INTRODUCTION

Biliary tract cancer (BTC) is relatively uncommon in most parts of the world [1], but is classified as a major cancer based on its incidence in certain countries such as Korea and India [2,3]. An increasing incidence of BTC has been observed at all 3 biliary tract subsites, specifically gallbladder cancer (GBC), extrahepatic bile duct cancer (EBDC), and ampulla of Vater cancer (AOVC), especially in high-risk areas [4,5]. The prognosis of BTC is generally poor, and the estimated 5-year survival rate is only approximately 5% [6]. Although surgery can be curative, only a small percentage of patients are candidates for surgery because a high proportion of patients are diagnosed at a late stage of the disease [7]. To improve the survival rate, early detection of the disease based on the
identification of risk factors is important.

Gallstones, concretions formed in the biliary tract, have been suggested as an important risk factor for BTC [5]. The carcinogenic mechanisms of BTC are poorly understood, but they may involve inflammatory changes near stones [8]. BTC could arise as a result of chronic inflammation associated with gallstones continuously irritating the gallbladder and bile duct [9]. While gallstones are a common condition [10], BTC rarely occurs, and most people with gallstones never end up developing cancer [11,12]. However, a significant number of BTC patients have gallstones [13], which leaves room for further investigations into the potential association between gallstones and the risk of BTC. One study attempted a systematic review [14], but it examined the literature on the association between benign gallbladder disease (the broader term used to represent gallstones) and the risk of BTC. There is a scarcity of reviews focusing on the relationship between gallstones and the risk of BTC.

We conducted a systematic review and meta-analysis of published cohort and case-control studies on associations between gallstone characteristics and the risk of BTC. This study aimed to update the latest studies through a systematic review and to provide a better description of the association of gallstones with the risk of BTC, encompassing its known subtypes GBC, EBDC, and AOVC [15], while intentionally excluding intrahepatic bile duct cancer (IBDC) and intrahepatic cholangiocarcinoma. The objective of this study is to synthesize data from the vast populations included in various studies (case-control and cohort studies) throughout the world to determine to what extent patients with gallstones are more likely to develop BTC and each of its subtypes than hospital-based or community-based control groups. In this study, gallstones were characterized in terms of their presence, size, number, and duration, and detailed subgroup analyses were also performed stratified by the study design, sex, geographic area, study period, measurement of exposure, study quality score, and adjustment of confounders.

MATERIALS AND METHODS

The study protocol followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines.

Data sources and searches

The first and second reviewers (DH and HJ) searched the PubMed, Embase and Cochrane Library databases for epidemiological studies with the following keywords: (“gallstone” OR “calculi” OR “cholelithiasis” OR “cholecystolithiasis” OR “choledocholithiasis”) AND (“biliary tract cancer” OR “biliary tract neoplasms” OR “gallbladder cancer” OR “gallbladder neoplasms” OR “gallbladder carcinoma” OR “cholangiocarcinoma” OR “extrahepatic bile duct cancer” OR “extrahepatic cholangiocarcinoma” OR “ampulla of vater cancer”). Medical subject headings (MeSH) terms were used for the PubMed search, and Emtree explode terms were used for the Embase search when available. The last search was conducted on Aug 9, 2018. The language was restricted to English in PubMed and Embase, but not in the Cochrane Library database. In terms of publication status, our search was confined only to published human studies. Papers published before April 9, 2018, were reviewed. Duplicates were excluded, and additional papers obtained by manually searching the references of the selected articles were included.

Study selection

The inclusion criteria for eligible studies were as follows: (1) cohort or case-control studies on the association between gallstones and the risk of BTC (GBC, EBDC, or AOVC); (2) gallstones (presence, size, number, or duration) as the exposure of interest; (3) studies in which the primary outcome was the occurrence of BTC (GBC, EBDC, or AOVC); and (4) studies that reported risk estimates (rate ratio [RR], odds ratio [OR], or hazard ratio [HR]) and their 95% confidence intervals (CIs). Studies were excluded if any of the following criteria were met: (1) non-human studies; (2) non-observational studies or observational studies without an analytic epidemiologic approach; (3) irrelevant exposure or outcome variables (hepatolithiasis or intrahepatic cholangiocarcinoma); (4) duplication or unobtainable abstract/full-text; (5) the absence of a risk estimate that was either reported or could be calculated by the given information.

Data extraction

The first and third reviewers (DH and WK) (under the supervision of AS) independently screened the titles and abstracts of included articles. The full texts were reviewed by 2 independent reviewers (DH and HJ) and the supervisor (AS). The 2 independent reviewers (DH and HJ) extracted data using a standardized extraction form. When discrepancies arose, a fourth investigator (NS) made the final decision for study eligibility and data extraction. The relevant data included the last name of the first author, publication year, study country, study design (cohort or case-control study), study period, sex, sample size (number of cohorts and incident cases for cohort studies or number of cases and controls for case-control studies), exposure variables (presence, size, number, and duration of gallstones), measurement of exposure (with or without imaging studies), outcome variables (occurrence of GBC, EBDC, AOVC), duration of follow-up for cohort studies, adjustment variables in the statistical analysis, and risk estimates, such as OR, RR, and HR with corresponding 95% CIs.

Quality assessment

Quality assessment data were extracted using the Newcastle-Ottawa scale (NOS), which contains 9 items, with 8 items receiving 1 point and 1 item accounting for 2 points, leading to a maximum of 10 points [16]. A quality score equal to or greater than the median value was judged as indicating high quality.
Statistical analysis

In this study, the summary risk estimates and their corresponding 95% CIs were calculated using a random-effects model [17]. Selected studies reported different types of risk estimates, such as ORs, RRs, and HRs. RRs and HRs were treated as equivalent to ORs. We compared gallstone characteristics as follows: presence (present vs. absent), size (≥ 1 cm vs. < 1 cm, ≥ 2 cm vs. < 2 cm), number (> 1 vs. 1), and duration. For studies reporting multiple risk estimates according to the subsites of BTC (GBC, EBDC, and/or AOVC), the pooled risk estimates and their corresponding 95% CIs that were adequate for meta-analysis were taken as representative risk estimates.

Statistical heterogeneity across studies was appraised using the I^2 statistic and the chi-square-based Q tests. I^2 values of 25%, 50%, and 75% indicated low, moderate, and high heterogeneity, respectively [18]. For the Q statistic, a p-value < 0.10 was considered to indicate statistically significant heterogeneity. To perform subgroup analyses, we stratified studies by study design, sex, geographic area (Asia and non-Asia), study period (before, around, and after 2000; around 2000 refers to studies where the starting point was before 2000 but the ending point was after 2000), measurement of exposure, study quality according to the NOS, and whether the analysis adjusted for confounders (such as age, sex, comorbidity, lifestyle factors, education, and/or geographic areas). Sensitivity analyses [19] were conducted by sequentially excluding 1 study at a time to evaluate the influence of individual studies on the stability of the pooled results. Forest plots were used to present results graphically. Publication bias was investigated through funnel plots [20] with the Egger test [21], and p-values < 0.01 indicated statistical significance. All statistical analyses were performed using Stata version 15.0 (StataCorp., College Station, TX, USA). A 2-tailed p-value < 0.05 was considered to indicate statistical significance, except as otherwise specified.

Ethics statement

Informed consent was waived due to the study design (systematic review and meta-analysis).

RESULTS

Study selection and characteristics

Figure 1 shows the process of study selection for the meta-analysis. Initially, we retrieved a total of 5,005 articles, including 1,941 from MEDLINE, 3,027 from Embase, and 37 from the Cochrane Library. We excluded 1,082 duplicate studies. Based on reviewing the titles and abstracts, 3,751 other studies were excluded for various reasons. After full-text review, 27 eligible full-text articles were included in the meta-analysis. Based on reviewing the titles and abstracts, 3,751 other studies were excluded for various reasons. The final number of included studies was 30, comprising 23 case-control studies and 7 cohort studies.

Figure 1. Flow chart of study selection for the meta-analysis.
Gallstones and the risk of biliary tract cancer

A total of 26 studies presented associations between the presence of gallstones and the risk of BTC (Figure 2). Among these studies, only 2 studies referred to BTC specifically, and the remaining 24 studies described the risk estimates according to the subsites of BTC (GBC, EBDC, and/or AOVC).

We identified 7 cohort studies and 19 case-control studies that presented associations between the presence of gallstones and the risk of BTC. When we examined the results stratified by the study design, a statistically significant positive association was shown in both case-control studies (OR, 5.04; 95% CI, 3.36 to 7.56; 

= 90.5%; p < 0.001) and cohort studies (OR, 3.17; 95% CI, 2.28 to 4.39; 

= 79.0%; p < 0.001). The pooled risk estimate was also statistically significant (OR, 4.38; 95% CI, 3.23 to 5.93), with high heterogeneity across the studies (I

= 91.2%; p < 0.001).

In subgroup meta-analyses, all results showed statistical significance, regardless of sex, geographic area, study period, measurement of exposure, study quality, and adjustment for confounders, as shown in Table 2. The magnitudes of the associations were larger in females (OR, 4.26; 95% CI, 2.75 to 6.59; 

= 84.5%; p < 0.001) than in males, larger in Asia (OR, 5.25; 95% CI, 3.50 to 7.86; 

= 82.4%; p < 0.001) than outside of Asia, larger in studies conducted before 2000 (OR, 5.39; CI, 2.57 to 11.34; 

= 95.5%; p < 0.001) than in studies conducted around and after 2000, larger in studies with imaging studies (OR, 7.09; 95% CI, 3.87 to 12.98; 

= 64.5%; p = 0.004) than in studies without imaging studies, and larger in low-quality studies (OR, 4.81; 95% CI, 2.87 to 8.05; 

= 94.9%; p < 0.001) than in high-quality studies. The meta-analysis indicated that the

| Study | Sample size | Country | Outcome | Study period | Study design | Gallstone characteristics | Adjustment |
|-------|-------------|---------|---------|--------------|--------------|--------------------------|------------|
| Bansal et al., 1996 [23] | 88,178 (104) | USA | GBC | 1981-1993 | Medical records | Present vs. absent | Age, sex |
| Chow et al., 1999 [24] | 1,775 (12) | USA | EBDC | 1977-1993 | Medical records | Present vs. absent | Age, sex |
| Ishiguro et al., 2008 [26] | 103,682 (142) | Japan | EBDC | 1990-1994 | Medical records | Present vs. absent | Age, sex |
| Lai et al., 2013 [27] | 214,179 (206) | Taiwan | GBC | 2005-2008 | Medical records | Present vs. absent | Age, sex |
| Maringhini et al., 1987 [22] | 2,583 (5) | Italy | EBDC | 1980-1970 | Medical records | Present vs. absent | Age, sex |
| Nordenstedt et al., 2012 [12] | 206,860 (147) | Sweden | GBC | 1967-2008 | Medical records | Present vs. absent | Age, sex |
| Zou et al., 2000 [25] | 10,059 (142) | China | EBDC | 1990-1992 | Medical records | Present vs. absent | Age, sex |

Table 1. Characteristics of the studies included in the meta-analysis.
| Study                        | Country | Study period | Sample size | Outcome | Gallstone characteristics | Gallstone measurement | Matching                                      | Adjustment                                      | NOS |
|------------------------------|---------|--------------|-------------|---------|--------------------------|-----------------------|-----------------------------------------------|-----------------------------------------------|-----|
|                              |         |              |             |         |                          |                       |                                               |                                               |     |
| **Case-control studies**     |         |              |             |         |                          |                       |                                               |                                               |     |
| Diehl, 1983 [28]             | USA     | 1976-1980    | 45/66       | GBC     | Size (≥1 vs. <1)          | Medical records       | Age, sex, hospital, screening year           |                                               | 7   |
|                              | USA     | 1976-1980    | 45/66       | GBC     | Size (≥2 vs. <2)          | Medical records       | Age, sex, hospital, screening year           |                                               | 7   |
| Lowenfels et al., 1985 [29]  | USA     | 1976-1980    | 74/2,013    | GBC     | Present vs. absent       | Medical records       | Age                                           |                                               | 5   |
| Lowenfels et al., 1989 [30]  | USA     | 1976-1980    | 57/386      | GBC     | Size (≥1 vs. <1)          | Medical records       | Age, race                                     |                                               | 7   |
|                              |         | 1976-1980    | 15/386      | GBC     | Size (≥2 vs. <2)          | Medical records       | Age, race                                     |                                               | 7   |
| Moerman et al., 1993 [31]    | Netherlands | 1966-1969 | 43/98       | GBC     | Size (≥1 vs. <1)          | Medical records with imaging | Age, sex, hospital, date of admission |                                               | 7   |
|                              |         | 1976-1980    | 41/96       | GBC     | Size (≥2 vs. <2)          | Medical records       | Age, sex, hospital, date of admission       |                                               | 7   |
| Khan et al., 1999 [32]       | USA     | 1980-1994    | 38/138      | GBC     | Present vs. absent        | Medical records       | Age, sex, ethnicity, socioeconomic status, smoking |                                               | 6   |
| Okamoto et al., 1999 [33]    | Japan   | 1986-1993    | 19/94,478   | GBC     | Present vs. absent        | Medical records with imaging | Age, sex, race, geographic region, state buy-in status |                                               | 6   |
| Welzel et al., 2007 [34]     | USA     | 1993-1999    | 549/102,782 | EHC     | Present vs. absent        | Medical records       | Age, sex, race, geographic region, state buy-in status |                                               | 6   |
| Hsing et al., 2007 [35]      | China   | 1997-2001    | 368/902     | GBC     | Present vs. absent        | Medical records with imaging | Age                                           | Age, sex, education                          | 8   |
|                              |         | 191/959      | BDC         | Present vs. absent        | Medical records with imaging | Age                                           |                                               |                                               |     |
| Ahrens et al., 2007 [36]     | Europe  | 1995-1997    | 153/1,421   | BTC     | Present vs. absent        | Self-reported         | Age, sex, region                             | Age, country, next-of-kin status            | 7   |
|                              |         | (45)         | GBC         | Present vs. absent        | Self-reported         | Age, sex, region                             |                                               |                                               |     |
| Grainge et al., 2009 [37]    | UK      | 1987-2002    | 372/5,760   | CCA     | Present vs. absent        | Medical records       | Sex, age, GP practice                        | Alcohol, smoking, BMI                        | 5   |
| Taot et al., 2010 [38]       | China   | 1998-2008    | 129/380     | EHC     | Present vs. absent        | Medical records with imaging | Age, sex                                     |                                               | 8   |
| Cai et al., 2011 [39]        | China   | 2000-2005    | 31/608      | EHC     | Present vs. absent        | Medical records with imaging | Age, sex                                     | Age, sex                                     | 7   |
| Alvi et al., 2011 [40]       | Pakistan | 1988-2007   | 60/120      | GBC     | Size (≥1 vs. <1)          | Medical records with imaging | Age, sex                                     | Age, parity, BMI, stone characteristics     | 5   |
|                              |         |              |             |         | n (>1 vs. 1)              |                       |                                               |                                               |     |

*(Continued to the next page)*
Table 1. Continued

| Study | Country | Study period       | Sample size¹ | Outcome | Gallstone characteristics | Gallstone measurement | Matching       | Adjustment                    | NOS² |
|-------|---------|--------------------|--------------|---------|--------------------------|------------------------|-------------------|-------------------------------|------|
| Wu et al., 2012 [41] | China | 1998-2010 | 93/809 (86/835) | GBC | Present vs. absent | Medical records with imaging | Age, sex | Age, sex, HBV, DM, TC, HDL-C | 9    |
| Onal et al., 2012 [42] | Turkey | 2006-2010 | 99/48 | EBDC | Present vs. absent | Self-reported | Age, sex | Age, sex, HBV, alcohol, smoking | 6    |
| Chang et al., 2013 [43] | Taiwan | 2004-2008 | 2,179/8,716 | CCA | Present vs. absent | Medical records with imaging | Age, sex, date of diagnosis | Cholangitis | 5    |
| Nogueira et al., 2014 [44] | USA | 1992-2005 | 3,681/100,000 (3,664) | CCA | Present vs. absent | Medical records with imaging | Age, sex, calendar year | 6    |
| He et al., 2014 [13] | China | 2006-2010 | 210/620 | AOVC | Present vs. absent | Medical records with imaging | Age, sex | Hypertension, DM, VOD, alcohol, smoking, BMI, PLG | 8    |
| Cha, 2015 [45] | Korea | 2008-2013 | 78/78 | GBC | Present vs. absent | Medical records with imaging | Age, sex | 8    |
| Lee et al., 2015 [46] | Korea | 2007-2013 | 276/552 (193/386) | CCA | Present vs. absent | Medical records | Age, sex, date of diagnosis | Alcohol, DM, HBV, LFI | 8    |
| Lee et al., 2015 [47] | Korea | 2007-2013 | 81/162 | EBDC | Present vs. absent | Medical records | Age, sex, date of diagnosis | DM, smoking | 8    |
| Rosato et al., 2016 [48] | Italy | Study 1: 1983-1992 Study 2: 1994-2009 | 159/795 | BTC | Present vs. absent | Self-reported | Age, sex, study center | Year of interview, education, BMI, alcohol, smoking | 8    |
| Tamrakar et al., 2016 [49] | Nepal | 2012-2013 | 100/100 | GBC | Present vs. absent | Self-reported | Age, sex, marital status | Education, hospital, smoking, fruit consumption, residence | 6    |

NOS, Newcastle-Ottawa scale; AOVC, ampulla of Vater cancer; BDC, bile duct cancer; BMI, body mass index; BTC, biliary tract cancer; CCA, cholangiocarcinoma; DM, diabetes mellitus; EBDC, extrahepatic bile duct cancer; EHC, extrahepatic cholangiocarcinoma; GBC, gallbladder cancer; GP, general physician; HBV, hepatitis B virus; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LFI, liver fluke infestation; PLG, polypoid lesion of gallbladder; VOD, vascular occlusive disease.  
¹Number of participants (cases) for cohort studies and cases/controls (cases of subsites) for case-control studies.  
²NOS for assessing the quality of non-randomized studies in a meta-analysis.  
³Gallstone presence of up to 3 years before the diagnosis of cancer.  
⁴Gallstone presence of up to 1 year before the diagnosis of cancer.  
⁵Gallstone presence of up to 1 year before the diagnosis of cancer.  
⁶History of gallstones (ever vs. never).
The heterogeneity varied substantially as the stratification method changed, and subgroup analysis with the studies that reported the outcomes of only the male patients exhibited the lowest level of heterogeneity ($I^2 = 35.8\%$; $p = 0.132$) among the subgroups including more than 2 studies.

**Gallstones and the risk of gallbladder cancer**

Among the 20 studies on associations between gallstones and the risk of GBC, 16 studies presented associations between the presence of gallstones and the risk of GBC, as shown in Supplemental Material 1A. A total of 5 cohort studies and 11 case-control studies were included in the meta-analysis of cancer at this subsite. When we analyzed the results according to the study design, statistically significant positive associations were shown in both case-control studies (OR, 9.60; 95% CI, 4.45 to 20.70; $I^2 = 95.4\%$; $p < 0.001$) and cohort studies (OR, 4.54; 95% CI, 2.62 to 7.87; $I^2 = 72.5\%$; $p = 0.006$). The pooled risk estimate including case-control and cohort studies was also statistically significant (OR, 7.26; 95% CI, 4.33 to 12.18), with high heterogeneity across the studies ($I^2 = 93.6\%$; $p < 0.001$).

Meta-analyses were stratified by diverse subgroups, as presented in Supplementary Material 2. Regardless of the subgroups, all the results of meta-analyses were statistically significant with little differences in the magnitude of risk estimates. However, some differences in the risk estimates according to the subgroup analyses were statistically significant, as follows: geographic areas in Asia (OR, 12.72; 95% CI, 6.35 to 25.46; $I^2 = 86.2\%$; $p < 0.001$) versus non-Asian areas (OR, 3.59; 95% CI, 2.68 to 4.81; $I^2 = 56.0\%$; $p = 0.026$), measurement of exposure with imaging studies (OR, 4.67; 95% CI, 3.29 to 6.61; $I^2 = 76.1\%$; $p < 0.001$), and adjustment for education (OR, 23.80; 95% CI, 17.00 to 33.32) ver-
Table 2. Meta-analysis results for the association between the presence of gallstones and the risk of BTC by subgroups

| Subgroup                       | No. of studies | OR (95% CI) | I² value (%) | p for heterogeneity |
|--------------------------------|----------------|-------------|--------------|---------------------|
| All studies                    | 26             | 4.38 (3.23, 5.93) | 91.2          | <0.001              |
| Study design                   |                |             |              |                     |
| Cohort study                   | 7              | 3.17 (2.28, 4.39) | 79.0          | <0.001              |
| Case-control study             | 19             | 5.04 (3.36, 7.56) | 90.5          | <0.001              |
| Sex                            |                |             |              |                     |
| Male                           | 9              | 3.40 (2.70, 4.28) | 35.8          | 0.132               |
| Female                         | 9              | 4.26 (2.75, 6.59) | 84.5          | <0.001              |
| Geographic area                |                |             |              |                     |
| Asia                           | 15             | 5.25 (3.50, 7.86) | 82.4          | <0.001              |
| Non-Asia                       | 11             | 3.58 (2.17, 5.91) | 95.1          | <0.001              |
| Study period                   |                |             |              |                     |
| Before 2000                    | 8              | 5.39 (2.57, 11.34) | 95.5          | <0.001              |
| Around 2000                    | 7              | 2.67 (2.10, 3.39) | 38.6          | 0.135               |
| After 2000                     | 7              | 5.21 (2.13, 12.74) | 85.7          | <0.001              |
| No records                     | 4              | 5.73 (2.61, 12.61) | 87.3          | <0.001              |
| Measurement of gallstones      |                |             |              |                     |
| Medical records with imaging   | 8              | 7.09 (3.87, 12.98) | 64.5          | 0.004               |
| studies                        |                |             |              |                     |
| Medical records without        | 16             | 3.81 (2.48, 5.85) | 93.9          | <0.001              |
| imaging studies                |                |             |              |                     |
| No records                     | 2              | 3.47 (2.88, 4.18) | 17.1          | 0.272               |
| Study quality                  |                |             |              |                     |
| High NOS                       | 14             | 3.99 (2.85, 5.59) | 75.6          | <0.001              |
| Low NOS                        | 12             | 4.81 (2.87, 8.05) | 94.9          | <0.001              |
| Adjustment for age, yes        | 21             | 3.71 (2.66, 5.16) | 90.6          | <0.001              |
| Adjustment for sex, yes        | 20             | 3.92 (2.77, 5.55) | 91.2          | <0.001              |
| Adjustment for comorbidities,   | 7              | 3.05 (1.84, 5.05) | 75.7          | <0.001              |
| yes                            |                |             |              |                     |
| Adjustment for lifestyle        | 7              | 4.84 (1.95, 11.98) | 85.5          | <0.001              |
| factors, yes                   |                |             |              |                     |
| Adjustment for education, yes  | 1              | 9.42 (3.56, 24.91) | -            | -                   |
| Adjustment for geographic areas, | 4              | 7.34 (2.28, 23.62) | 87.6          | 0.000               |

BTC, biliary tract cancer; OR, odds ratio; CI, confidence interval; NOS, Newcastle-Ottawa scale.
*OR refers to a summary estimate of effects based on a random-effects model.
*Non-Asia including USA and European areas.
*Study period was defined by the study’s starting point (a) and ending point (b). Before 2000, (a) and (b) are both before 2000; around 2000, (a) is before 2000 but (b) is after 2000; after 2000, (a) and (b) are both after 2000.
*Quality scores greater than or equal to the median value were judged as a high NOS (≥7).
*Adjustment for lifestyle factors such as alcohol, smoking, body mass index, etc.

sus the original summary risk estimates (OR, 7.26; 95% CI, 4.33 to 12.18; I² = 93.6%; p < 0.001).

With regard to gallstone characteristics, we found that the risk of GBC was associated with gallstone size (>1 vs. < 1 cm: OR, 1.88; 95% CI, 1.10 to 3.22; I² = 35.2%; p = 0.201) (> 2 vs. < 2 cm: OR, 2.62; 95% CI, 0.90 to 7.60; I² = 73.8%; p = 0.022) [28,30,31,40] and gallstone number (> 1 vs. 1: OR, 2.10; 95% CI, 0.80 to 5.47; I² = 63.8%; p = 0.096) [31,40].

Gallstones and the risk of extrahepatic bile duct cancer

A total of 17 studies presented associations between the presence of gallstones and the risk of EBDC in its broadest sense (a concept embracing EBDC, EHC, CCA, and BDC), as shown in Supplementary Material 1B. We identified 4 cohort studies and 13 case-control studies that presented associations between the presence of gallstones and the risk of EBDC. Among the 17 studies, 12 studies reported the risk of EBDC (or EHC), while the remaining 6 studies investigated the risk of CCA (or BDC) [23,35,37,42,44,46], with 1 study [46] describing the risk of both EBDC and CCA. The summary risk estimate for the association between gallstone presence and the risk of cancer was stronger within the studies on EBDC (or EHC) (OR, 2.87; 95% CI, 2.06 to 3.99; I² = 95.0%; p < 0.001) than the studies on CCA (or BDC) (OR, 2.12; 95% CI, 1.35 to 3.33; I² = 92.7%; p < 0.001) without statistical significance.

In the comprehensive meta-analysis of EBDC, when we analyzed the results according to the study design, a statistically significant positive association was shown in both case-control studies (OR, 3.67; 95% CI, 2.26 to 5.95; I² = 96.0%; p < 0.001) and cohort studies (OR, 2.33; 95% CI, 2.00 to 2.72; I² = 21.4%; p = 0.282). The pooled risk estimate was also statistically significant (OR, 3.17; 95% CI, 2.24 to 4.50), with high heterogeneity across the studies (I² = 95.2%; p < 0.001).

In the subgroup meta-analyses, all results showed statistical significance regardless of sex, geographic area, study period, measurement of exposure, and study quality, as presented in Supplementary Material 3. However, the differences between the magnitudes of the effect sizes did not have statistical significance in any of the stratifications.

Gallstones and the risk of ampulla of Vater cancer

Five studies presented associations between gallstone characteristics and the risk of AOVC. Among these studies, 1 study reported the duration of gallstones [35], and all 5 studies reported the presence of gallstones [13,24,35,36,44]. Due to the limited number of eligible studies, we only conducted a meta-analysis according to the presence of gallstones, as shown in Supplementary Material 1C. The result still showed a significant association between the presence of gallstones and the risk of AOVC (OR, 3.28; 95% CI, 1.33 to 8.11; I² = 93.3%; p < 0.001). In the subgroup analyses, the magnitudes of association were significantly higher in Asian studies (OR, 7.23; 95% CI, 2.49 to 21.00; I² = 88.0%; p = 0.004) than in non-Asian studies (OR, 1.57; 95% CI, 1.28 to 1.92; I² = 0.0%; p = 0.608) and in studies that measured gallstones by an imaging modality (OR, 7.23; 95% CI, 2.49 to 21.00; I² = 88.0%; p = 0.004) than the
studies that did not (OR, 1.57; 95% CI, 1.28 to 1.92; $I^2 = 0.0\%$; $p = 0.608$) (Supplementary Material 4).

**Sensitivity analysis and publication bias**

The sensitivity analyses for the relationships between the presence of gallstones and the risk of BTC are given as Supplementary Material 5. We found similar results to those of the original meta-analysis, with the same directions and magnitudes of effects (ORs ranging from 3.96 to 4.72 and each OR with a 95% CI, embodying the original OR, of 4.38) when we sequentially excluded every study one by one. The funnel plots for the association between the presence of gallstones and the risk of BTC by each subsite revealed no evidence of publication bias (Supplementary Material 6). The Egger test did not identify publication bias in the overall meta-analysis, including all the subsites of BTC (BTC: $t = 0.79$, $p = 0.421$; GBC: $t = 1.98$, $p = 0.068$; EBDC: $t = 0.41$, $p = 0.688$; AOVC: $t = 1.13$, $p = 0.340$) (Supplementary Material 6A-D).

**DISCUSSION**

Our systematic review and meta-analysis provided the most comprehensive evidence to date on the associations between gallstones and the risk of BTC, including GBC, EBDC, and AOVC. This study showed that the risks of GBC, EBDC, and AOVC increased with gallstone presence, and statistically significant associations were observed both in 7 cohort studies and in 19 case-control studies. In terms of gallstone size and number, the meta-analyses revealed that only size (> 1 vs. < 1 cm) was significantly associated with the risk of GBC. Sensitivity analyses of studies restricted according to the study quality or adjustments, as well as sequentially excluding studies one by one, supported the stability of the results.

Based on the meta-analysis results for the BTC subsites, specifically GBC and AOVC, a common trend of significantly stronger summary effect sizes on the association between the presence of gallstones and the risk of BTC is much more complex, and the effect size of each study may tend to vary substantially. In the stratification by sex, wherein the degree of heterogeneity decreased in the studies that reported the outcomes of only the male group, a possible explanation may be rooted in the unique epidemiological nature of cholelithiasis and BTC, as female sex and its related attributes (sex hormones, parity, and the number of pregnancies) are well-known risk factors for both diseases [10]. Unlike male patients, female patients are impacted by additional potential confounders, which were mostly unadjusted in previous studies. Thus, determining the association between the presence of gallstones and the risk of BTC is much more complex, and the effect size of each study may tend to vary substantially.

The biological mechanisms linking gallstones to the risk of BTC are not well known. One hypothesis suggests that gallstones dropped down from the upstream biliary tract might result in chronic inflammation of the bile duct epithelium as an underlying condition for tumor development. That is, gallstones could lead to EBDC by causing inflammation of the bile duct wall [11]. In addition, approximately 35% of patients with stones develop complications such as cholecystitis or cholangitis [50], which may contribute to carcinogenesis in the gallbladder or bile ducts. Another possible hypothesis of the pathogenesis assumes that hormonal or repro-
ductive factors might play a role in tumor development [51]. The increased exposure to endogenous estrogen and progesterone during pregnancy or exogenous estrogen seems to promote the formation of biliary stones. Under hormonal exposure, cholesterol saturation of bile mounts, leading to impaired contractility of the smooth muscles of the biliary tract [52]. Therefore, biliary status and gallstone formation easily occur, which might be the key steps in the process of carcinogenesis in the biliary tract [52]. Our meta-analysis results are not contradictory to either of these hypotheses.

There are several limitations to this systematic review and meta-analysis. First, we tried to capture the association between gallstones and the risk of BTC, thereby inevitably excluding some other studies [53-56] that investigated the association between gallbladder disease (or condition), not gallstones, and the risk of BTC. Second, the definition of EBDC used in our study encompassed not only EBDC and its equivalent term, EHC, but also CCA and its equivalent term, BDC. CCA (or BDC) is an overlapping term with EBDC, as approximately 90% of CCA is EBDC, but the remaining 8% to 10% comprises IBDC, which is usually not a subsite of BTC [15,57]. Third, although we extracted the risk estimates considering adjustments for potential confounders, the scope of adjusted confounders varied across the studies, which could have caused deviations in the meta-analysis results. Finally, there was significant heterogeneity across the studies, which might cast some doubts on the reliability of the summary risk estimates. This high heterogeneity may have originated from the obscurity in defining gallstones’ presence in previous studies, as most studies lacked concrete information about the duration of gallstones. This implies that the interval between the presence of gallstones and the diagnosis of BTC is inconsistent among studies, leaving the same limitations for the meta-analyses. Therefore, future research needs to implement clear criteria for gallstone presence, assuming that differences in the definition are a plausible source of heterogeneity. Another reason for this phenomenon is that our meta-analyses combined all eligible studies, which in fact, had distinct natures. In our subgroup meta-analyses, the groups that shared a common study design (cohort study), sex (male), and measurement of exposure (imaging study) showed less heterogeneity, respectively, compared to each of their counterparts. Further research with a more sophisticated approach is needed to narrow these specific groups to secure a lower level of heterogeneity when synthesizing the risk estimates on the association between gallstones and BTC.

Despite these limitations, our study has several strengths. To the best of our knowledge, this study is the first systematic review and meta-analysis of the associations between gallstone characteristics and the risk of BTC. Unlike a previous systematic review [14], we reported the characteristics of gallstones (presence, size, and number), not gallbladder disease as a whole, in association with the risk of BTC. Moreover, we conducted meta-analyses stratified by each subsite of BTC (GBC, EBDC, and AOVC) and other diverse factors, including the study design, sex, geographic area, study period, measurement of exposure, study quality, and whether analyses were adjusted for various confounders. In this study, we attempted to explore all the relevant studies and to reflect the findings and achievements hitherto established to the greatest extent possible.

CONCLUSION

We found statistically significant associations between gallstones and an increased risk of BTC through systematic reviews and meta-analyses. We verified that the presence of gallstones is a critical risk factor for BTC as well as for GBC, EBDC, and AOVC. Our study provides a better description of the association between gallstones and the risk of BTC.

SUPPLEMENTARY MATERIALS

Supplementary materials are available at http://www.e-epih.org/.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare for this study.

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AUTHOR CONTRIBUTIONS

Conceptualization: DH, HJ, SC, NS, AS. Data curation: DH, HJ, NS, WK. Formal analysis: HJ, DH, NS, SC, AS. Funding acquisition: AS. Methodology: DH, HJ, NS, SC. Project administration: AS. Visualization: DH, HJ, AS. Writing – original draft: HJ, DH. Writing – review & editing: HJ, DH, NS, SC, WK, AS.

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence
and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Forman D, Bray F, Brewster DH, Gombe Mbalawa C, Kohler B, Piñeros M, et al. Cancer incidence in five continents Vol. X; 2014 [cited 2020 Dec 1]. Available from: https://ci5.iarc.fr/CI5I-X/old/vol10/CI5vol10.pdf.
3. Jung KW, Won YJ, Kong HJ, Lee ES. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2016. Cancer Res Treat 2019;51:417-430.
4. Shaib Y, El-Serag HB. The epidemiology of cholangiocarcinoma. Semin Liver Dis 2004;24:115-125.
5. Randi G, Malvezzi M, Levi F, Ferlay J, Negri E, Franceschi S, et al. Epidemiology of biliary tract cancers: an update. Ann Oncol 2009;20:146-159.
6. Anderson CD, Pinson CW, Berlin J, Chari RS. Diagnosis and treatment of cholangiocarcinoma. Oncologist 2004;9:43-57.
7. Misra S, Chaturvedi A, Misra NC, Sharma ID. Carcinoma of the gallbladder. Lancet Oncol 2003;4:167-176.
8. Lee SH, Park SW. Inflammation and cancer development in pancreatic and biliary tract cancer. Korean J Gastroenterol 2015;66:325-339 (Korean).
9. Schottenfeld D, Beebe-Dimmer J. Chronic inflammation: a common and important factor in the pathogenesis of neoplasia. CA Cancer J Clin 2006;56:69-83.
10. Stinton LM, Shaffer EA. Epidemiology of gallbladder disease: cholecystitis and cancer. Gut Liver 2012;6:172-187.
11. Flood TA, Jain D, Marginen EC. Malignant tumours of gallbladder and extrahepatic bile ducts. Diagn Histopathol 2010;16:360-370.
12. Nordenstedt H, Mattsson F, El-Serag H, Lagergren J. Gallstones and cholecystectomy in relation to risk of intra- and extrahepatic cholangiocarcinoma. Br J Cancer 2012;106:1011-1015.
13. He XD, Wu Q, Liu W, Hong T, Li JJ, Miao RY, et al. Association of metabolic syndromes and risk factors with ampullary tumors development: a case-control study in China. World J Gastroenterol 2014;20:9541-9548.
14. Randi G, Franceschi S, La Vecchia C. Gallbladder cancer worldwide: geographical distribution and risk factors. Int J Cancer 2006;118:1591-1602.
15. de Groen PC, Gores GJ, LaRusso NF, Gunderson LL, Nagorney DM. Biliary tract cancers. N Engl J Med 1999;341:1368-1378.
16. Zeng X, Zhang Y, Kwong JS, Zhang C, Li S, Sun F, et al. The methodological quality assessment tools for preclinical and clinical studies, systematic review and meta-analysis, and clinical practice guideline: a systematic review. J Evid Based Med 2015;8:2-10.
17. Borenstein M, Hedges LV, Higgins JP, Rothstein HR. Introduction to meta-analysis. West Sussex: John Wiley & Sons; 2009. p. 69-75.
18. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557-560.
19. Patopoulos NA, Evangelou E, Ioannidis JP. Sensitivity of between-study heterogeneity in meta-analysis: proposed metrics and empirical evaluation. Int J Epidemiol 2008;37:1148-1157.
20. Sedgwick P, Marston L. How to read a funnel plot in a meta-analysis. BMJ 2015;351:h4718.
21. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629-634.
22. Maringhini A, Moreau JA, Melton LJ 3rd, Hench VS, Zinsmeister AR, DiMagno EP. Gallstones, gallbladder cancer, and other gastrointestinal malignancies. An epidemiologic study in Rochester, Minnesota. Ann Intern Med 1987;107:30-35.
23. Bansal P, Sonnenberg A. Comorbid occurrence of cholecystitis and gastrointestinal cancer. Eur J Gastroenterol Hepatol 1996;8:985-988.
24. Chow WH, Johansen C, Gridley G, Mellemkjaer L, Olsen JH, Fraumeni Jr JF. Gallstones, cholecystectomy and risk of cancers of the liver, biliary tract, and pancreas. Br J Cancer 1999;79:640-644.
25. Zou S, Zhang L. Relative risk factors analysis of 3,922 cases of gallbladder cancer. Zhonghua Wai Ke Za Zhi 2000:38:805-808.
26. Ishiguro S, Inoue M, Kurahashi N, Iwasaki M, Sasazuki S, Tsugane S. Risk factors of biliary tract cancer in a large-scale population-based cohort study in Japan (JPHC study); with special focus on cholecystitis, body mass index, and their effect modification. Cancer Causes Control 2008;19:33-41.
27. Lai HC, Chang SN, Lin CC, Chen CC, Chou JW, Peng CY, et al. Does diabetes mellitus with or without gallstones increase the risk of gallbladder cancer? Results from a population-based cohort study. J Gastroenterol 2013;48:856-865.
28. Diehl AK. Gallstone size and the risk of gallbladder cancer. JAMA 1983;250:2323-2326.
29. Lowenfels AB, Lindström CG, Conway MJ, Hastings PR. Gallstones and risk of gallbladder cancer. J Natl Cancer Inst 1985;75:77-80.
30. Lowenfels AB, Walker AM, Althaus DP, Townsend G, Domeløf L. Gallstone growth, size, and risk of gallbladder cancer: an interrational study. Int J Epidemiol 1989;18:50-54.
31. Morerman CJ, Lagerwaard FJ, Bueno de Mesquita HB, van Dalen A, van Leeuwen MS, Schrover PA. Gallstone size and the risk of gallbladder cancer. Scand J Gastroenterol 1993;28:482-486.
32. Khan ZR, Neugut AI, Ahsan H, Chabot JA. Risk factors for biliary tract cancers. Am J Gastroenterol 1999;94:149-152.
33. Okamoto M, Okamoto H, Kitahara F, Kobayashi K, Karikome K, Miura K, et al. Ultrasonographic evidence of association of polyps and stones with gallbladder cancer. Am J Gastroenterol 1999;94:446-450.
34. Welzel TM, Graubard BI, El-Serag HB, Shaib YH, Davi ML, Ahrens W, Timmer A, Vyberg M, Fletcher T, Guénel P, Merler E, et al. Risk factors for intrahepatic biliary tract carcinoma in men: medical conditions and lifestyle: results from a European multicentre case–control study. Eur J Gastroenterol Hepatol 2007;19:623-630.
37. Grainge MJ, West J, Solaymani-Dodaran M, Aithal GP, Card TR. The antecedents of biliary cancer: a primary care case-control study in the United Kingdom. Br J Cancer 2009;100:178-180.

38. Tao LY, He XD, Qu Q, Cai L, Liu W, Zhou L, et al. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma: a case-control study in China. Liver Int 2010;30:215-221.

39. Cai WK, Sima H, Chen BD, Yang GS. Risk factors for hilar cholangiocarcinoma: a case-control study in China. World J Gastroenterol 2011;17:249-253.

40. Alvi AR, Siddiqui NA, Zafar H. Risk factors of gallbladder cancer in Karachi—a case-control study. World J Surg Oncol 2011;9:164.

41. Wu Q, He XD, Yu L, Liu W, Tao LY. The metabolic syndrome and risk factors for biliary tract cancer: a case-control study in China. Asian Pac J Cancer Prev 2012;13:1963-1969.

42. Onal IK, Parlak E, Kekilli M, Kurt M, Alioglu H, Disibeyaz S, et al. Hepatitis B and C virus infection and cholangiocarcinoma: a case-control study in Turkey. Int J Hematol Oncol 2012;22:187-191.

43. Chang JS, Tsai CR, Chen LT. Medical risk factors associated with cholangiocarcinoma in Taiwan: a population-based case-control study. PLoS One 2013;8:e69981.

44. Nogueira L, Freedman ND, Engels EA, Warren JL, Castro F, Ko shioli J. Gallstones, cholecystectomy, and risk of digestive system cancers. Am J Epidemiol 2014;179:731-739.

45. Cha BH. Epidemiological characteristics of gallbladder cancer in Jeju Island: a single-center, clinically based, age-sex-matched, case-control study. Asian Pac J Cancer Prev 2015;16:8451-8454.

46. Lee BS, Park EC, Park SW, Nam CM, Roh J. Hepatitis B virus infection, diabetes mellitus, and their synergism for cholangiocarcinoma development: a case-control study in Korea. World J Gastroenterol 2015;21:502-510.

47. Lee BS, Cha BH, Park EC, Roh J. Risk factors for perihilar cholangiocarcinoma: a hospital-based case-control study. Liver Int 2015;35:1048-1053.

48. Rosato V, Bosetti C, Dal Maso L, Montella M, Serraino D, Negri E, et al. Medical conditions, family history of cancer, and the risk of biliary tract cancers. Tumori 2016;2016:252-257.

49. Tamrakar D, Paudel I, Adhikary S, Rauniyar B, Pokharel P. Risk factors for gallbladder cancer in Nepal a case control study. Asian Pac J Cancer Prev 2016;17:3447-3453.

50. Magouliotis DE, Tasiopoulou VS, Svoros AA, Svoros KA, Chatedaki C, Sioka E, et al. Ursodeoxycholic acid in the prevention of gallstone formation after bariatric surgery: an updated systematic review and meta-analysis. Obes Surg 2017;27:3021-3030.

51. Godrey PJ, Bates T, Harrison M, King MB, Padley NR. Gall stones and mortality: a study of all gall stone related deaths in a single health district. Gut 1984;25:1029-1033.

52. Moerman CJ, Berns MP, Bueno de Mesquita HB, Runia S. Reproductive history and cancer of the biliary tract in women. Int J Cancer 1994;57:146-153.

53. Zatonski WA, Lowenfels AB, Boyle P, Maisonneuve P, Bueno de Mesquita HB, Ghadirian P, et al. Epidemiologic aspects of gallbladder cancer: a case-control study of the SEARCH Program of the International Agency for Research on Cancer. J Natl Cancer Inst 1997;89:1132-1138.

54. Serra I, Yamamoto M, Calvo A, Cavada G, Báez S, Endoh K, et al. Association of chili pepper consumption, low socioeconomic status and longstanding gallstones with gallbladder cancer in a Chilean population. Int J Cancer 2002;102:407-411.

55. WHO Collaborative Study of Neoplasia and Steroid Contraceptives. Combined oral contraceptives and gallbladder cancer. Int J Epidemiol 1989;18:309-314.

56. Yagyu K, Lin Y, Obata Y, Kikuchi S, Ishibashi T, Kurosawa M, et al. Association of chili pepper consumption, low socioeconomic status and longstanding gallstones with gallbladder cancer in a Japanese population. Cancer Sci 2004;95:674-678.

57. Gupta A, Dixon E. Epidemiology and risk factors: intrahepatic cholangiocarcinoma. Hepatobiliary Surg Nutr 2017;6:101-104.