

## Association between *Helicobacter* spp. infections and hepatobiliary malignancies: A review

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cholangiocarcinoma, the gallbladder and the ampulla of Vater (collectively known as biliary tract cancers). These tumors account for approximately 13% of all annual cancer-related deaths worldwide and for 10%-20% of deaths from hepatobiliary malignancies. Cholangiocarcinoma (CCA) is a devastating disease that displays a poor survival rate for which few therapeutic options are available. Population genetics, geographical and environmental factors, cholelithiasis, obesity, parity, and endemic infection with liver flukes have been identified as risk factors that influence the development of biliary tract tumors. Other important factors affecting the carcinogenesis of these tumors include chronic inflammation, obstruction of the bile ducts, and impaired bile flow. It has been suggested that CCA is caused by infection with *Helicobacter* species, such as *Helicobacter bilis* and *Helicobacter hepaticus*, in a manner that is similar to the reported role of *Helicobacter pylori* in distal gastric cancer. Due to the difficulty in culturing these *Helicobacter* species, molecular methods, such as polymerase chain reaction and sequencing, or immunologic assays have become the methods of choice for diagnosis. However, clinical studies of benign or malignant biliary tract diseases revealed remarkable variability in the methods and the findings, and the use of uniform and validated techniques is needed.

**Key words:** *Helicobacter bilis*; *Helicobacter hepaticus*; *Helicobacter species*; Extrahepatic cholangiocarcinoma; Cholangiocarcinoma; Biliary tract cancer

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### Abstract

Hepatobiliary cancers are highly lethal cancers that comprise a spectrum of invasive carcinomas originating in the liver hepatocellular carcinoma, the bile ducts intrahepatic cholangiocarcinoma and extrahepatic

**Core tip:** Hepatobiliary cancers are highly lethal cancers which are difficult to diagnose early and for which few therapeutic options are available. Its etiological factors have been suggested to include chronic inflammation, obstruction of the bile ducts, and impaired bile flow, as well as infection with *Helicobacter* species, such

as *Helicobacter bilis* and *Helicobacter hepaticus*, in a manner that is similar to the reported role of *Helicobacter pylori* in distal gastric cancer. Population genetics, geographical and environmental factors, cholelithiasis, obesity, parity, and endemic infection with liver flukes have been identified as risk factors that influence the development of biliary tract tumors.

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## INTRODUCTION

Hepatobiliary cancers are highly lethal cancers that comprise a spectrum of invasive carcinomas originating in the liver [hepatocellular carcinoma (HCC)], the bile ducts intrahepatic cholangiocarcinoma (ICC) and extrahepatic cholangiocarcinoma (ECCA), the gallbladder and the ampulla of Vater (collectively known as biliary tract cancers)<sup>[1]</sup>. These tumors account for approximately 13% of all annual cancer-related deaths worldwide and for 10%-20% of deaths from hepatobiliary malignancies<sup>[2]</sup>. The incidence and mortality of CCA has increased dramatically in North America and Europe in recent decades<sup>[3,4]</sup>. The CCA prevalence in North America is heterogeneously distributed among different racial and ethnic groups, with the highest age-adjusted prevalence in Hispanics (1.22/100000) and the lowest in African Americans (0.17-0.50/100000)<sup>[2]</sup>. In 2012, the worldwide incidence of hepatobiliary cancer was 960552 cases, resulting in 888330 deaths, whereas in the United States, an estimated 39880 cases of liver or other biliary tract cancer were diagnosed, including approximately 24312 deaths from liver cancer or CCA and 3845 deaths from gallbladder cancer<sup>[5]</sup>.

In 2012, the worldwide mortality of gallbladder and biliary tract cancers was estimated to be 42813 cases, which represents 1.7% of deaths due to all cancers<sup>[5]</sup>. The regional prevalence of these tumors is highly variable; they are rare in most regions of Europe and North America but are exceptionally high in regions such as Chile, Thailand, Japan, central Europe, Northeastern India and Pakistan, with a 5-year survival rate of less than 10% in most cases. These contrasting rates are partially explained by differences in the prevalence of risk factors of this disease, such as population genetics, geographical and environmental factors, cholelithiasis, obesity, and parity, as well as endemic infection with liver flukes such as *Opisthorchis viverrini* or *Clonorchis sinensis*, which occurs in some regions of Asia<sup>[6-14]</sup>. Other important

factors affecting hepatobiliary carcinogenesis include chronic inflammation, obstruction of the bile ducts, and impaired bile flow<sup>[15,16]</sup>.

CCA is a devastating disease that displays a poor survival rate for which few therapeutic options are available. Although surgical resection has been considered to be the best option for localized CCA, local recurrence is very common because of persistent micro-metastatic disease in the regional lymph nodes or at the surgical margins<sup>[17]</sup>. Multiple studies have reported a survival rate of 20%-40% after 10 years for patients with resectable tumors localized to the hilar or distal third of the ductus choledochus, although the survival rate is decreased in advanced stages of this disease. Previous reports have concluded that tumor extension directly correlates to the mortality rate<sup>[2,18-24]</sup>. Another therapeutic option is liver transplantation, for which the reported 5-year survival rate is 38%-50%<sup>[17,25]</sup>. For patients with unresectable disease, the placement of a self-expandable biliary stent is a widely accepted palliative procedure because it improves the quality of life by improving biliary drainage and by reducing the frequency of cholangitis and its complications<sup>[26,27]</sup>.

The etiology of most of these tumors remains unknown, although the role of an infectious agent has been suggested, and studies of patients and animal models have indicated that *Helicobacter* species might play this role. Because clinical and experimental evidence for a role of *Helicobacter* spp. infection in the carcinogenesis of CCA has been accumulating in recent years, we considered it to be appropriate to review and summarize the relevant studies addressing the probable participation of *Helicobacter* species infection in the development of these tumors.

## EVIDENCE SUGGESTING A ROLE OF *HELICOBACTER* SPP. INFECTION IN CCA

CCA displays the histological characteristic of adenocarcinoma of the bile-duct epithelial cells, and it has been suggested that the progression to cancer is similar to that which is observed in the intestinal type of gastric cancer: from hyperplasia to metaplasia, dysplasia, and, ultimately, the development of adenocarcinoma. In agreement with this proposal, it has been suggested that CCA may be caused by infection with *Helicobacter* species, such as *Helicobacter bilis* (*H. bilis*) and *Helicobacter hepaticus* (*H. hepaticus*), in a manner that is similar to the reported role of *Helicobacter pylori* (*H. pylori*) in distal gastric cancer; in fact, *H. pylori* was classified as a type I carcinogen by the International Agency for Research on Cancer (IARC)<sup>[9-11,28-30]</sup>.

The enterohepatic *Helicobacter* spp., such as *H. bilis*, *H. hepaticus*, and *H. cholecystus*, have

been isolated from the human gallbladder, liver tissue and bile juice and are suggested to express genes that facilitate their colonization in this hostile environment, which is highly osmotic and filled with biliary salts. The pathogenicity of *H. bilis* and *H. hepaticus* has been studied using various animals infected experimentally or naturally, and *Helicobacter* infection has been found to induce chronic active hepatitis, hepatocellular and biliary tract carcinomas, typhlocolitis, and lower bowel cancer in susceptible strains of inbred and genetically engineered mice<sup>[29-39]</sup>.

*H. hepaticus* may establish a chronic infection in A/JCr mice by colonizing the lower bowel and, sporadically, the liver, particularly in the bile canaliculi<sup>[40]</sup>. This persistent infection caused chronic inflammation and necrosis in the hepatic parenchyma and portal triads, and infected animals exhibited oval cell, Kupffer cell, and Ito cell hyperplasia, hepatocytomegaly, and bile duct proliferation. Notably, hepatic adenomas were observed only in male animals. Thus, persistent *H. hepaticus* infection was associated with chronic proliferative hepatitis and hepatomas in exclusively male mice, suggesting that *H. hepaticus* may cause an increased risk for hepatic cancer. The constitutive androstane receptor (CAR) regulates the metabolic detoxification of endobiotics, and CAR knockout (KO) mice were used to examine tumor progression after *H. hepaticus* infection<sup>[41]</sup>. Although *H. hepaticus* infection induced chronic hepatitis in both wild type and CAR KO mice, the CAR KO mice exhibited increased numbers of liver lobes displaying dysplasia and neoplasia. The authors found that this enhanced tumor promotion was associated with decreased hepatic expression of the P450 enzymes Cyp2b10 and Cyp3a11, increased expression of Camp, and increased serum concentrations of chenodeoxycholic acid. Thus, liver tumor promotion in mice infected with *H. hepaticus* might be enhanced by impaired metabolic detoxification.

A study of aged hamsters detected liver infection with *Helicobacter* of the *H. Bilis* cluster<sup>[42]</sup>, and the infected animals exhibited predominant portocentric and, to a lesser extent, perivenular fibrosis and nodular dysplasia. Lesion formation was associated with chronic active portal/interface and lobular inflammation, along with significant portal hepatitis. These results suggest the participation of *H. bilis* in hepatobiliary disease in both animals and humans. Enterohepatic *Helicobacter* infection might also contribute to the development of cholesterol gallstones and intrahepatic cholelithiasis, as suggested by recent studies of animal models<sup>[12,13,39,43-52]</sup>.

The geographical distribution of *H. bilis* and other enteric *Helicobacter* species among the human population is unknown, although it is presumed that their prevalence is low in populations which display low risk for biliary duct malignancies and nonviral

hepatitis. Similarly, its increased prevalence may contribute to the unexplained increase in biliary cancer incidence in specific regions of East and South Asia<sup>[53]</sup>. Because of the difficulty in culturing these *Helicobacter* spp., molecular techniques, such as polymerase chain reaction (PCR) and sequencing, have become the methods of choice to identify these species in clinical specimens<sup>[54,55]</sup>.

### ***H. hepaticus***

In 1994, spiral bacilli were isolated from the liver, the colon and the cecum of A/JCr mice and were identified as a novel species of *Helicobacter* termed *H. hepaticus*<sup>[40]</sup>. *H. hepaticus* is a microaerophilic, 1.5- to 5- $\mu$ m-long, gram-negative, spiral-shaped bacterium that displays positive urease, catalase, and oxidase activities. It reduces nitrate, grows at 37 °C but not at 25 °C or 42 °C, is resistant to cephalothin and nalidixic acid but is sensitive to metronidazole, and as expected, exhibits resistance to bile. *H. hepaticus* must express genes that enable it to colonize the hostile, highly osmotic environment of the biliary duct. It lacks the genes expressed by *H. pylori* which are required for the colonization of the stomach, but it shares genes with *Campylobacter* spp. that may assist in the colonization of other enteric regions. The current availability of its genomic sequence provides investigators with the opportunity to examine the mechanisms underlying its tissue tropism and carcinogenesis<sup>[56]</sup>. *H. hepaticus* contains two flagella that are localized in a characteristic bipolar manner and possesses a flagellar sheath. These flagellar genes are closely related to those of *H. pylori*, and as in *H. pylori*, these genes are scattered throughout the chromosome, with few small clusters of functionally linked genes<sup>[57]</sup>. The *flaA* and *flaB* genes likely encode the major and minor flagellin subunits, which form the multimeric flagellar filament. One particular characteristic that is common to all of the *H. hepaticus* genomes analyzed is the presence of two identical copies of the *flaA* gene, including its promoter region (*flaA\_1* and *flaA\_2*), which are present at two separate locations in the genome<sup>[58]</sup>. The expression of flagella and their regulatory genes are essential for the motility required for the intestinal colonization of *H. hepaticus*. Motility and chemotaxis could play a predominant role in enabling these bacteria to locate their appropriate intestinal habitat, particularly during the initial steps of colonization<sup>[59]</sup>. Cytolethal-distending toxin (CDT) is a multimeric cytotoxin that displays nuclease activity which may contribute to the exposure of endogenous antigens to the immune system following infection with *H. hepaticus*. CDTs are a family of proteins that cause cell cycle arrest and cellular distention, leading to the enlargement of both cells and nuclei followed by chromatin fragmentation<sup>[46,57,58]</sup>. These CDTs are composed of three subunits: a catalytic subunit, CdtB, which displays DNase I-like activity, and CdtA

and CdtC, which bind to the cell membrane and deliver CdtB to the target cells<sup>[39,59]</sup>. The translocation of CdtB to the nucleus exerts genotoxic effects on the host DNA, triggering DNA repair cascades that induce cell cycle arrest and, ultimately, cell death. It was recently shown that *H. hepaticus* CDT plays a crucial role in promoting the progression of hepatitis to pre-malignant, dysplastic lesions *via* the activation of pro-inflammatory NF- $\kappa$ B and the increased proliferation of hepatocytes<sup>[60-63]</sup>. These results represent the first evidence that CDT displays carcinogenic potential *in vivo*. The urease enzyme is an important virulence factor involved in gastric colonization by *H. pylori* and *H. mustale*, although the contribution of urease to the development of *H. hepaticus*-associated disorders has yet to be demonstrated. Although urease expression by *H. hepaticus* is lower than that by *H. pylori*, its expression remains relatively high compared to that in other urease-positive pathogens. Active urease converts urea into ammonia and bicarbonate, and the produced ammonia mediates protection against acidic microenvironments and may also serve as a nitrogen source<sup>[64]</sup>. In contrast to *H. pylori*, the urease activity of *H. hepaticus* is not induced at acidic pH, and *H. hepaticus* does not grow or survive at pH 3.0. This finding suggests that the urease system is not a universal acid-resistance factor throughout ureolytic *Helicobacter* species and that its functions may differ between gastric and enterohepatic *Helicobacter* species; in fact, urease expression in *H. hepaticus* may respond to distinct environmental stimuli<sup>[64,65]</sup>.

The complete genomic sequence of *H. hepaticus* strain ATCC51449 was published in 2003<sup>[56]</sup>. This sequence consists of 1779146 bp and shares certain characteristics with those from *H. pylori*, *C. jejuni*, and other enteric bacteria, such as *V. cholerae* and *E. coli*. Thus, approximately 50% of *H. hepaticus* open reading frames (ORFs) have orthologs in the sequences of the *H. pylori* 26695 and J99 strains, and 50.8% have orthologs in the sequence of *C. jejuni* NCTC 11168. It was also found that 821 *H. hepaticus* proteins have the same ortholog in both *H. pylori* and *C. jejuni* and that 109 *H. hepaticus* proteins have orthologs in both *H. pylori* genomes but not in *C. jejuni*. In addition, 130 *H. hepaticus* ORFs have an ortholog in *C. jejuni* but not in *H. pylori*<sup>[40,57,58,66,67]</sup>. The *H. hepaticus* genome contains one large region and many small regions that differ from the rest of the chromosome with respect to their G + C content, suggesting that these regions may have been generated *via* horizontal gene transfer. The largest region is a 71 Kb region termed genomic island 1 (HHGI1), which displays a G+C content of 33.2% and contains 70 ORFs (HH0233-HH0302). Most genes in HHGI1 encode hypothetical proteins, although it also encodes three proteins that are homologous to the structural components

of the type IV secretion system, which is present in several *H. pylori* strains. HHGI1 also contains a gene that displays homology to *V. cholerae* *hcp*, which encodes a secreted protein that is co-regulated with *V. cholerae* hemolysin, and a gene cluster (HH244 to HH251) that displays significant homology to a cluster of genes of unknown function on the small chromosome of *V. cholerae* (VCA0107 to VCA0115) and on the *Yersinia pestis* genome. Additionally, the HH252 gene displays high similarity to the *IcmF* gene of *V. cholerae* and *Legionella pneumophila*, which encodes a protein that is involved in macrophage killing and bacterial conjugation. These data suggest that HHGI1 might be involved in the virulence of *H. hepaticus*<sup>[49,68]</sup>. The genome also contains a *katA* gene (HH0043), which encodes a catalase that is homologous to that of *Bordetella pertussis* (65.95%) and *H. pylori* (62.5%)<sup>[49]</sup>. *katA* may confer protection against hydrogen peroxide and other ROS produced as a result of the inflammatory reaction induced by *H. hepaticus* infection. In fact, there is evidence that the levels of 8-oxo-guanine and lipid peroxidation are significantly increased in *H. hepaticus*-infected mice and that these oxidized macromolecules increase with the duration of infection<sup>[60,69]</sup>.

The epidemiology of *H. hepaticus* infection is unknown; although the persistent infection of the lower intestine suggests that the fecal-oral route, particularly in mice considering their coprophagic habits, may transmit infection<sup>[40,48,70-72]</sup>. *H. hepaticus* is routinely cultured from the colon and the liver of mice suffering from various hepatobiliary disorders<sup>[31,40,67]</sup>. In contrast, attempts to culture these bacteria from specimens of patients suffering from various hepatobiliary disorders have largely been unsuccessful, although *H. hepaticus* infection has been detected *via* PCR or immunologic assays<sup>[6,13,14,28,29]</sup>. This difference suggests that characteristics such as the host hepatobiliary environment play a role in determining the adaptability and cultivability of this bacterium *in vitro*<sup>[72]</sup>.

### **Chronic inflammation as the mediator of tumor promotion by *H. hepaticus***

Similar to *H. pylori*, *H. hepaticus* can establish a persistent infection in its host, inducing chronic inflammation that may represent the primary risk factor of the progression to carcinogenesis<sup>[58]</sup>. Nod2-deficient mice challenged with *H. hepaticus* secrete these bacteria in feces for longer periods than wild type mice, and an increased number of *H. hepaticus* was detected in the terminal ilea, suggesting that Nod2 protects the host from intestinal colonization. However, intestinal pathology was not detected following infection of either WT or Nod2-deficient mice. In contrast, in mice exhibiting altered immune function, the typhlocolitis/colitis lesion can be severe, leading to rectal prolapse, weight loss, and

death<sup>[44,73]</sup>. Mice lacking IL-10 and infected with *H. hepaticus* developed spontaneous colitis, which was less severe when the mice were housed under specific-pathogen-free (SPF) conditions, suggesting enhanced susceptibility to pathogen-induced inflammation<sup>[69]</sup>. Inflammation was associated with a Th-1-related cytokine profile, including increased mRNA expression of IFN- $\gamma$  and IL-17 in the colon. These results indicate that *H. hepaticus* infection induces Th1 and Th17 responses in mice exhibiting an impaired anti-inflammatory response or IL-10 deficiency<sup>[74]</sup>. Early studies demonstrated that in regulatory T-lymphocyte-deficient mice, *H. hepaticus* induced colitis and the progression to colonic carcinoma, including neoplastic peritoneal invasion<sup>[75,76]</sup>. Recombinase-activating gene-2-deficient (*Rag2*<sup>-/-</sup>) mice, which lack functional lymphocytes, represent a useful model of chronic inflammatory bowel disease. Infection of *Rag2*<sup>-/-</sup> mice with *H. hepaticus* led to the accumulation of macrophages and neutrophils and the up-regulation of inducible nitric oxide synthase (iNOS) expression in tissue, along with increased nitric oxide (NO) production in the colonic wall. Increasingly severe inflammation led to metaplasia, hyperplasia, dysplasia, and cancer. Concurrent administration of an iNOS inhibitor prevented NO production and abrogated this epithelial pathology and the onset of cancer. The presence of Gr-1<sup>+</sup> cells and elevated TNF- $\alpha$  expression in the colon were also required for increased iNOS expression and for cancer development; alternatively, anti-inflammatory CD4<sup>+</sup> T<sub>reg</sub> lymphocytes and IL-10 down-regulated the expression of TNF- $\alpha$  and iNOS and suppressed cancer. Collectively, these results confirm the essential roles of elevated NO production and TNF- $\alpha$  expression in carcinogenesis in this experimental model<sup>[77,78]</sup>. Chronic *H. hepaticus* infection is characterized by the infiltration of neutrophils and macrophages in the mouse cecum and liver, where they secrete ROS that may contribute to tumor promotion by damaging DNA in hepatocytes or intestinal epithelial cells, as suggested by the increased levels of oxidized nucleoside 8-hydroxydeoxyguanosine (8-oxo-dG) in liver DNA after *H. hepaticus* infection<sup>[79]</sup>. Orogastric infection of C57BL/6 *Apc*<sup>Min/+</sup> (*Min*) mice with *H. hepaticus* rapidly promoted extraintestinal tumors in mammary tissue<sup>[45]</sup>, and breast cancer has been suggested to arise from intestinal bacteria-induced inflammation. The pro-inflammatory cytokine TNF- $\alpha$  plays a prominent role in this tumor model, as it participates in cancer progression in the bowel, the liver, and other sites in mice<sup>[49,80,81]</sup>.

### *H. bilis*

*H. bilis* is a gram-negative, opportunistic, non-sporulating, microaerophilic, fusiform bacteria that measures 0.5 nm in width and 4 to 5 nm in length.

This bacterium produces 3 to 14 bipolar-sheathed flagella and periplasmic fibers that surround the cell. Comparative analysis of its 16S rRNA sequences revealed that it displays a similarity of 98% to *H. cinaedi*, 97.4% to *H. hepaticus* and 93.4% to *H. pylori*<sup>[30,42,82,83]</sup>. It was originally isolated from the colonic crypts, the cecum, the bile and the liver of mice with hepatitis<sup>[30]</sup>, and because it has not been found in the stomach, it is also referred to as non-gastric *Helicobacter*. *H. bilis* is an enterohepatic *Helicobacter* species which is endemic in most mouse facilities that may induce disease in susceptible animals<sup>[84]</sup>. *H. bilis* infection has been associated with an increased incidence of typhlocolitis<sup>[85,86]</sup> inflammatory bowel disease<sup>[33]</sup>, hepatitis<sup>[30]</sup>, and cholecystitis<sup>[85]</sup>.

In humans, *H. bilis* has been associated with chronic liver disease<sup>[55]</sup>, biliary tract and gallbladder cancer<sup>[87,88]</sup>, chronic diarrhea<sup>[89]</sup>, and pyoderma gangrenosum-like ulcers<sup>[90]</sup>. *H. bilis* has not been cultured from human specimens, and it was first detected *via* PCR in the gallbladder and the bile of Chilean patients with chronic cholecystitis<sup>[91]</sup>. *H. bilis* antibodies have been identified in sera from patients with chronic liver disease and cirrhosis<sup>[88]</sup>, and *H. bilis* infection has also been demonstrated *via* PCR in patients with benign and malignant liver neoplasm-like hepatoma and HCC<sup>[36,37]</sup>.

In mice, *H. bilis* is known to be responsible for chronic hepatitis and hepatocellular tumors and may play a major role in cholesterol gallstone formation<sup>[30,54,87,88,92,93]</sup>. However, a direct association between *H. bilis* and bile duct cancer has yet to be established.

Due to the presence of enterohepatic *Helicobacter* in gallbladder carcinoma, this species has been considered as a cofactor that contributes to biliary carcinogenesis, although the mechanisms responsible for the processes that lead to cancer remain unclear<sup>[94]</sup>. Enterohepatic *Helicobacter* infection has been suggested to participate in the development of cholesterol gallstones and intrahepatic cholelithiasis, which may subsequently lead to carcinogenesis<sup>[54,95]</sup>. Its promotion of stone formation has been suggested to be due to its serving as a foreign body nest around which the stone develops, likely by producing hydrolyzing enzymes or nucleating proteins, such as immunoglobulins<sup>[54,96]</sup>. The risk for lithogenicity might also be associated with the modulation of enterohepatic cycling of conjugated bile acid, including the transit time through the gut<sup>[54,82,87,97-101]</sup>. It was recently confirmed that *H. bilis* infection activates NF- $\kappa$ B in bile duct carcinoma cells and increases the expression of the angiogenic factor VEGF, elevating the angiogenic potential of these cells *via* CRE-binding protein<sup>[83,97]</sup>. Recently, gamma-glutamyl transpeptidase (gGT) has been reported as a novel *H. bilis* virulence factor (HBgGT). The *H.*

*bilis* genome encodes two putative *gGT* sequences, one of which was found to be functionally active, inhibiting the activity of T cells and suppressing the proliferation of epithelial AGS cells, in a manner that is similar to *H. pylori* *gGT* (HPgGT)<sup>[102]</sup>. HBgGT has been implicated as an important regulator of inflammation in *H. bilis*-infected intestinal epithelial cells that may be responsible for the inflammatory disorders observed in experimental animal models<sup>[103]</sup>.

### **Clinical evidence for the role of *Helicobacter* spp. in biliary diseases**

Currently, multiple human clinical studies have associated *Helicobacter* spp. infection with biliary diseases. *H. pylori* has been suggested to infect the biliary tract and cause cancer. Although *H. pylori* DNA has been detected in human bile, whether this organism rarely colonizes the bile duct epithelium was not clearly documented. In this infection, carcinogenesis includes the activity of the virulence factor CagA, an oncoprotein that interferes with signal transduction pathways, and the host response to *Helicobacter* antigens in the form of cytokines and other inflammatory mediators<sup>[101,104,105]</sup>. However, based on the currently available evidence, inflammation followed by epithelial cell proliferation and interference with cell cycle *via* alterations in signal transduction appear to be the most plausible explanations of carcinogenesis<sup>[42,57,97,98]</sup>. As different *Helicobacter* spp. have been successfully identified in the biliary system, these organisms have been suggested to participate in the development of malignant and benign biliary diseases. A meta-analysis based on a systematic review of 18 studies published between 1998 and 2011 revealed significantly higher frequencies of *H. pylori* and *H. hepaticus* infection in patients with lithiasis<sup>[99]</sup>. Other studies have focused on patients with chronic liver disease, particularly cholelithiasis, or cholecystitis and demonstrated a significantly higher prevalence of *H. hepaticus* in samples from these patients than in samples from patients with other diseases<sup>[105,106]</sup>. Increased concentrations of anti-*H. Hepaticus* antibodies were found in sera from patients with liver disease compared to those suffering from other diseases, especially HBV- and/or HCV-infected patients<sup>[13]</sup>.

Based on a meta-analyses of 10 case-control studies that 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. The authors concluded that there is sufficient evidence to suggest a role of *Helicobacter* spp. in hepatobiliary tract cancers, although there was some variation in the results from different regions of the world and in the methods used to detect *Helicobacter*<sup>[96]</sup>. Another meta-analysis explored the association between the *Helicobacter*

species *H. pylori*, *H. bilis*, *H. hepaticus*, and *H. ganmani* and biliary tract cancer. The presence of *Helicobacter* spp. was determined *via* PCR or immunohistochemical analysis of specimens from bile and biliary tissues. A significantly higher pooled infection rate of *Helicobacter* spp. (predominantly *H. pylori* and *H. bilis*) was observed in the biliary tract of the cancer group ( $P = 0.0001$ ) and in the benign biliary disease group ( $P = 0.0001$ ) than in the asymptomatic group<sup>[53]</sup>. In another case-control study of patients with HCC, the authors searched for *Helicobacter* spp. in liver tissue. PCR detected *Helicobacter* spp. in 60.7% of the cases, whereas the controls displayed no infection ( $P < 0.01$ ), suggesting that *Helicobacter* spp. colonized the liver tissues of HCC patients<sup>[107]</sup>.

## **CONCLUSION**

The high mortality rates observed in biliary tract cancer demands further investigation to elucidate the etiology and carcinogenesis of these tumors. Population genetics, geographical and environmental factors, cholelithiasis, obesity, parity, and endemic infection with liver flukes (*Opisthorchis viverrini* or *Clonorchis sinensis*), particularly in certain regions of Asia, have been identified as risk factors that influence the development of biliary tract tumors. Other important factors in the carcinogenesis of these tumors include chronic inflammation, obstruction of the bile ducts, and impaired bile flow. CCA has been suggested to be caused by infection with *Helicobacter* spp., such as *H. bilis* and *H. hepaticus*, in a manner that is similar to the reported role of *H. pylori* in distal gastric cancer. 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. The availability of the entire genomic sequence of *H. hepaticus* has facilitated the identification of potential virulence factors and the elucidation of their mechanisms of action, including their participation in inflammation, cell proliferation and interference with the cell cycle.

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. The geographical distribution of enteric *Helicobacter* species has been suggested to be low in populations that display a low incidence of biliary duct malignancies. Because of the difficulty in culturing these *Helicobacter* species, molecular methods, such as PCR and sequencing, and im-

munologic assays have become the methods of choice for diagnosis. However, clinical studies of benign or malignant biliary tract diseases revealed remarkable variability in the methods and the findings, and the use of uniform and validated techniques is needed.

Because the biliary tract is only accessible *via* invasive procedures or surgery, it is necessary to develop PCR assay protocols, more suitable antigens for immunohistochemistry, and easy and effective serological methods for the early detection of *Helicobacter* spp. to help reduce the incidence of biliary diseases, as well as the morbidity and mortality of this group of patients.

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