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Microbiome dysbiosis in gallbladder cancer: A systemic review

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ABSTRACT

Gallbladder cancer (GBC) starts in the epithelial tissue (lining of the bile duct and gallbladder). It is a type of aggressive cancer called adenocarcinoma that can spread to other tissues. Among all cases of biliary tract cancer, 50% is from GBC. It is a deadly cancer with a survival rate of 17.6% between 2007 and 2013. GBC is rarely found in the Western world, but it is commonly found in South Asia. In Southeast Asian countries, GBC plays a significant role in cancer-related morbidity and mortality. GBC incidence exhibits marked regional variability, a rare condition in the western population but having a higher frequency in India, especially the Indo-Gangetic belt and some northeast districts excluding Nagaland. This might be attributed to the differences in environmental factors and genetic predisposition modulating carcinogenesis. In GBC, only 10% of cases are identified in the early stages. The low rate of early detection is due to the lack of screening techniques and the aggressive characteristics of the tumor. Various risk factors are associated with GBC, for example, chronic cholecystitis with or without gallstones, obesity, exposure to heavy metals such as lead and arsenic, bacterial infection, congenital biliary cysts, and abnormal pancreaticobiliary duct junction. The risk factors can cause chronic gallbladder mucosa irritation, leading to dysplasia and neoplasia. GBC can form metaplasia to dysplasia in a time span of 5–15 years, then to carcinoma *in situ*, and finally to invasive cancer. Dysbiosis is responsible for various diseases, including cancer. Multiple triggers can cause dysbiosis, for example, environmental changes, inflammation, infection, medications, dietary changes, or genetic predisposition. Various researches show that *Helicobacter pylori*, human papillomavirus, Hepatitis B virus, and Hepatitis C virus microbial species can cause cancer. They are the major species responsible for 90% of infection-associated cancers. Various studies demonstrate that the strains of *Salmonella* and *Helicobacter* colonize are linked to developing GBC. While the mechanisms linking gut microbiota to GBC are not fully understood, several studies have suggested a potential association. According to a study, certain gut microbiomes, such as *Fusobacterium nucleatum*, found glut in GBC tissues, compared to adjacent normal tissues. By evaluating gut microbiome dysbiosis, we can see the potential link between gut microbiome dysbiosis and GBC; it may provide valuable insights into the development and progression of GBC. It could lead to the identification of new diagnostic markers and the development of novel therapeutic strategies. In GBC, the evaluation of gut microbiome dysbiosis (involving evaluating the composition, diversity, and functional capacity of the gut microbiome in patients) has emerged as a promising method for understanding the molecular mechanisms and identifying biomarkers for early prevention and detection of GBC and also investigating the possibility of any link between the gut microbiome and host immune response. In conclusion, evaluating gut microbiome dysbiosis in GBC is a promising direction for identifying potential early detection and prevention biomarkers. Additional investigation is therefore needed to determine the role of gut microbiome dysbiosis in the development and progression of GBC and to identify reliable biomarkers for clinical use.

Key words: Microbiome; Gallbladder cancer; Dysbiosis; Gall stones; Inflammation; Infection

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INTRODUCTION

The gallbladder is a small and sac-shaped organ located under the liver. It is known as the biliary tree of the biliary track. It stores and concentrates bile produced by the liver and is released into the small intestine. The gallbladder and biliary system develop by the end of the 4th week of embryogenesis, and a structure called the hepatic diverticulum appears. It is involved in the digestion of food containing fat. The gallbladder has three sections: The fundus, body, and neck. It can vary in size and shape among different people. Anatomy of gallbladder and its relation to the hepatobiliary system can be seen in Figure 1.

Gallbladder cancer (GBC) epidemiology

GBC is one of the most common cancers found in Asian countries and Latin America. In Latin America, Bolivia and Chile are the two major countries where cases of GBC are high. According to GLOBOCAN 2022 data, GBC accounts for 0.612% of all global cancer diagnoses but 10.91% of all cancer deaths.[1] Number of the incidence of gallbladder cancer in both sexes can be seen in Figure 2.

GBC incidence

World

According to the World Health Organization (WHO) Absolute numbers, the incidence for both sexes in 2022 is 122,491; Bolivia and Chile are the major countries. Region grouping versus incidence of gallbladder cancer can be seen in Figures 3 and 5.

Asia

According to the WHO absolute number, the incidence of both sexes in 2022 is 88,095. Number of the incidence of gallbladder cancer in both sexes can seen in Figure 2.

China is in the top with 35.3% (31,132) cases, followed by India 24.7% (21,780), Japan (12.3%), Bangladesh (9.5%), Korea (3.7%), and Pakistan (3.2%).

India

GBC is a common cancer in the Indo-Gangetic belt and northeast India. GBC is seen more often in women. India contributes 24.7% of total cases in Asia. According to GLOBOCAN 2022, GBC is in 19th position with 21,780 new cases and 15th position by death due to cancer. The total number of prevalent cases in India in 5 years is 36,170. Age-standardized rate incidence in gallbladder cancer seen in Figure 4.

GBC risk factors

I. Gallstones
   Inflammation and gallstones (cholecystic) are the most common risk factors for GBC present (approximately 85%) of GBC patients.
II. Obesity
   People with body mass index >30 kg/m^2 are at higher risk of developing GBC.
III. Family history
   History of GBC in the family can increase the risk of developing GBC.
IV. Gallbladder polyps
   The extended growth of the gallbladder mucosal membrane may further develop into non-caners to cancers.
V. Smoking
   Smoking increases the risk of developing GBC.
VI. Bile duct abnormality
   Obstruction of the bile flow due to stones and cysts can increase the risk of GBC.
VII. Age
   GBC risk increases with advancing age.
VIII. Gender
   Women are more commonly affected (2–6 times) than males. Estrogen and progesterone receptor expression levels in GBC are similar between genders; females exhibit increased co-expression of both receptors, highlighting a potential treatment target.
IX. Alcohol
   Drinking alcohol can increase the risk of developing GBC; regular and high amounts of alcohol consumption increase the risk of developing GBC.
X. Diabetes
   People with diabetes at 45–50% are at higher risk for developing GBC.
XI. Infection
   A person with a bacterial (*Salmonella*) infection is at a clear risk of developing GBC.
XII. Ethnicity
   For different ethnicities, GBC is very around the world due to different factors.

CLINICAL PRESENTATION

Symptoms

The symptoms are often non-specific, with pain in the upper right side of the abdomen, abdominal bloating, loss of appetite, fever, weight loss, and obstructive jaundice.

The clinical diagnosis involves ultrasound sonography, computed tomography, magnetic resonance imaging, endoscopic retrograde cholangiopancreatography, magnetic resonance cholangiopancreatography, laparoscopic biopsy, and fine needle aspiration cytology.

Understanding the depth of the human gut microbiome

The term microbiome comes from two words: "Micro and biome," which denotes a specific microbial community

with different physical and chemical properties that inhibit clearly defined habitats.[2] The human microbiome, for example, gut bacteria, eukaryotes, archaea, and specific viruses, is responsible for diverse synthetic and metabolic processes and detoxifying various xenobiotics.[3] They can form bidirectional communication between the gut and brain ("gut-brain axis"). It regulates satiety and appetite, mood elevation, cognitive development, and neuroprotection.[4] Some studies have shown a link between gut microbiome and immune homeostasis. The development of the adaptive and innate immunity of the individual is a result of the host immune and gut microbiome bidirectional interaction.[5,6] The microbiota act as a first line of defense; it prevents the overgrowth of pathogenic bacteria and maintains mucosal integrity.[7] Hence, any imbalances in the gut microbiota are responsible for various autoimmune diseases. The increase of harmful bacteria (enterobacteria) is a result of "dysbiosis or an "imbalance" in gut microbiota. Various factors, such as dietary changes, inflammatory conditions, and exposure to drugs and toxins, are responsible for dysbiosis.[8,9] Microbiome dysbiosis in gallbladder cancer seen in Figure 6.

The gut microbiome has been linked to a wide variety of diseases, such as obesity, inflammatory bowel disease, irritable bowel syndrome, and some neuropsychiatric diseases like depression.[10] However, what fascinates researchers is the role of gut microbiota in developing GBC.

Process of carcinogenesis

Changing in cells at cellular, genetic, or epigenetic levels may lead to the development of cancerous cells by a process known as cell transformation.[11] Tumor protein 53 (TP53) is a gene that encodes a tumor suppressor protein, P53, found in chromosome 17. It plays a key role in maintaining genomic stability by regulating the cell cycle and DNA repair, and the cells that cannot repair undergo apoptosis. P53 is activated by conditions such as DNA damage, oncogene activation, or hypoxia; after the activation of P53, it can induce a cellular response, that is, cell cycle arrest, DNA repair, or apoptosis. Chronic inflammation is responsible for the alteration in the TP53 gene and is further responsible for the inactivation of the P53, which leads to cancer development.[12] Gallbladder cancer Figure 7. TP53 is the most commonly mutated gene, followed by PIK3CA, SMAD4, ARID1A, and KRAS.[13-15]

Role of gut microbiota in cancer development

Russell was the first person who gave the proposition of the role of gut microbiome in cancer in 1890. A group of scientists from NCI, USA, claimed in 1963 that the bacteria in the cancer tissues were probably contaminants.[16] Marshall

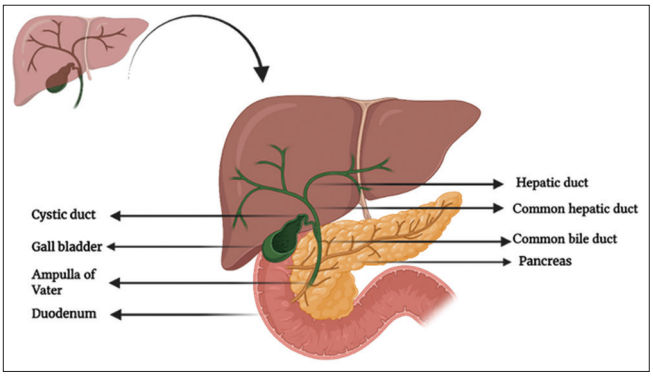

Figure 1: Anatomy of gallbladder and its relation to the hepatobiliary system (Image made using Biorender: www.bioender.com)

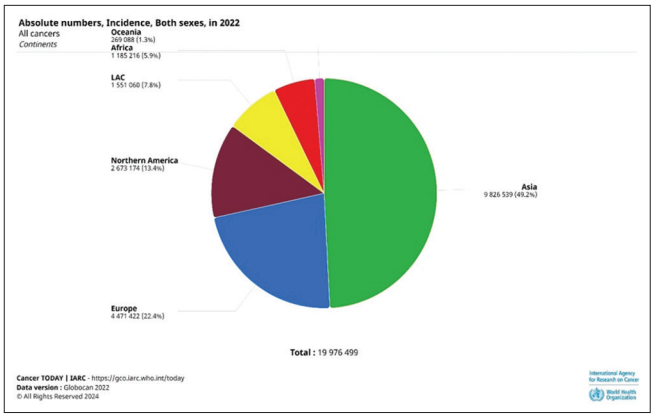

Figure 2: Number of the incidence of gallbladder cancer in both sexes (Bray et al., 2024)

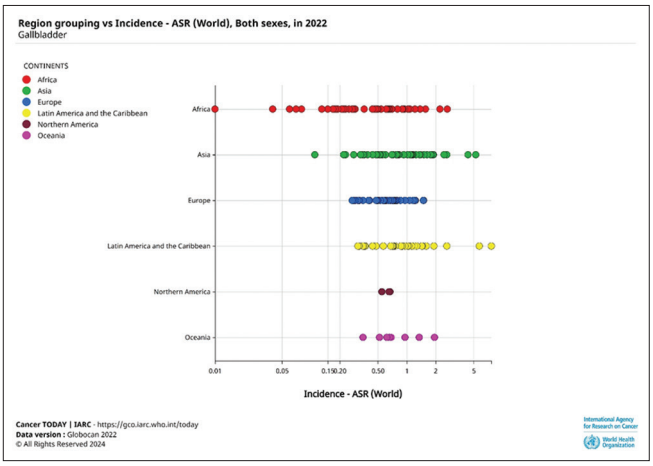

Figure 3: Region grouping versus incidence of gallbladder cancer (Bray et al., 2024)

and Windsor later solved this controversial statement; his study defined the association between *Helicobacter pylori* and gastric adenocarcinoma.[17] After that, different studies found that many bacteria are associated with different carcinogenesis.[18,19]

Microbiota is a carcinogen through chronic inflammation

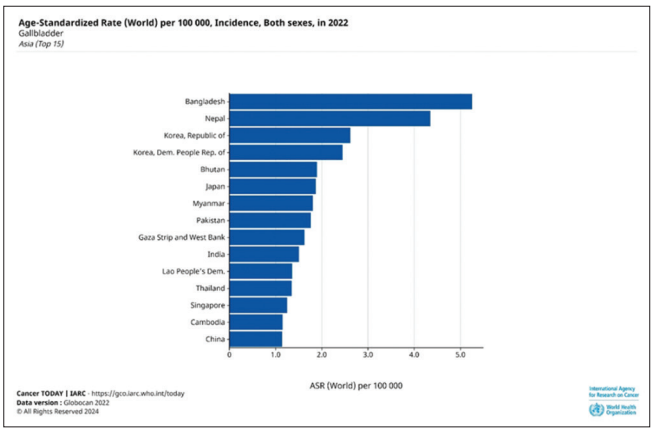

Figure 4: Age-standardized rate incidence in gallbladder cancer (Bray et al., 2024)

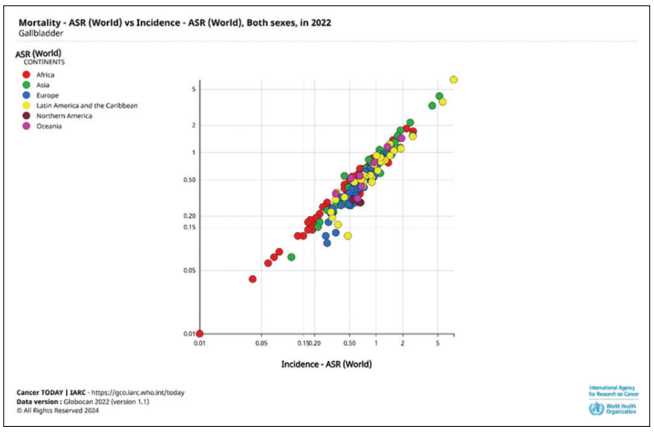

Figure 5: Age-standardized rate, mortality versus incidence (Bray et al., 2024)

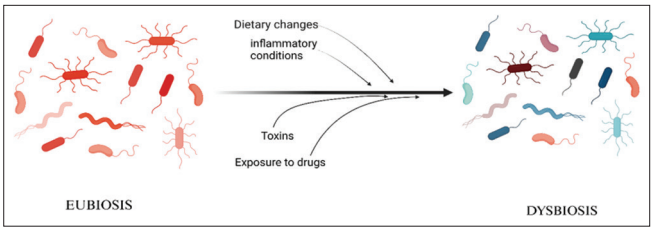

Figure 6: Microbiome dysbiosis in gallbladder cancer

and direct injury by toxins and metabolites.[16,20,21] The generation of reactive oxygen species and proinflammatory mediators such as tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-1 stimulates lymphoepithelial proliferation and cell division. This leads to immune dysregulation, resulting tumorigenesis.[5] It causes changes in the cell cycle, directing immunosuppression.[22] The bacterial toxin can cause DNA damage, which can cause cancer and, thus, chronic bacterial infection. Thus, chronic bacterial infections play a dual role in carcinogenesis by stimulating and inhibiting the immune system. Chronic bacterial infection plays a dual role in carcinogenesis by inhibiting and stimulating the immune system.

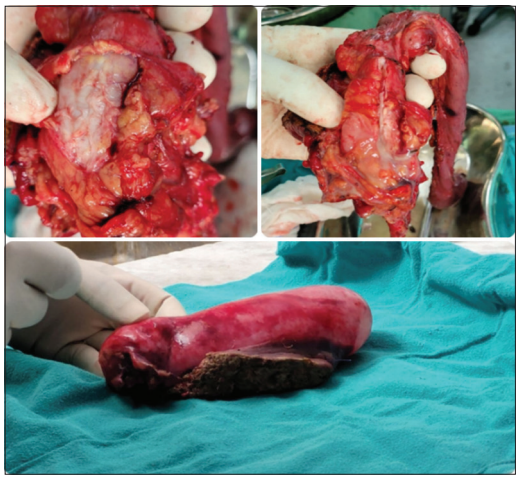

Figure 7: Gallbladder cancer. Mass removed post-surgery

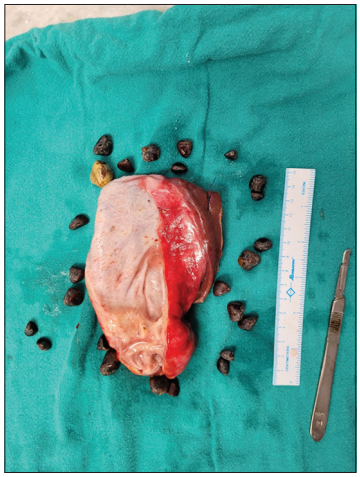

Figure 8: Gallbladder with gallstones

Significance of the biliary microbiome

In the earlier biliary tract, it is considered sterile due to its antimicrobial properties. Recent studies show the diversity of microorganisms in the bile involved in its regulation, composition, and metabolism.[23] Gut microbiome dysbiosis is involved in developing and progressing various cancers such as gastric,[24,25] colorectal,[26] and oral cancers.[27,28] The intrinsic connection between microbiome and bile acid metabolism indicates that dysbiosis could contribute to biliary tract disorders such as gallstone formation and cancer development. Gallbladder with gallstones can be seen in Figure 8.

Mechanistic pathways of biliary tract carcinogenesis

Cholangiocytes are known as the cells of the origin for biliary tract cancer, including GBC.[29] Cholangiocytes release proinflammatory mediators such as IL-6 and IL-1β, prompting the differentiation of T helper cells, cholangiocytes recognized bacteria by pathogen-associated molecular pattern present in the bile; this interaction occurs with recognition receptors such as Toll-like receptors and

NOD-like receptors, prompting their activation.[30] Resulting in fibrosis and deposition of collagen, IL-17, TNF-α, and transforming growth factor-beta are responsible for causing genetic alteration in tumor suppressor genes and oncogenes, resulting in cell transformation.[31]

Association between GBC and enteric bacteria

In biliary tract cancer, the gallbladder is the most common cancer. GBC has significant geographical variation. In the southern districts of northeast India, cases of GBC are very high, with a poor survival rate. Only 10% of cases of GBC are detected early due to a lack of screening techniques and aggressive tumor properties. Gallstones chronic inflammation plays a key role in the development of GBC.

Various culture-independent methods show organisms linked to the formation of gallstones (pigmented and cholesterol).[32,33] Enteric bacteria is one of the dysbiosis organisms that can form biofilm through resisting cellular and DNA damage caused by bile, for example, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus* spp., *Acinetobacter* spp.,[34-36] *Clostridium*, *Bifidobacterium*, *Peptostreptococcus*, *Bacteroides*, and other bacterial spp. They are responsible for the formation of gallstones by application of polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis, a range of uncultivable bacteria, such as *Staphylococcus haemolyticus*, *Enterobacter* or *Citrobacter* spp., *Morganella* spp., *Salmonella* spp., *Capnocytophaga* spp., *Lactococcus* species, *Bacillus* spp., and *H. pylori*, found in varying compositions.[37,38] Some studies have shown positive cultures of enteric bacteria in GBC patients, indicating a direct association of the gut flora with GBC; however, evidence is low.[34] *Helicobacter* species and *Salmonella* typhi have been significantly studied and are vigorously implicated in the formation of GBC.[39,40]

Correlation between GBC, Salmonella Typhi, and Helicobacter species

By the mechanism of inflammation-induced tumorigenesis, *H. pylori* is responsible for the causation of gastric as well as intestinal cancers, now associated with the development of hepatobiliary cancers.[41] *H. pylori* releases proinflammatory mediators such as TNF-α, IL-1, and IL-6 by its ability to induce a chronic inflammatory state.[42] They also inhibit cell adhesion and guide the migration of cells.[43] Production of IL-8 promotes inflammation and causes changes in cellular proliferation and apoptosis.[44] Cag-A protein is an extensively studied virulence factor secreted by *H. pylori* responsible for causing chronic inflammatory states and increasing the risk of gallstones.[33] Kawaguchi et al., were the first to discover *H. pylori* in the gallbladder mucosa.[45] Kuroki et al.,[46] showed a higher biliary epithelial proliferation rate in patients infected with *Helicobacter* species compared to the control group. In this study, bacterial isolation is done using nucleic acid isolation. Dewhirst and Fox[47] identified five strains of *Helicobacter bilis*, two strains of Flexisipira rapini, and one strain of *Helicobacter pullorum* using PCR analysis in patients with gallbladder diseases and GBC. Various studies have shown high positivity of *H. bilis* in patients with biliary tract and GBC, suggesting an association of *H. bilis* with GBC.[48-50] Several meta-analyses link *Helicobacter* infection and biliary tract cancer.[39] Studies limited to observational research or small-scale meta-analyses show the imperative for higher-tier evidence to establish a definitive consensus.

One of the Gram-negative, bacilli, flagellated bacteria known as *Salmonella enterica* serovar Typhi is responsible for typhoid fever. It inhibits the gallbladder and is responsible for the chronic inflammation leading to the formation of gallstones.[51] *Salmonella* Typhi produces biofilm, which protects against antibacterial response, resulting in a chronic state of the gallbladder, several studies have shown the potential link between GBC and *Salmonella* Typhi.

The toxin produced by *Salmonella* Typhi is carcinogenic and can cause alteration in the cell cycle and DNA damage.[52] *Salmonella* produced a protein called AvrA, which belongs to pathogenicity Group 1 through the Type III secretion system. It dampens the host's inflammatory response and inhibits autophagy, thereby enabling bacterial persistence and the development of a chronic carrier state. Cases of GBC and typhoid are common in Gangetic and northeast regions of India, which strongly links GBC and *Salmonella* Typhi.

A study conducted in north India shows patients with GBC exhibited elevated levels of Vi-polysaccharide compared to the control group; the risk of developing GBC among patients with typhoid carrier was found to be 8.47 times higher than in non-carriers, leading to the identification of the chronic typhoid carrier state as a risk factor for GBC; these finding further supported by different studies. However, evidence is deficient for the link between GBC and *Salmonella* Typhi; data are minimal. Table 1 summarizes the various studies showing an association of gut microbiota with GBC.

Further research is needed to elucidate the exact role of the gut microbiome in GBC development and progression and to identify reliable biomarkers for clinical use.

Table 1: Sharma et al., 2022

Study	Sample	Bacterial strain	Isolation technique	Inference
Welton et al.,[53]	Deceased typhoid carriers	*Salmonella* Typhi	Record registers	Chronic typhoid patients are 6 times more likely to die of hepatobiliary cancer than controls (P<0.001)
Caygill et al.[54]	Chronic typhoid carriers	*Salmonella* Typhi	Record registers	167-fold higher risk of GBC in chronic typhoid carriers. Chronic infection is a risk factor for GBC
Csendes et al.[55]	Tissue, bile	*Escherichia coli*, *Enterococcus faecalis*, *Klebsiella* Enterobacter	Culture	Both aerobic and anaerobic Gram-negative bacteria were found and may have a link to GBC.
Shukla et al.[56]	Serum	*Salmonella* Typhi	IHA Vi antigen	Significantly high Vi positivity in patients with gallbladder carcinoma compared to controls. The risk of developing GBC is 8.47 times higher in culture-positive typhoid carriers than the non-carriers
Dutta et al.[57]	Serum	*Salmonella* Typhi	ELISA Vi antigen	Chronic typhoid carrier state is a risk factor for GBC
Dewhirst et al.[58]	Multiple sources	*H. bilis*, Flexisipira rappini, *H. pullorum*	PCR (16S rRNA)	Correlation of *Helicobacter* species with GBC and other biliary tract diseases. Identified five strains of *H. bilis*, two strains of Flexisipira rapini, and one strain of *H. pullorum*
Matsukura et al.[59]	Bile	*H. bilis*	PCR (16S rRNA)	*H. bilis* infection in bile was associated with gallbladder cancer in Japanese and Thai patients
Fukuda et al.[60]	Bile, tissue	*Helicobacter*	PCR, histology, IHC	Significantly higher positivity of *Helicobacter* DNAs in 52.6% of patients with hepatobiliary cancer than that in the benign cases (P=0.03)
Lu et al.[61]	Tissue	Colibacillus *Bacteroides fragilis*, *Klebsiella*, *Clostridium perfringens*, *Clostridium*	PCR 16S rRNA	Possible association of both aerobic and anaerobic bacteria with GBC
Murata et al.[62]	Tissue	*H. bilis*	Nested PCR (16S rRNA)	4 out of 14 cases with biliary tract cancer were positive for *H. bilis*, which may indicate their role in GBC
Kobayashi et al.[63]	Bile	*H. pylori*, *H. hepaticus*, *H. bilis*	PCR	*Helicobacter* DNA was found in the bile of 86% of malignant biliary diseases. DNA fragments of *Helicobacter* species other than *H. pylori*, *H. hepaticus*, and *H. bilis* were commonly detectable
Bohr et al.[64]	Tissue	*Helicobacter* spp.	Culture, IHC, PCR (16S rRNA)	*Helicobacter* species do not play a predominant role in the German population's pathogenesis of GSD and GBC.
Shimoyama et al.[65]	Blood	*H. hepaticus*	ELISA	*H. hepaticus*-specific antigen was significantly higher in patients with biliary tract cancer (P<0.05)
Iyer et al.[66]	Tissue	143 HPV *Salmonella* Typhi Ty2 *Salmonella* Typhi CT18 *Salmonella* Typhimurium-LT2 *Salmonella choleraesuis*-SCB67 *Salmonella* Paratyphi-TCC *Salmonella* Paratyphi SPB7	PCR analysis	Association of non-typhoidal *Salmonella* species with GBC along with typhoidal strains. Chronic carrier state is a risk factor for GBC.
Tsuchiya et al.[67]	Blood	*H. pylori*	ELISA	No significant differences in antibody titers or *H. pylori* infection positivity rates between cases and controls
Song et al.[68]	Tissue	*Peptostreptococcus stomatitis Enterococcus faecium*	DNA extraction and metagenomic sequencing	The existence of an altered microbiota in GBC

GBC: Gallbladder cancer, IHA: Indirect hemagglutination test, ELISA: Enzyme-linked immunosorbent assay, *H. bilis*: *Helicobacter bilis*, *H. pullorum*: *Helicobacter pullorum*, PCR: Polymerase chain reaction, rRNA: Ribosomal ribonucleic acid, IHC: Immunohistochemistry, *H. hepaticus*: *Helicobacter hepaticus*, *H. pylori*: *Helicobacter pylori*, GSD: Glycogen storage diseases, HPV: Human papillomavirus

CONCLUSION

Gut microbiome dysbiosis may lead to GBC by altering the local microenvironment through bacterial-produced metabolites and small molecules.

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