MINNI-REVIEW

Helicobacter Species are Possible Risk Factors of Cholangiocarcinoma

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Abstract

Several infectious agents are considered to be causes of cancer in human, mainly hepatitis B and C viruses, high-risk human papilloma viruses, Helicobacter pylori, Clonorchis sinensis, and Opisthorchis viverrini. Here we described the evident research and the association between Helicobacter spp. and biliary tract cancer particularly cholangiocarcinoma (CCA). Global epidemiological studies have suggested that Helicobacter spp. are possible risk factors for biliary tract diseases. Molecular studies support a linkage of Helicobacter spp. with CCA development. H. pylori, H. bilis, and H. hepaticus, are found in CCA, but the most common species are H. pylori and H. bilis. The type of CCA are associated with Helicobacter spp. include extrahepatic CCA, and common bile duct cancer. Up to the present, however, the results from different regions, materials and methods, sub-sites of cancer, and controls have not been consistent, thus introducing heterogeneity. Therefore, a comparison between co-Helicobacter spp.-CCA in the countries with low and high incident of CCA is required to settle the question. Furthermore, clarifying variation in the role of Helicobacter species in this CCA, including pathogenesis of CCA through enhanced biliary cell inflammation and proliferation, is necessary.

Keywords: Helicobacter - cholangiocarcinoma - biliary tract cancer - possible risk

Introduction

Infection is one of the main contributors to cancer development particularly the chronic infection mainly hepatitis B and C viruses, Helicobacter pylori, Clonorchis sinensis, and Opisthorchis viverrini, the biological agents have been identified as group 1 carcinogens by the International Agency for Research on Cancer Monographs (Oh and Weiderpass, 2014).

Recently, infection with Helicobacter spp. plays a role in the development of various cancer have been reported including biliary tract carcinoma mainly cholangiocarcinoma (CCA). There has been a strong, positive correlation between opisthorchiasis-associated CCA and infection with Helicobacter. Infection with H. bilis and H. hepaticus species can cause biliary cancer (Chang and Parsonnet, 2010). Recently, Deenonpoe et al (2015) reported liver fluke O. viverrini in the biliary tree of the hamsters harbors H. pylori and Helicobacter-like bacteria. Accordingly, the association between O. viverrini and H. pylori may be an obligatory mutualism and possible risk of CCA. Boonyanugomol et al. (2012b; 2012c) reported an association between H. pylori and hepatolithiasis or CCA in people in northeast Thailand, a region endemic for opisthorchiasis. Molecular mechanisms integral to H. pylori induced hepatobiliary diseases have also been reported (Boonyanugomol et al. 2011; 2012a).

Therefore, update on the association of Helicobacter spp. and CCA is required, mini-review critically analyzed the literature through the PubMed and Ovid MEDLINE databases searched, using a combination of relevant text words and MeSH terms: Helicobacter and/or cholangiocarcinoma, bile duct neoplasms, intra-extrahepatic, common bile duct, gall bladder cancer and biliary tract cancer.

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Cholangiocarcinoma and its Associated Risk Factors

Cholangiocarcinoma (CCA) a neoplasm that involves epithelial cells of the bile duct, also known as one of the most aggressive malignant tumors associated with local invasiveness and a high rate of metastasis. CCA originated in the bile duct which drained bile from the liver into the small intestine. Other biliary tract cancers include pancreatic cancer, gall bladder cancer, and cancer of the ampulla of Vater. It is also known to be one of the most common causes of cancer related to death in Thailand and it has been reported that Thailand is the highest incident of the world (Green et al., 1991; Sripa et al., 2007; Shin et al., 2010). It has an annual incidence rate of 1-2 cases per 100,000 in the Western world, but rates of CCA have been rising worldwide over the past several decades (Landis et al., 1998; Patel, 2002). This disease is difficult to have early diagnosis, as most symptoms present late in the disease course. In addition, the specific anatomic position can cause periductal extension and result in a very low radical excision rate and a very poor prognosis. Furthermore, CCA is considered to be an incurable and rapidly lethal disease unless all the tumors can be fully resected. Three-year survival rates of 35% to 50% are achieved only in a subset of patients who have negative histological margins at the time of surgery (Akamatsu et al., 2011). Survival of CCA patients in northeastern Thailand after supportive treatment was reported and indicated that the stage of disease was an important prognosis factor affecting survival of CCA patients who had diagnosis in late stage. To encourage patients to see health personnel at early stage is very important (Thunyaharn et al., 2013). Palliative therapeutic approaches, consisting of percutaneous and endoscopic biliary drainage, have usually been used for these patients because there is no effective chemotherapeutic treatment for this type of cancer.

A number of risk factors for the development of CCA have been described and multifactorial is associated to develop CCA. The 3 main factors have been hypothesized, including carcinogenic agents, infection, and other factors. Caroli’s disease, choledococal cyst, liver fluke infection, gallstones, hepatolithiasis, sclerosing cholangitis, thorotrust, and ulcerative colitis, are strongly associated with CCA development. While, asbestos, isoniazid, methyldopa, oral contraceptive, polychlorinated biphenyls are the possible association to the development of CCA (Yeo et al., 1990; Sripa et al., 2005). Infections are associated with the development of CCA, mainly liver flukes, *Opisthorchis viverrini* (Watanapa and Watanapa, 2002; Sripa et al., 2007; Kaewpitoon et al., 2008; Sripa et al., 2010), O. felineus (Maksimova et al., 2015), *Clonorchis sinensis* (Hong and Fang, 2012; Rustagi and Dasanu, 2012), and viral hepatitis (e.g. hepatitis B or hepatitis C) (Kobayashi et al., 2000; Lu et al., 2000; Yamamoto et al., 2004). In Thailand, the experimental and epidemiological evidences strongly indicated that *O. viverrini* infection in the etiology of CCA (Thamavit W., et al., 1978; IARC, 1994; Sripa B., et al., 2007). There has been a strong, positive correlation between opisthorchiasis-associated CCA and infection with *Helicobacter*. Infection with *H. bilis* and *H. hepaticus* species can cause biliary cancer (Chang and Parsonnet, 2010).

**Helicobacter Species are Causes of Cancer**

Several infectious agents are considered to be causes of cancer in humans. The estimated total of infection-attributable cancer in the year 2002 is 1.9 million cases, or 17.8% of the global cancer burden. The principal agents are the bacterium *H. pylori* (5.5% of all cancer) (Parkin, 2006). Therefore, here is described about *Helicobacter* species that caused of cancer. *Helicobacter* is a Gram-negative bacteria possessing a characteristic helical shape and to be a new genus name of *Helicobacter* (Goodwin et al., 1989). The *Helicobacter* genus contains about 35 species (Vandamme et al., 1991; Yamaoka 2008; Boyanova, 2011), the most widely known species of the genus is *H. pylori*, which infects up to 50% of the human population (Yamaoka 2008). *Helicobacter* species have been reported that found living in the lining of the upper gastrointestinal tract of mammals, birds, and naturally inhabit mammals (except humans) and birds (Ryan and Ray, 2004). They are *H. suis*, *H. bacluliformis*, *H. equorum*, *H. hepaticus*, *H. mustelae*, *H. bilis*, *H. felis*, *H. bizzozeronii*, *H. salmonis*, *H. ganmani*, *H. pullorum*, *H. anseris*, *H. brantae*, *H. cinaedi*, *H. canis*, *H. fennelliae*, *H. parmetensis*, *H. candensis*, *H. rodentium*, *H. typhlonicus*, *H. cholecystus*, *H. mesocricetorum* *H. muridurum*, *H. rappini*, and *H. trogontum* (Vandamme et al., 1991; Hua et al., 1999; Yamaoka 2008; Boyanova, L, 2011; Mateos-Muñoz et al., 2013).

Infection with *H. pylori* causes chronic inflammation and significantly increases the risk of developing duodenal and gastric ulcer disease and gastric cancer. Infection with *H. pylori* is the strongest known risk factor for gastric cancer, which is the second leading cause of cancer-related deaths worldwide (Wroblewski et al., 2010). Many studies show the link between *H. pylori* and gastric cancer, mainly gastric cancer developed in approximately 3% of *H. pylori*-infected patients, compared to none of the uninfected patients. These indicated that *H. pylori* infection significantly increases gastric cancer risk (Uemura et al., 2001). Eradication significantly reduced the presence of premalignant lesions, providing additional evidence that this organism has an effect on early stages of gastric carcinogenesis (Wong et al., 2004; Mera et al., 2005). *H. pylori* or mixed *H. pylori* and *H. bilis* infection had a significant association between and CCA in patients from northeast Thailand (Boonyanugomol et al., 2012a; 2012b). While, other known *Helicobacter* species have been reported the associated to cancer in human mainly *H. hepaticus* and *H. bilis* associated to hepatocellular carcinoma (Avenaud et al., 2000; Nilsson et al., 2001; Dore et al., 2002; Coppola et al., 2003; Huang et al., 2004; Ito et al., 2004; Pellicano et al., 2004; Zhang et al., 2004; Rocha et al., 2005; Li et al., 2006; Pellicano et al., 2008). In addition, the association between *Helicobacter* species and CCA has been reported mainly *H. bilis* (Matsukura et al., 2002; Kobayashi et al., 2005; Bohr et al., 2007), and *H. ganmani* (Bohr et al., 2007). Form above reports indicate
that *Helicobacter* species are possible risk of cancer.

**Helicobacter spp. Infection in Biliary Tract Cancer Including Cholangiocarcinoma**

*Helicobacter* spp. have been known to be a causative factor of gastric adenocarcinoma, hepatobiliary disease, and CCA (Bouvard et al., 2009; Zhou et al., 2013; Mateos- Muñoz et al., 2013; Murphy et al., 2014). The *Helicobacter* spp., such as *H. pylori*, *H. bilis*, *H. hepaticus*, and *H. cholecystis*, have been isolated from the human gallbladder, liver tissue and bile juice and *Helicobacter* infection has been found to induce chronic active hepatitis, hepatocellular and biliary tract carcinomas in susceptible strains of inbred and genetically engineered mice (Fox et al., 1994; Fox and Lee, 1997; Shomer et al., 1997; Young et al., 2000; Fukuda et al., 2002; Fox et al., 2004; Kobayashi et al., 2005; Abu Al-Soud et al., 2008; Casswall et al., 2010; Kosaka et al., 2010; Boonyanugomol et al., 2012; Fowsantear et al., 2014). It has been suggested that CCA is caused by infection with *Helicobacter* species (Segura-López et al., 2015).

Pradhan and Dali (2004) have been reported that the various histopathological changes in the gallbladder with cholelithiasis and to correlate them with *Helicobacter* hepaticus infection. A total of 380 cholecystectomy specimens were received and found that among the study group, 43% cases were found to have chronic cholecystitis, 17% adenomyomiasis, 13% cholesterolosis, 9% low grade dysplasia, 9% metaplasia, 7% malignancy, 1% carcinoma in situ and 1% xanthogranulomatous change. All the malignant cases were found to be adenocarcinoma. Out of total 100 cases, 82% cases were found to have *H. hepaticus* infection. Only one out of 7 malignant cases (14.29%) was found to be negative for *H. hepaticus* infection. Gallbladder neoplasm was found to be common in Nepal comprising 2.63%. *H. hepaticus* infection was found in 82% of gallbladders and it was found in 87.5% of malignant cases. Whether *H. hepaticus* that might be the number one cause for the gallstone formation that ultimately leads to malignancy or itself acts as a risk factor for the pathogenesis of carcinoma gallbladder is yet to be determined. Meanwhile, Fox et al. (2009) reported hamsters naturally infected with *H. bilis* and that aged animals showed chronic hepatitis, hepatic dysplasia, fibrosis, and biliary hyperplasia. Furthermore, Murata et al. (2004) has been found *H. bilis* infection in the gallbladder in patients with biliary tract disease. Archival gallbladder specimens from 34 patients (14 males and 20 females) with an average age of 61.4 +/- 12.2 years (mean +/- SE) were retrieved, consisting of 11 cases of gallbladder cancer, three of bile duct cancer, 16 of cholecystolithiasis and four of pancreatic cancer. Amplification was observed in 3 of 11 gallbladder cancer cases (27.2%) and one of three cases with biliary duct cancer (33.3%). In total, four of 14 cases with biliary tract cancer were positive for *H. bilis* (28.6%). This study suggested that *H. bilis* infection may play a role in biliary tract disease, particularly in biliary tract cancer. During other liver fluke infections, *H. bilis* has been identified in the intrahepatic bile ducts of rats experimentally infected with Fasciola hepatica (Foster, 1984). Deenoonpe et al (2015) reported the liver fluke *O. viverrini*; an agent caused of CCA, in the biliary tree of the hamsters harbors *H. pylori* and Helicobacter-like bacteria. Accordingly, the association between *O. viverrini* and *H. pylori* may be an obligatory mutualism and possible risk of CCA. *H. pylori* presented in the biliary tract of patients with hepatolithiasis, while *H. pylori* promotes the formation of stones in the biliary tract. The development of intrahepatic CCA might therefore be linked to the presence of *H. pylori* because of the accelerated activity of cell kinetics in the epithelium of the biliary tract (Kuroki et al., 2002). Roe et al (1999) have been reported that the *Helicobacter* found in the biliary tract diseases of humans. *Helicobacter* DNA was detected in 37.5%, and 31.3% by PCR with urea gene, and 16S rRNA, respectively. In addition, Fukuda et al (2002) investigated whether *Helicobacter* species possess a causative potential for human hepatobiliary disease, especially for hepatobiliary carcinogenesis, and found that *Helicobacter* DNA were positive in 10 (52.6%) of the 19 patients with hepatobiliary cancer. The incidence was significantly higher than that (15.7%) in the benign cases (P = 0.03). The findings suggest that *Helicobacter* species may play a role in the pathogenesis of hepatobiliary cancer through an acceleration of biliary cell kinetics.

In addition, Kobayashi et al (2005) have been shown the *Helicobacter* species in the bile to know their participation in the development of extrahepatic biliary diseases. DNA was extracted from 57 bile samples from 30 patients with benign biliary diseases (cholecystolithiasis and choledochocystolithiasis), 6 malignant biliary diseases (gallbladder cancer and common bile duct cancer), and 21 non-biliary diseases. The presence of *Helicobacter* genus-, *H. pylori*-, *H. hepaticus*-, and *H. bilis*-specific 16S rRNA genes, the *H. pylori* urease A gene, and the *H. pylori* 26K protein gene in the bile was determined by PCR and sequencing analysis. *Helicobacter* genus DNA (shorter amplicons, 400 bp) was statistically frequently detected in bile from 53% (16/30) and 86% (5/6) of benign and malignant biliary diseases, compared with 9% (2/21) of non-biliary diseases, but longer amplicons (1200 bp) were not detectable in any samples. The *H. pylori* urease A gene (nested amplicon) was also frequently found in bile, whether benign, malignant, or control, though neither *H. pylori* 16S rRNA nor the 26K protein gene was detectable in any bile samples. *H. bilis*-16S RNA genes were detectable in only two cases. *H. hepaticus* was not detectable in any samples. DNA fragments of *Helicobacter* species other than *H. pylori*, *H. hepaticus*, and *H. bilis* are commonly detectable in the bile of patients with extrahepatic biliary diseases, whether benign or malignant, implying that the *Helicobacter* genus may be directly or indirectly involved in the pathogenesis of these diseases. Abu Al-Soud et al (2008) reported a retrospectively investigated the presence of DNA of *Helicobacter* species in samples of the cancer and the surrounding tumour-free liver tissues of patients with hepatocellular carcinoma (n=12) and CCA (n=13). The patients were from an area with low liver cancer incidence and with low hepatitis B and C prevalence. Patients with a benign liver disease (n=24) were included as controls. PCR assay detected
Helicobacter DNA in seven of 12 (58%) and eight of 13 (62%) normal liver tissue specimens from HCC and CC patients, respectively. The study suggested that presence of DNA of Helicobacter species in liver specimens, but not of other common gut bacteria, was associated with human hepatic carcinogenesis. Boonyanugomol et al (2012a; 2012c) reported an association between H. pylori and hepatolithiasis or CCA in people in northeast Thailand, a region endemic for opisthorchiasis. In addition, H. pylori was found in 66.7%, 41.5% and 25.0% of the patients in the CCA, cholelithiasis and control groups (P < 0.05), respectively. By comparison, H. bilis was found in 14.9% and 9.4% of the patients with CCA and cholelithiasis, respectively (P > 0.05), and was absent in the control group. The cagA gene of H. pylori was detected in 36.2% and 9.1% of the patients with CCA and cholelithiasis, respectively (P < 0.05). Among patients with CCA, cell inflammation and proliferation in the liver and gall bladder were significantly higher among those DNA H. pylori positive than negative. These findings suggest that H. pylori, especially the cagA-positive strains, may be involved in the pathogenesis of hepatobiliary diseases, especially CCA through enhanced biliary cell inflammation and proliferation (Boonyanugomol et al., 2012a). Boonyanugomol et al (2012c) investigated cag genes; virulent gene related to cancer, and the association of those and the clinical outcomes in hepatobiliary diseases, and found that the vacAs1a + c/m1, iceA1 and babA2 genes were the most predominant genotypes in both CCA and cholelithiasis patients. The cagA and cagE genes were found significantly more frequently in patients with CCA than those with cholelithiasis (P < 0.05). The cagA positive samples were the Western-type cagA and showed that almost all of the detected sequences in Thai hepatobiliary and Thai gastric cancer patients were classified in the same cluster but separated from the cluster of Japan and other countries. The authors suggested that cagA and cagE genes may be associated in the pathogenesis of hepatobiliary diseases, especially of CCA. Besides the bacterial variation, other host factors may be involved in the pathogenesis of hepatobiliary cancer. However, Bohr et al (2007) reported that low prevalence of Helicobacteraceae in gall stone disease and gall bladder carcinoma (GBC) in the German population. Gall-bladder tissue from 99 patients who had undergone cholecystectomy was tested, including 57 cases of gall stone disease (GSD), 20 cases of GBC, and 22 control patients. The presence of Helicobacter spp. was investigated by culture, immunohistochemistry and a group-specific PCR targeting the 16S rRNA gene of all currently known Helicobacteraceae. Of the 99 cases investigated, only one patient with GSD was PCR-positive for Helicobacteraceae. For this individual, sequence analysis of the 16S rRNA gene showed that it had homology closest to the 16S rRNA sequence of H. gamnani. Helicobacteraceae were not detected by culture or immunohistochemistry. The low prevalence of Helicobacteraceae in the gall bladders investigated suggests that Helicobacteraceae do not play a predominant role in the pathogenesis of GSD and GBC in the German population. The low prevalence could be a possible explanation for a relatively low incidence of GBC in the German population, despite the fact that GSD, the major risk factor for GBC, is highly prevalent.

Epidemiological Studies Revealed Helicobacter Species as a Possible Risk of Biliary Tract Cancer Including Cholangiocarcinoma

Many studies of humans have evaluated the presence of Helicobacter species in biliary tracts of cancer patients versus controls. Base on a meta-analysis study of 10 case-control studies contained a cumulative sample size of 205 cases, 115 cases (56%) were positive for Helicobacter spp. infection, whereas among the 263 controls, 53 (20%) were positive for Helicobacter spp. infection. Overall meta-analysis favoured a significant association between Helicobacter species infection and CCA (cumulative OR 8.88, 95% CI 3.67-21.49). Subgroup analysis based on geographic distribution indicated that Helicobacter species infection may serve as a risk factor not only in a region with high CCA incidence (Asia, OR 6.68, 95% CI 2.29-19.49) but also in low incidence region (Europe, OR 14.90, 95% CI 4.79-46.35). The reports included suggested that the possible association between Helicobacter species infection and CCA (Xiao et al., 2014). Another meta-analysis of 10 studies published between 2002 and 2011 was reported that the association between Helicobacter pylori, Helicobacter bilis, Helicobacter hepaticus, and Helicobacter gnamani and CCA. A significantly higher pooled infection rate of Helicobacter spp. was observed in the biliary tract cancer group compared with the normal group (P=0.0001) and the benign biliary disease group, respectively (P=0.0001). Studies from East Asia and South Asia showed a higher prevalence of Helicobacter spp. in the malignant group. Evidence supporting the higher presence of Helicobacter spp. in the cancer group was obtained using PCR and immunohistochimical analysis of specimens from bile and biliary tissues. This meta-analysis suggests a trend of a higher presence of Helicobacter spp. in patients with biliary tract cancers compared with normal controls or those with benign biliary diseases (Zhou et al 2013).

In addition, a meta-analysis of 5 single group and 10 case control contained the cumulative sample size of cases was 205, of which 115 were positive (56%) for Helicobacter, while among 263 controls 53 (20%) were found to be positive for Helicobacter infection. The positivity rate in case control studies was higher than that observed in single group studies. The cumulative odds ratio for the study sample was 8.72 (95% CI 4.78-15.91) (Z=7.07; p<0.00001). There is enough evidence to suggest a possible role of Helicobacter species in hepatobiliary tract cancers. However, the results from different regions of the world differ. Studies also differ on method of Helicobacter detection, subsite of cancer with in the hepatobiliary tract and choice of controls thus introducing heterogeneity. The authors suggest that further case control studies with larger sample size are required to settle the question (Pandey and Shukla, 2009).

Meanwhile, case-control studies have been published mainly, Segura-López et al, (2015) reported that 44/103
cancer with (P < 0.01) and the odd ratios for bile duct or gallbladder cancer showed significantly higher positive rates for \( H. \) bilis (29%) subjects without biliary disease tested positive for cholecystitis tested positive for \( H. \) bilis. Only 4 out of 14 (29%) subjects without biliary disease tested positive for \( H. \) bilis among the Japanese. Bile duct and gallbladder cancer showed significantly higher positive rates for \( H. \) bilis than did the non-biliary diseases among the Japanese (P < 0.01) and the odd ratios for bile duct or gallbladder cancer with \( H. \) bilis in comparison with gallstone and / or cholecystitis tested positive for \( H. \) bilis. Only 4 out of 14 (29%) subjects without biliary disease tested positive for \( H. \) bilis among the Japanese. Bile duct and gallbladder cancer showed significantly higher positive rates for \( H. \) bilis than did the non-biliary diseases among the Japanese (P < 0.01) and the odd ratios for bile duct or gallbladder cancer with \( H. \) bilis in comparison with gallstone and / or cholecystitis were 6.50 (95%CI 1.09 - 38.63) in the Japanese and 5.86 (1.31 - 26.33) in the Thai patients. This study suggested that \( H. \) bilis infection in bile was associated with biliary tract and gallbladder cancers in the highly risk populations of Japanese and Thai. Furthermore, Bulajic et al (2002) reported the \( H. \) pylori and the risk of benign and malignant biliary tract disease and found that there was a strong association between the presence of \( H. \) pylori in the stomach and in the bile (P < or = 0.01). Biliary \( H. \) pylori was associated with age but not with gender, and it was associated strongly with the clinical diagnosis. Patients with gallstones were 3.5 times as likely to have \( H. \) pylori in the bile compared with patients in a control group [95%CI], 0.8-15.8; P = 0.100), and \( H. \) pylori was 9.9 times more frequent in patients with biliary tract carcinoma compared with patients in the control group (95%CI, 1.4-70.5; P = 0.022). There is a strong association between biliary tract carcinoma and \( H. \) pylori in bile. Recently, CCA has been suggested to be cause by infection with \( Helicobacter \) spp., such as \( H. \) bilis, and \( H. \) hepaticus. The pathogenicity of \( H. \) bilis and \( H. \) hepaticus has been studied in various animals infected experimentally or naturally, in which \( Helicobacter \) infection has been found to cause chronic active hepatitis, hepatocellular and biliary tract carcinoma, typhlocolitis, and lower bowel cancer. These infections can also promote the development of cholesterol gallstones and intrahepatic cholelithiasis, which is another risk factor for CCA. Multiple clinical studies have identified different \( Helicobacter \) spp. in the biliary tract, including \( H. \) pylori, and have associated these infections with the development of benign and malignant biliary diseases (Segura-López et al., 2015). The association of possible risk between \( Helicobacter \) spp. and biliary tract malignancy is included in Table 1.

### Helicobacter Species are Associated with Possible Increase in Risk of Cholangiocarcinoma

Sixth studies have been reported that there are CCA types which are associated to infect \( Helicobacter \) spp. Type of CCA is included one extrahepatic CCA (Segura-López et al., 2015), two intrahepatic CCA (Abu Al-Soud et al., 2008; Boonyanugomol et al., 2012), three CCA types which were associated to infect \( Helicobacter \) spp. Type of CCA is included one extrahepatic CCA (Segura-López et al., 2015), two intrahepatic CCA (Abu Al-Soud et al., 2008; Boonyanugomol et al., 2012), three CCA

### Table 1. Possible Risk of Helicobacter Species and Bile Duct Malignancy

| Type of bile duct malignancy | Helicobacter species | Increased risk | Number of recruited studies | References |
|-----------------------------|----------------------|---------------|----------------------------|------------|
| Extrahepatic CCA            | \( H. \) bilis        | 2.83(1.49, 5.32)\(^a\) | 103 cases and 91 controls (Case-control study) | Segura-López et al., 2015 |
| CCA                         | Helicobacter species | 8.88(3.67, 21.49)\(^a\) | 245 cases and 244 controls (Case-control study) | Xiao et al., 2014 |
| Intrahepatic CCA            | \( H. \) pylori, \( H. \) bilis | 744(2.19, 25.28)\(^a\) | 149 cases and 20 controls | Boonyanugomol et al., 2012 |
| Bile duct cancer            | \( H. \) pylori, \( H. \) hepaticus | 1.69(0.78, 3.66)\(^a\) | 58 cases and 167 controls | Shimoyama et al., 2010 |
| Intrahepatic CCA            | \( H. \) pylori, \( H. \) hepaticus | 11.20(2.16, 58.13)\(^a\) | 21 cases and 27 controls | Abú Al-Soud et al., 2008 |
| Common bile duct cancer     | Helicobacter species | 47.50(3.55, 363.17)\(^a\) | 11 cases and 23 controls | Kobayashi et al., 2005 |
| Bile duct cancer            | \( H. \) bilis        | 3.86(0.17, 87.65)\(^a\) | 17 cases and 6 controls | Murata et al., 2004 |
| CCA                         | \( H. \) pylori        | 15.36(0.70, 338)\(^a\) | 18 cases and 4 controls | Chen and Chen, 2003 |
| Bile duct cancer            | \( H. \) bilis        | 12.00(2.66, 54.19)\(^a\) | 18 cases and 4 controls | Matsukura et al., 2002 |
| CCA                         | \( Helicobacter \) spp | 95.67(4.69, 1950.42)\(^a\) | 24 cases and 20 control | Nilsson et al., 2001 |

\(^a\) Odds ratio

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**Conclusion**

Global epidemiological studies have suggested that *Helicobacter* spp. are possible risk of biliary tract diseases including CCA. The molecular studies from other investigators support the linkage of *Helicobacter* spp. and CCA. Up to the present, however, the results from different regions, material and method, subsite of cancer, and controls thus introducing heterogeneity, therefore, case control studies with larger sample size are required. The comparison between co-*Helicobacter* spp.-CCA in the countries with low and high incident of CCA is required to settle the question. Furthermore, clarify the variation and role of *Helicobacter* species in this CCA, including pathogenesis of CCA through enhanced biliary cell inflammation and proliferation.

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(Nilsson et al., 2001; Chen and Chen, 2003; Xiao et al., 2014), three bile duct cancer (Matsukura et al., 2002; Murata et al., 2004; Shimoyama et al., 2010; Boonyanugomol et al., 2012), followed by *H. bilis* (Matsukura et al., 2002; Murata et al., 2004; Boonyanugomol et al.; Segura-López et al., 2015), and *H. hepaticus* (Chen and Chen, 2003; Abu Al-Soud et al., 2008; Shimoyama et al., 2010) have been reported in each study. This reveals suggested that *Helicobacter* spp. are associated with possible increase in risk of CCA, particularly *H. and H. bilis*. The association of possible risk between *Helicobacter* spp. and CCA is included in Table 1.

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**Conclusion**

Global epidemiological studies have suggested that *Helicobacter* spp. are possible risk of biliary tract diseases including CCA. The molecular studies from other investigators support the linkage of *Helicobacter* spp. and CCA. Up to the present, however, the results from different regions, material and method, subsite of cancer, and controls thus introducing heterogeneity, therefore, case control studies with larger sample size are required. The comparison between co-*Helicobacter* spp.-CCA in the countries with low and high incident of CCA is required to settle the question. Furthermore, clarify the variation and role of *Helicobacter* species in this CCA, including pathogenesis of CCA through enhanced biliary cell inflammation and proliferation.

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