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 pylori 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-1 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
3 additional articles identified through other sources

420 articles identified through database searching

423 articles screened

120 duplicated articles

303 full-text articles retrieved for detailed assessment

290 articles excluded, with reasons

222 not a case-control study

13 articles included in the meta-analysis

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 (cagaA, 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, Frozen tissue | Culture, IHC, PCR (rRNA 16s)     | GBC                | 0/20                            | 0/22                              |
| Bohr et al. [24]    | 2007 | Germany   | FFPE, FFPE    | PCR (VacA)                        | CC                 | 0/6                            | 5/7                               |
| Leelawat et al. [21]| 2007 | Thailand  | FFPE          | PCR (Urease A, 26K protein)       | GBC, CC            | 2/6                             | 12/21                             |
| Kobayashi et al. [20]| 2005 | Japan     | Bile          | PCR (Urease A, 26K protein)       | CC                 | 2/6                            | 3/11                              |
| Chen et al. [27]    | 2003 | China     | Fresh tissue  | PCR (16s rRNA)                    | CC                 | 6/11                            | 0/6                               |
| Bulajic et al. [25] | 2002 | Yugoslavia| Bile sample   | 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.
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].
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 tissues and serum reached 15.36 (95% CI = 0.70–338.48, *P* = 0.08) and 3.08 (0.50, 19.12), 0.23 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 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).

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 cases with cholangiocarcinoma (58% vs 37%, *P* = 0.0001) [16, 19, 20, 22–24, 26, 27, 34] and other biliary tract cancers

| 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* ≤ 0.001 |
| North America                 | 2              | 137/164                                  | 250/316                                | 2.51 (1.43–4.42) | *P* ≤ 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 (26kDa 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            |                |                                          |                                        |             |           |
| Cholangiocarcinoma            | 8              | 96/166                                   | 54/147                                 | 4.18 (2.03, 8.58) | *P* ≤ 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.

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