers [5]. The global epidemiology of biliary tract cancer is complex and varies according to the anatomical location and geographic regions that are related to risk factor distribution. The carcinogenesis of biliary tract malignancies is still unclear although several known established risk factors were elucidated such as chronic inflammation, cholestasis, and primary sclerosing cholangitis; however, higher scientific evidence is missing [6].

Since Kawaguchi et al. [7] detected *H. pylori* in the gallbladder's mucosa of a patient with cholecystitis, it has been suggested a potential link between *H. pylori* infection and biliary tract carcinogenesis. In fact, clinical and epidemiological studies have reported an association between GBC, CC, and previous infection with *H. pylori* [8, 9]. Kuroki et al. [10] showed that epithelial proliferation is higher in biliary epithelium infected with *Helicobacter* compared to a control group.

In addition, it has been demonstrated that *H. pylori* might infect the biliary tract and cause hepatobiliary pathologies ranging from chronic cholecystitis and primary sclerosing cholangitis to GBC and CC [11, 12]. To date, several meta-analyses were conducted in order to explore the association between *Helicobacter spp.* infection and biliary tract cancer, but the results are conflicting with prevalences ranging from 0% to 71.2% [13, 14]. However, the relationship between *H. pylori* infection and biliary tract cancer remains controversial. Several studies supported a cause-effect link, whereas others failed to find a statistically significant association. Therefore, an update on the association of *H. pylori* and BTC is required to quantitatively assess a possible association of *H. pylori* with BTC.

2. Materials and Methods

2.1. Literature Research. Literature research was performed by two investigators (S. C. and A. A.) using MeSH search terms "*Helicobacter pylori,*" "*H. pylori,*" or "*Helicobacter*" combined with "biliary tract cancer," "gallbladder cancer," or "cholangiocarcinoma" in PubMed, Embase, and Cochrane databases. The identified studies were examined to determine their relevance and their eligibility in this meta-analysis. This systematic review and meta-analysis met the PRISMA statement guidelines.

2.2. Inclusion Criteria and Data Extraction. The studies scrutinized for the meta-analysis were case-control or cross-sectional studies of *H. pylori* prevalence and biliary tract cancer. Studies were considered only if they reported the detection of *H. pylori* prevalence in biliary tract cancer and included more than five cases of biliary tract cancer. If more than one article showed data for the same studied population, only the most recent publication was included.

Two independent authors (S. C. and A. A.) scrutinized and extracted data from the included studies. The data retrieved were: name of the first author, year of publication, country of the study, year of publication, sample size, the location of the malignancy, *H. pylori* detection method, specimen type, and number of *H. pylori*-positive cases in cancer and control group.

Any disagreements on study inclusion or data extraction were resolved according to a third reviewer's opinion.

2.3. Statistical Analysis. Statistical analyses were performed on RevMan 5.1 software. A meta-analysis was performed using a random-effects model if the heterogeneity was significant ($I^2 \geq 50\%$); otherwise, the fixed-effect model was used. Calculations of the odds ratio (OR) and corresponding 95% confidence interval (CI) were carried out as the summary statistics. Statistical heterogeneity among studies was assessed with the chi-square statistic and measured by I^2 statistic.

Subgroup analysis was carried out to explore the possible influence of the study characteristics on the pooled outcome.

2.4. Assessment of Bias and Sensitivity Analysis. To detect publication bias, a visual inspection of funnel plot, Begg's rank correlation test, and Egger's regression test were generated. A two-sided P value less than 0.05 was deemed statistically significant.

3. Results and Discussion

3.1. Description of the Literature Search Strategy. A total of 423 publications yielded from the systematic search. A diagram schematizing the selection process is displayed in Figure 1. Of these, 120 were duplicated and excluded, and 303 were reviewed for detailed assessment. Two hundred and ninety papers that were not case-control studies, sixty-five publications with an inappropriate number of cases, and 3 articles with duplicated data were excluded. Finally, thirteen articles published from 2001 to 2018 were included in our meta-analysis and involved 473 cancer cases and 596 controls. The main characteristics of the included studies are summarised in Table 1.

3.2. Association between H. pylori Infection and Biliary Tract Carcinoma. The link between *H. pylori* infection and BTC is still under debate. Studies carried out so far yielded inconsistent conclusions [14, 28–31]. Also, there is a lack to determine the pathways of *H. pylori* in the hepatobiliary tract, either arriving from the duodenum or passing via the portal system from the liver [32]. Among diverse *Helicobacter* species, *H. pylori* is the most studied one in the hepatobiliary tract. In our meta-analysis, a slightly higher infection rate was noted in the biliary tract cancer group compared with the control group (60% vs 58%, $P = 0.006$), with a pooled OR of 2.57 (95% CI, 1.35–4.91) (Figure 2). Thus, the overall meta-analysis favored an association between *H. pylori* and biliary tract cancer. Due to the significant heterogeneity among studies ($I^2 = 58\%$; $P < 0.05$), our meta-analysis was done in a random-effect model.

Diverse possible mechanisms might explain this association, such as promoting cell inflammation (IL-8 production) and disturbing cell proliferation and apoptosis [18]. The perigenetic pathway was also proposed, by inducing inflammation and enhanced production of TNF-α and IL-6, which alter cell adhesion and lead to dispersion and migration of mutated epithelial cells [28]. In addition, Cag PAI is one of the most studied *H. pylori* virulence factors is activating the proinflammatory signaling pathways in hepatobiliary cells, and the same effect was already reported in the gastric epithelial cell [33].

3.3. Assessment of Bias. An asymmetrical appearance was observed in the funnel plot (Figure 3), and the P value of 0.05 by Egger's regression test and a P value of 0.3 by Begg and Mazumdar rank correlation suggest that there is evidence of publication bias in our study.

3.4. Subgroup Analysis. A subgroup analysis was performed to examine the influence of potential factors on the overall

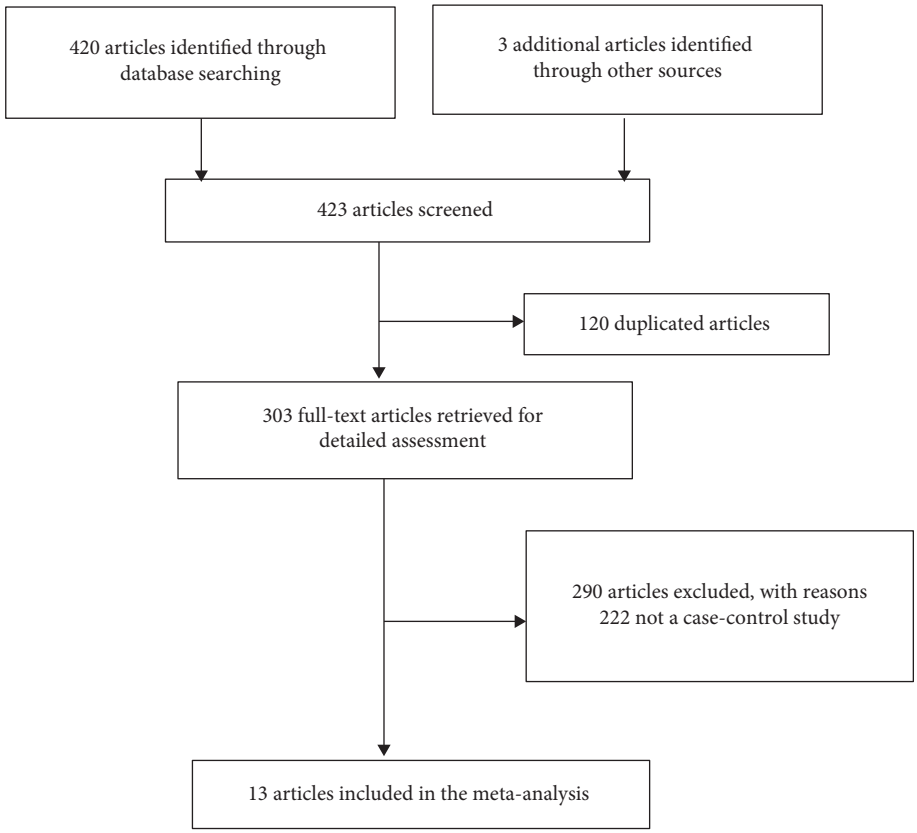

Figure 1: Flow diagram of the literature research.

Table 1: Characteristics of the studies included in the meta-analysis.

Study	Year	Country	Specimen type	Detection method	Type of malignancy	H. pylori (+) in the case group	H. pylori (+) in the control group
Tsuchiya et al. [26].	2018	India	Blood	ELISA	GBC	41/100	42/100
Avilés-Jiménez et al. [19]	2015	Mexico	Frozen tissue	PCR (cagA, vacA)	CC	75/100	52/92
Murphy et al. [23]	2014	USA	Blood	ELISA	BTC	62/64	198/224
W. Boonyanugomol et al. [18]	2011	Thailand	Bile samples	PCR (Urease A)	CC	58/87	4/16
Shimoyama et al. [22]	2010	Japan	Blood	ELISA	CC, GBC	14/18	29/34
Abu Al-Soud et al. [15]	2007	Sweden	FFPE	PCR (26 kDa protein gene)	CC	07/13	03/24
Bohr et al. [24]	2007	Germany	FFPE, frozen tissue	Culture, IHC, PCR (rRNA 16s)	GBC	0/20	0/22
Leelawat et al. [21]	2007	Thailand	FFPE	PCR (VacA)	CC	6/6	5/7
Kobayashi et al. [20]	2005	Japan	Bile	PCR (Urease A, 26K protein)	GBC, CC	2/6	12/21
Chen et al. [27]	2003	China	Fresh tissue	PCR (16S rRNA)	CC	6/11	0/6
Bulajic et al. [25]	2002	Yugoslavia	Bile	PCR (Urease A)	BTC	12/15	3/11
Fukuda et al. [17]	2002	Japan	Bile sample, FFPE	PCR (Urease A), histology, IHC	CC, GBC	1/19	1/19
Nilson et al. [16]	2001	Sweden	FFPE	PCR (16s rRNA)	CC	3/14	0/20
Total						287/473	349/596

FFPE: formalin-fixed paraffin-embedded tissues, IHC: immunohistochemistry, ELISA: enzyme-linked immunosorbent assay, PCR: polymerase chain reaction, H. pylori: Helicobacter pylori, Cag A: cytotoxin-associated gene A, Vac A: Vacuolating cytotoxin A, CC: cholangiocarcinoma, GBC: gall bladder cancer, and BTC: biliary tract cancer.

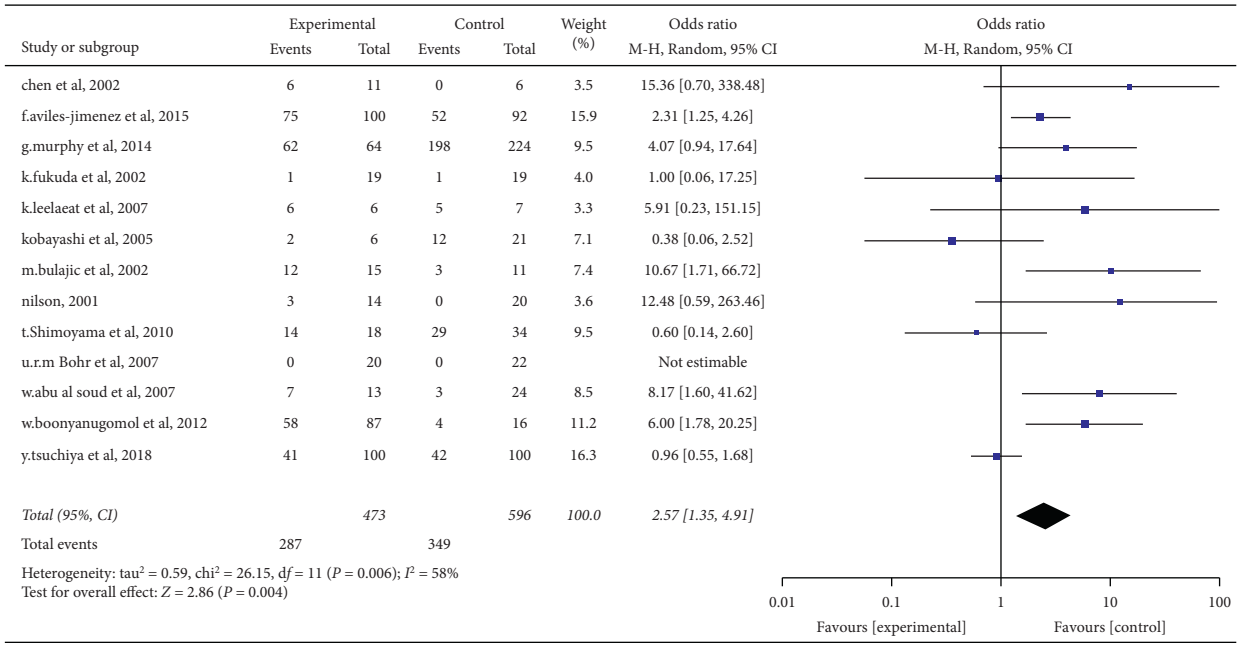

Figure 2: Forest plot for the pooled odd ratio and 95% confidence interval.

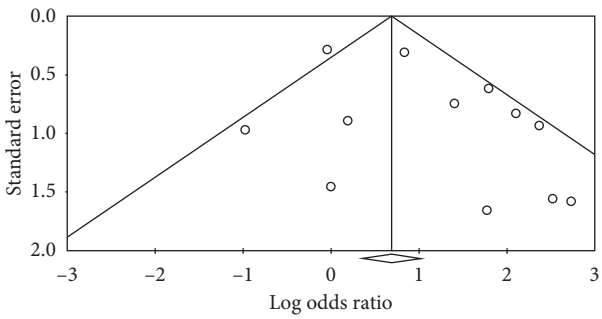

Figure 3: Funnel plot to examine publication bias in this study.

results. The most important concerns are the potential differences in the geographic location, specimen types, detection method, and the type of malignancy.

The infection rate of *H. pylori* varies according to the geographic location. Stratifying by geographic location, the ORs were 1.54 (95% CI, 0.62–3.86) for 7 studies in Asia (Japan [19, 23, 26], Thailand [22, 34], India [15], and China [24]), 9.58 (95% CI, 3.09–29.6) in Europe (Germany [21], Sweden [20, 27], and Yugoslavia [25]), and 2.51 (95% CI, 1.43–4.42) in North America (USA [17] and Mexico [16]); respectively (Table 2). In our study, the prevalence of *H. pylori* was higher in Asian and North American countries. A comparable prevalence was found by Zhou et al. [28] in a biliary lithiasis patients. Normally, the low hygiene and socioeconomic standards in developing countries is linked to a higher infection rate of *Helicobacter spp.* [29, 30].

However, in some developed countries where the environmental hygiene is higher, an important infection rate of *Helicobacter* reaching 60% in biliary lithiasis patients is reported [28]. And this could be explained by the implementation of screening and eradication programs in a few Asian countries, while in Europe, the efforts are limited [31].

Nowadays, PCR, IHC, ELISA, specific stainings, and culture are the most used techniques for the detection of *H. pylori*. The range of detection rates varies from 0 to 64% due to the different methods and specimen types used. PCR has been recognized as a highly sensitive method for the detection of *H. pylori* [30]. A wide range of primers was designed to identify the presence of *H. pylori* such as 16S rRNA, 26 kDa protein gene, Urease A, Cag A, and Vac A. However, a difference in the detection rate was observed in our meta-analysis (Table 2). Out of five primers, three reported a higher positive rate in the malignant group than the control group, of which 16s rRNA and 26 kDa noted a significant difference ($P = 0.02$ and $P = 0.01$, respectively). Most of the studies, utilize 16S rRNA because of its high specificity to identify the whole *Helicobacter* genome in addition to the *H. pylori* specific subunits of urease [32] . Although a false positive result may yield from using urease A gene, that might cross-react with the urease gene of other microorganisms [33]. Moreover, the positive rate of Cag A and Vac A in the malignant group was less than the control group (Table 2). These results highlight the genetic variability of *H. pylori*. We should note that the substantial intraspecies variability in *H. pylori* may act on the detection by a single set of PCR primers [35]. Therefore, the capability of PCR primers to distinguish *Helicobacter* species is questionable, especially in patients from different geographic regions. Also, the sensitivity of PCR is inversely proportional to the length of the amplicon. Especially, in formalin-fixed paraffin-embedded tissues that could risk a DNA damage.

The ELISA based approach to identify the presence of *H. pylori* is still limited when it comes to specificity due to the cross-reactivity between *Helicobacter* and *Campylobacter*. In our study, no significant difference was found ($P = 0.68$). Recently, there has been a decrease in the usage of *H. pylori*

Table 2: Subgroup analysis of *H. pylori* prevalence in the malignant group compared with the control group.

Subgroup	No. of studies	*H. pylori* (+) in malignant group n/N (%)	*H. pylori* (+) in control group n/N (%)	OR (95% CI)	*P* value
Geographic distribution					
Asia	7	128/247	93/203	1.54 (0.62–3.86)	0.36
Europe	4	22/62	6/77	9.58 (3.09–29.6)	$P \leq 0.001$
North America	2	137/164	250/316	2.51 (1.43–4.42)	$P \leq 0.001$
Specimen types					
FFPE	5	17/72	9/92	5.77 (1.75, 19.02)	0.004
Fresh tissue	1	6/11	0/6	15.36 (0.70, 338.48)	0.08
Frozen tissue	2	75/120	52/114	2.31 (1.25, 4.26)	0.007
Bile	4	72/108	19/48	3.08 (0.50, 19.12)	0.23
Serum	3	117/182	296/358	1.22 (0.49, 3.05)	0.68
Detection method					
PCR (16s rRNA)	3	9/51	0/69	13.83 (1.58, 121.27)	0.02
PCR (Urease A)	4	73/127	20/67	2.57 (0.56, 11.86)	0.23
PCR (26 kDa protein gene)	2	7/19	3/45	8.17 (1.60, 41.62)	0.01
PCR (Cag A)	1	46/100	52/92	0.66 (0.37, 1.16)	0.15
PCR (Vac A)	2	56/103	57/99	1.33 (0.27, 4.73)	0.87
ELISA	3	117/182	269/358	1.22 (0.49, 3.05)	0.68
Culture	2	0/26	0/43	NA	NA
IHC	2	0/39	0/41	NA	NA
Histology	1	0/19	0/19	NA	NA
Type of malignancy					
Chanlogiocarcinoma	8	96/166	54/147	4.18 (2.03, 8.58)	$P \leq 0.001$
Gallbladder cancer	7	66/226	89/321	1.36 (0.34, 5.44)	0.67
Other biliary tract cancers	2	74/79	201/235	5.93 (1.89, 18.63)	0.002

FFPE: formalin-fixed paraffin-embedded tissues, IHC: immunohistochemistry, ELISA: enzyme-linked immunosorbent assay, PCR: polymerase chain reaction, *H. pylori*: *Helicobacter pylori*, Cag A: cytotoxin-associated gene A, and Vac A: vacuolating cytotoxin A.

cultivation because of technical difficulties in culture maintenance. It is a time-consuming method, demanding a skilled operator to confirm the diagnosis. In parallel with this tendency, there is an increase in *H. pylori* resistance to antibiotics because of the difficulty to establish its susceptibility profile by cultivation. Also, the diagnosis of *H. pylori* can be performed in hematoxylin and eosin (H&E) staining; however, the specificity can be improved by special stains such as Giemsa, Warthin–Starry silver, and immunohistochemical (IHC) stains. Nevertheless, it has several limitations, including high cost, sampling error, and interobserver variability in the assessment [36]. In our meta-analysis, no study confirmed the presence of *H. pylori* by culture, histology, or IHC.

On stratification by specimen types collected, the ORs were 5.77 (95% CI = 1.75–19.02, $P = 0.004$) in studies using FFPE blocks [20–22, 26, 27], 3.08 (95% CI = 0.50–19.72, $P = 0.23$) in four studies conducted on bile samples [23, 25, 26, 34], and 2.31 (95% CI = 1.25–4.26, $P = 0.007$) in studies where frozen tissues were used [16, 21]. These results indicate higher detection rates in the malignant group. Besides, fresh [24] tissues and serum [15, 17, 19] reached ORs of 15.36 (95% CI = 0.70–338.48, $P = 0.08$) and 1.22 (95% CI = 0.49–3.05, $P = 0.86$).

Finally, as shown in Table 2, the detection of *H. pylori* in cases with cholangiocarcinoma (58% vs 37%, $P < 0.0001$) [16, 19, 20, 22–24, 26, 27, 34] and other biliary tract cancers (93% vs 85%, $P = 0.002$) [17, 25] indicated a high infection rate in the malignant group than the control group. In studies carried out on gallbladder cancer cases [15, 19, 21, 23, 26], the OR was 1.36 (95% CI, 0.34–5.44; $P = 0.67$).

Previously, it was found that *Helicobacter spp*. including *H. pylori* was often detected in benign biliary tract diseases that are known as risk factors for the development of BTC, as well as with BTC and GBC [13, 14, 37]. However, studies have showed a variability in methods and results. Our findings suggested that a significantly higher presence of *H. pylori* was detected in cholangiocarcinoma (58% vs 37%, $P < 0.0001$) [16, 19, 20, 22–24, 26, 27, 34] and other biliary tract cancer (93% vs 85%, $P = 0.002$) [17, 25].

Regarding the limitations of this meta-analysis, there is a lack in the selection of controls and detection, which may introduce heterogeneity. In addition, publication bias might be explained by the fact that published studies in journals are more likely to report statistically significant results than studies that report a nonsignificant outcome. Also, none of the studies included only mentioned whether antibiotics were used which might produce a false negative result.

4. Conclusion

In summary, this meta-analysis showed a higher presence of *H. pylori* in patients with BTCs compared with control

group, and this result was in accordance with the geographical distribution of this pathogen. Further investigation is required with a large-scale study in order to clarify the relationship between *H. pylori* infection and BTC.

Conflicts of Interest

The authors have no conflicts of interest to disclose.

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