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EDITORIAL

Role of bacteria in carcinogenesis, with special reference to carcinoma of the gallbladder

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Abstract

Carcinoma of the gallbladder (CaGB) is the fifth commonest gastrointestinal tract cancer and is endemic in several countries. The interplay of genetic susceptibility, infections, and life style factors has been proposed to be responsible for carcinogenesis of gallbladder. Persistence of infection leading to chronic inflammation, and production of certain toxins and metabolites with carcinogenic potentials, by certain bacteria has been speculated to be involved in the transformation of the gallbladder epithelium. Therefore, any bacteria that have evolved to acquire both of the above carcinogenic mechanisms can cause cancer. *Salmonella typhi* has been found to be prominently associated with CaGB. Chronic typhoid carriage (persistence) and production of mediators of chronic inflammation and a genotoxic toxin (cytotoxic distending toxin, CdtB) are also known for this bacterium. Furthermore, the natural concentrating function of the gallbladder might amplify the carcinogenic effect of the mediators of carcinogenesis. In addition to *S. typhi*, certain species of *Helicobacter* (*H. bilis* and *H. hepaticus*) and *Escherichia coli* have also been implicated in carcinogenesis. As the isolation rate is very poor with the presently available culture techniques, the existence of bacteria in a viable but non-cultivable state is quite likely; therefore, sensitive and specific molecular techniques might reveal the etiological role of bacterial infection in gallbladder carcinogenesis. If bacteria are found to be causing cancers, then eradication of such infections might help in reducing the incidence of some cancers.

Key words: Bacteria; Chronic inflammation; Carcinogen; Bacterial toxins; Carcinoma gallbladder; DNA damage

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INTRODUCTION

Carcinoma of the gallbladder (CaGB) is the fifth commonest cancer of the gastrointestinal tract and it is one of the commonest biliary tract (BT) malignancies[1]. Although, gallbladder cancer was described as early as 1777[2], for the majority of patients late diagnosis and lack of effective treatment is a typical feature of the disease even today[2-4]. Carcinoma of the gallbladder is an aggressive disease with dismal prognosis and has marked ethnic and geographical variations in incidence. CaGB is more common in females than males[5], except in Far East Asian countries like Japan and China. The highest documented incidence rate was shown in women from

Delhi, India (21.5/100 000); followed by Karachi, Pakistan (13.8/100 000), and Quito, Equador (12.9/100 000)[5]. High incidence rates have also been reported from Far East Asia, Eastern Europe, South America, and Spain (Granada), the incidence of CaGB has also been reported to be high. However, the incidence rate of CaGB in North Europe and North America has been observed to be quite low (< 3/100 000)[6].

A number of factors, such as genetics, infections, and life style have been reported to be associated with CaGB. Genetic etiology might be more important in Japan, Korea, and China where the sex ratio for CaGB is close to unity. This cancer has also been linked with certain genetic disorders, such as multiple familial polyposis/Gardener syndrome[7], Peutz-Jegher syndrome[8], porcelain gallbladder[9], and anomalous pancreatico- biliary ductal union[10]. The interplay of genetic susceptibility, infections, and life style factors in gallbladder carcinogenesis is still poorly understood[6]. Despite recent insights into the possible mechanisms involved in biliary carcinogenesis, the key events and specific links in this multistage cascade that leads to transformation of gallbladder epithelial cells remain unknown and deserve further investigation. In this review, we have specifically focused on the association of chronic bacterial infection with CaGB.

CHRONIC MICROBIAL INFECTIONS AND CARCINOGENESIS

When bacteria were discovered to be the cause of many infectious diseases, it was accepted that cancer does not behave as an infectious or contagious disease. Thus, the notion of involvement of bacteria in carcinogenesis was rejected. In 1890, Russel[11] for the first time on the possibility of bacteria-induced carcinogenesis. A few years later, Thomas Glover[12] in 1926 stated that specific bacteria could be isolated consistently from neoplastic tissues. In 1931, Hodgkin's disease was found to be associated with acid fast bacteria[13]. Later, in 1941, George Mazet[14] reported that both leukemia and Hodgkin's diseases were consistently associated with bacteria. From 1936-1955, Crofton[15], Livingston *et al*[16], and Villesquez[17] also reported the presence of microbes in cancer tissues. In 1953, White[18] made a claim that antiserum raised against cancer bacteria had a protective effect. Diller[19] in 1953 reported the isolation of extremely polymorphic bacteria from cancer tissues.

However, in 1963, a group of scientists from National Cancer Institute (NCI), USA, rejected the hypothesis of association of bacteria with carcinogenesis. These bacteria were considered simply either as contaminants or having secondarily infected the cancer growth. In addition, this hypothesis could not withstand Koch's postulate. In contrast, a few years later (1969), another group of scientists from NCI, USA, reported positive associations between bacteria and cancers. Barile *et al*[20] in 1965 observed latent infection of Mycoplasma in leukemia cases. Later, Lo[21] in 1992 reported multistage malignant transformations due to Mycoplasma infection, which could be reversed by antibiotic therapy. The best studied relationship between bacterial infection and cancer is that of *Helicobacter pylori* implicated in two different forms of gastric cancers: MALT lymphoma and gastric adenocarcinoma[22]. *Streptococcus bovis* has been implicated in colon cancer, *Chlamydiae pneumonie* in lung cancer and *Bartonella species* in vascular tumor formation[22-25].

Certain animal studies have shown the involvement of *Helicobacter hepaticus* in chronic active hepatitis that progressed to hepatocellular carcinoma in A/JCr mice[26]. Chronic infection with *Citrobacter rodentium*, a mouse pathogen, which is genetically similar to enteropathogenic *Escherichia coli*, can result in colon cancer[27]. Recently, *H. hepaticus* has been stated to promote cancer formation indirectly in the mammary gland of mice[28].

For many years, chronic inflammation has been reported to be associated with a variety of epithelial malignant tumors. Chronic osteomyelitis has been found to be associated with the development of squamous cell carcinoma along the draining sinus of osteomyelitis[29], and chronic inflammatory bowel disease has been associated with increased risk of development of adenocarcinoma[30,31]. *Schistosoma haematobium,* a water-borne parasite that causes a secondary bacterial infection of the urinary tract due to its persistence, is an important cause of squamous cell carcinoma of the urinary tract in the Middle East and Northern Africa[32]. Furthermore, increase risk of developing cancer of the urinary bladder due to chronic inflammation has been confirmed by epidemiological data and by animal experiments[33-35].

MECHANISMS OF BACTERIAL CARCINOGENESIS

Understanding bacteria-induced carcinogenesis might enable us to prevent and cure some forms of cancers[36]. The involvement of bacteria in carcinogenesis is still not without controversy because no clear agreement has been achieved on the molecular mechanism/s by which they might promote carcinogenesis. In the 21st century, scientists started hypothesizing that: (1) Chronic inflammation caused by persistent bacterial infections might lead to carcinogenesis[37-39]; and (2) Bacterial toxins and secondary metabolites produced by the chronic bacterial infection might induce carcinogenesis[37].

Chronic inflammation due to bacteria and carcinogenesis

Bacterial infections are usually believed to cause acute disease, but it has now been accepted that many bacteria can cause chronic infections and diseases, including cancers[37,40].

There may be various mechanisms of carcinogenesis induced by chronic bacterial infections (Table 1[41-50] and Figure 1). Continuous release of mediators of inflammation is a common feature of chronic infections[37,38]. The nuclear factor-κB (NF-κB) family of transcription factors are linked to inflammation driven carcinogenesis[38]. The NF-κB activation pathway is triggered by microbial infections and also by proinflammatory cytokines, such as TNF-α and IL-1. This pathway leads to activation of IKK

Table 1 Chronic inflammatory mechanisms involved in carcinogenesis

Signaling	Sub categories	Role in inflammation assumed cancer
Pro-inflammatory cytokines and immunosuppressant cytokines	ILS: Pro-inflammatory (IL-1, IL-6, IL-8, IL-17); immunosuppressor (IL-10); TNF-α plays dual role in carcinogenesis, usually it is tumor promoter	Over expressed in inflamed and hyperplastic, metaplastic tissues and adenocarcinoma; Induce DNA damage; Pro-angiogenic molecule such as VEGF, VEGFR, IL-8, NO, ICAM-1 VCAM-1; Activation of pro-inflammatory signals mediated *via* JAK-STAT and NF-κB; Maintain inflammatory tumor microenvironment; Stimulate cell proliferation and inhibit apoptosis
Chemokines	Four major groups: CXC, CC, XC, CX3C (primary function is to recruit leucocytes at the site of inflammation)	Responsible for attraction to inflammatory and immune cells to tumor microenvironment; Promotion of tumor cell migration, facilitation of invasion and metastasis; Stimulation of inflammatory angiogenesis
COX-2 and prostaglandins	An inducible form of cyclooxygenase, serves as interface between inflammation and cancer[41-44]	Causes promotion of: cellular proliferation, suppression of apoptosis, enhancement of invasiveness, angiogenesis
iNOS	Expression of iNOS is elevated in various precancerous lesions and carcinomas[45]	Elevated in precancerous and cancerous lesions and cause: DNA damage by nitrosation/oxidative pathways; Produce proinflammatory mediators like NO by catalyzing Arginin metabolism; Acts as a downstream effector of NF-κB and inflammatory cytokines mediated signaling
NO	Elevated in precancerous and cancerous lesions[46]	Selects mutant p53 cells and contribute to tumorigenesis by upregulating VEGF; DNA damaged by nitrosation of nucleotide bases
NF-κB (The NF-κB/Rel family of proteins includes CRel, RelA (p65), RelB, NF-κB1 (p50/100), NF-κB2 (p52/p100)[47]	One of the DNA binding proteins that are aberrantly activated in response to inflammatory stimuli leading to induction of transcription of various proinflammatory genes in tumor cells[48]	Enhances expression/production of proinflammatory mediators: Amplifies inflammation signal transduction; Increased expression of anti-apoptotic protein; Help transformed cells to escape apoptosis
ErbB2 (a receptor strongly involved in carcinogenesis)	Inflammation induces the expression[49-50]	Binding of ErbB1 and ErbB2 to ligands results in prolong activation of intrinsic protein kinase activity, leading to activation of a biochemical cascade responsible for mitogenic cell signal transduction

ILS: Interleukins; IL: Interleukin; TNF: Tumor necrosis factor; CC: Chemotactic cytokine; NF-κB: Nuclear factor-κB; VEGFR: Vascular endothelial growth factor receptor; iNOS: Inducible nitric oxide synthetase; NO: Nitric oxide; VCAM-1: Vascular cell adhesion molecule 1; ICAM-1: Inter-cellular adhesion molecule 1.

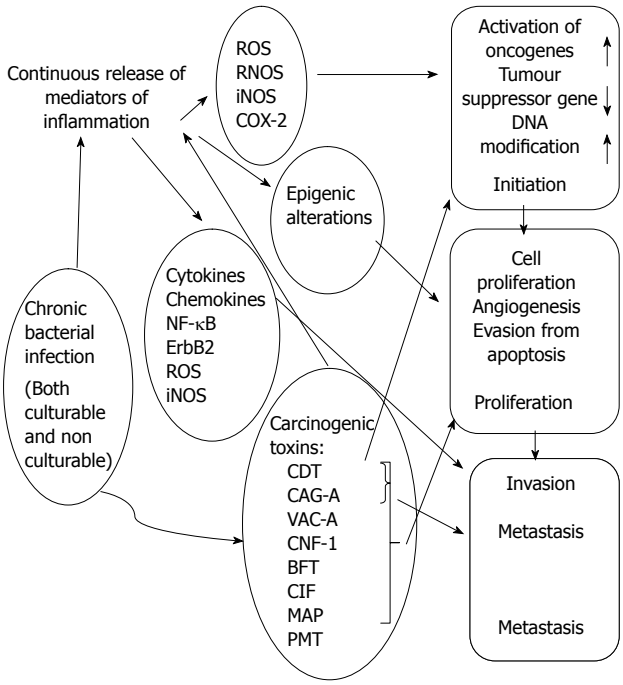

Figure 1 Mediators of inflammation and bacterial toxins in carcinogenesis caused by bacteria. ROS: Reactive oxygen species; RNOS: Reactive nitrogen oxide species; iNOS: Inducible nitric oxide synthetase; NF-κB: Nuclear factor-κB; CDT: Cytolethal distending toxin; CNF: Cytotoxic necrotizing factor; BFT: Bacteroides fragilis toxin; CIF: Cycle inhibiting factor; MAP: Mitochondrial associated protein; PMT: Pasturella multocida toxin.

complex[51] and to degradation of NF-κB inhibitors, thus freeing NF-κB to enter the nucleus and mediate transcription of target genes. Many of genes, such as cyclin D1, CDK2 kinase, c-myc (cell cycle regulators), involved in cell cycle control are upregulated, while the genes responsible for decreased apoptosis, such as p21, p53 and pRb, are downregulated by NF-κB. NF-κB also upregulates numerous cytokines, such as IL-1β, IL6, VEGF (proinflammatory and proangiogenic), but downregulates TNF, thus enhancing tumor growth. Genes responsible for invasion and metastasis are also upregulated by NF-κB. Moreover, downregulation of genes involved in suppression of apoptosis, i.e. Bcl-2 family members and IAP proteins, an important feature of cancer cells, is mostly due to deregulation by NF-κB. NF-κB not only helps in persistence of intracellular as well as extracellular infections, but also leads to suppression of cell death; thus creating a niche for bacterial survival defying the host immune response[52,53]. Survival of such partially transformed cells provides a chance for a higher level of transformation. Reactive oxygen species (ROS) and nitric oxide (NO) are produced by epithelial cells in response to inflammation. These compounds increase mutations in genes responsible for controlling malignant transformations. In particular, ROS can inhibit tyrosine phosphatases, causing overexpression of Mox1 (the catalytic subunit of NADPH oxidases). NO inhibits the Fpg protein, a DNA repair enzyme[54], leading to failure of damage control. HER-2/neu (also known

Table 2 Bacterial toxins and their possible roles in carcinogenesis

Toxin	Source	Activity and outcome
Potential genotoxins		
CDT (three subunits: CdtB is a functional unit, while CdtA and CdtC serve as accessory units for delivery into target cells)	*Haemophilus ducreyi, Helicobacter hepaticus, Salmonella typhi. Actinobacillus actinomycetemcomitans*	DNAase; DNA damage and cell cycle inhibitor[56,57]
Cytolethal distending toxin B	*Salmonella typhi*	DNAase activity, genotoxic by creating DNA lesions[58]
Colibactin	*Escherichia coli*	Mechanism unknown[59]
Potential pro-carcinogenic toxins		
Pasturella multocida toxin	*Pasturella multocida*	Modifies Gq proliferation[60]
CagA	*Helicobacter pylori*	Binds to SHP2 and c- Met cells scattering[61]
Vacuolating cytotoxin A	*Helicobacter pylori*	Upregulation of VEGF expression (seems to depend on the activation of EGFR, MAP kinase and COX-2 mediated)
Bacteroides fragilis toxin	*Bacteroides fragilis*	Cleaves E- cadherin proliferation[62]
Cytotoxic necrotizing factor-1	*Escherichia coli, Shigella dysenteriae, Campylobacter jejuni and Salmonella typhi, Helicobacter hepaticus, Actinobacillus actinomycetemcomitans*	Modifies Rho family proteins, inflammation and inhibition of cell cycle, blocks cytokines[39]
Cycle inhibiting factor	*Escherichia coli*	Inhibit cell cycle at G_2-M transition[63]
MAP	*Citrobacter rodentium*	Multifunctional effectors protein that target host cell mitochondria implicated in the disruption of epithelial barrier function both *in vitro* and *in vivo*[64]
VEGF	*Bartonella species*	Angiogenesis and proliferation[65]

MAP: Mitochondrial associated protein; VEGF: Vascular endothelial growth factor; EGFR: Epidermal growth factor receptor.

as ErbB2) stands for "human epidermal growth factor receptor 2" and is a protein conferring higher aggressiveness in breast cancers. It is a member of the ErbB protein family, more commonly known as the epidermal growth factor receptor family, which is involved in cell proliferation, differentiation, and oncogenesis. Overexpression of ErbB2 can occur due to chronic inflammation. Binding of ErbB1 and ErbB2 to ligands results in prolonged activation of intrinsic protein kinase activity, leading to activation of a biochemical cascade responsible for mitogenic cell signal transduction[49,50]. All these factors contribute to the multistage process of carcinogenesis[55]. These factors all cause oxidative damage to DNA of the cells (Figure 1).

Bacterial toxins implicated in carcinogenesis

The probable bacterial toxins implicated in carcinogenesis are listed in Table 2[39,56-65]. The pathways involved in carcinogenesis have been depicted in Figure 1. Bacterial toxins can either kill the cells or modify the cellular processes that control DNA damage, proliferation, apoptosis, and differentiation. These toxins interfere either with the key eukaryotic processes, such as cellular signaling components, or directly by attacking the DNA[62,66]. The damage to the host cells can be mediated either[67]: (1) directly by: (a) enzymatic attack; (b) DNA damage; or (c) by affecting DNA damage repair mechanisms; or (2) indirectly by: (a) provoking a chronic inflammatory reaction; or (b) producing free radicals.

These changes might be associated with carcinogenesis and might stimulate cellular aberrations, modify the immune response, or inhibit normal cell controls.

BACTERIOLOGY OF THE GALLBLADDER AND BILE

Lykkegaard *et al*[68] reported that the liver is normally sterile, as is bile from individuals with a normal biliary tree. Bile favors the growth of some organisms but inhibits others, such as *Streptococcus pyogenes* and *Streptococcus pneumoniae*.

A positive bile culture might not only be important in the genesis of biliary tree infection, but might also be a significant determining factor in the incidence of various short and long term consequences. The pathological process of cholecystitis can be acute, chronic, or more commonly, a combination of both types. This is evident from the observations that approximately 95% of gallbladders removed for acute cholecystitis exhibit fibrosis and other signs of chronic inflammation.

Routes of infection through which microbes may enter the biliary tract

(1) Ascending route - although the sphincter of Oddi, situated at the junction of the biliary tract and the gastrointestinal tract, forms an effective mechanical barrier to duodenal reflex vis-à-vis ascending bacterial infection, when the barrier mechanism is broken down either by surgical intervention or by certain pathology, microbes can enter the biliary system. It is interesting to note that the type of organisms recovered from bile are not those dominant in the sparse flora of the duodenum but are usually encountered in the ileum and colon[69] (Table 3); and (2) Descending route (hematogenous route) - As a part of the normal innate immune system, Kupffer cells prevent toxic metabolites and bacteria from entering the hepatobiliary system from the portal circulation. Added to this, the continuous flushing action of bile and the bacteriostatic effect of bile salts keep the biliary tract sterile under normal conditions. Moreover, secretory immunoglobulin A (SIgA), the predominant immunoglobulin in the bile and the mucous membrane and excreted by the biliary epithelium, probably acts by its anti-adherent function to prevent microbial

Table 3 Spectrum of bacteria isolated from bile (Brook *et al*[73])

Organism	No. of isolates	%
Aerobic bacteria		
Escherichia coli	71	32.9
Group D streptococci	42	19.4
Klebsiella species	29	15.3
Enterobacter species	26	12.5
Proteus species	15	6.9
α-haemolytic streptococci	11	5.1
Citrobacter species	8	3.6
Staphylococcus species	7	3.2
γ-haemolytic streptococci	5	2.3
Pseudomonas species	2	0.9
Anaerobic bacteria		
Clostridium perfringens	23	29.9
Bacteroides fragilis	9	11.7
Other *Bacteroides* species	5	6.5
B. thetaiotamicron	4	5.2
B. ovatus	2	5.2
B. distasonis	2	2.6
Propionibacterium acne	7	9.1

colonization. Despite these mechanisms, it is likely that organisms in the bile might be derived from blood.

The presence of bacteria in bile may not cause symptoms. In a series of cases, Flemming *et al*[70] observed that only 20 of 32 patients with positive cultures had symptomatic cholangitis, while six of 43 with negative cultures had had symptoms in the recent past. Further, out of 15 patients who had previous biliary intestinal anastomosis, 12 had positive cultures, but only seven had a history of cholangitis. However, efficiency of culture isolation techniques and the type of bacteria associated specifically with symptoms must be explored further. Gallstone formation has been reported to be predisposed by bactobilia[71]. Bacteria themselves might act as a nidus for gall stone formation or may alter the bile composition or damage the wall of the gallbladder. It is difficult to estimate the bacteriology of bile in an absolutely healthy population. The majority of the available reports regarding the microbial spectrum of infected bile are from individuals suffering from hepatobiliary diseases. Interestingly, most of these reports concur with one another[72].

Brook[73] reported the spectrum of anaerobic isolates from the biliary tract (Table 3). Anaerobic bacteria could be recovered from 48% of specimens. Anaerobic bacteria could be isolated exclusively in 3% and mixed in 49% of 123 bile specimens collected. The author suggested that lowering of oxygen tension and pH achieved by initial colonization of aerobic bacteria in acute infection paved the way for predominance of anaerobes in the chronic stage of illness. However, it is difficult to draw any conclusion without knowing the flora of gallbladder in individuals without any sign or symptom. Most studies have reported isolation of the bacteria in bile to be < 50%[74,75]. Lu *et al*[76] detected bacterial DNA in 78.3% of CaGB tissue samples. They used a single amplification cycle targeting 16S r DNA. However, detection rates will rise further if nested PCR (being more sensitive) is used. Therefore, the possibility of the existence of viable but non-culturable (VBNC) forms of bacteria cannot be ruled out in the gallbladder. However, the detection of bacteria causing persistent infection in biliary system is warranted.

Gallbladder cancer and Salmonella typhi

The interplay of genetic susceptibility, life style factors, and infections of the hepatobiliary system in carcinogenesis of the gallbladder is poorly understood; however, a link has been specifically proposed between chronic bacterial infections of the biliary tree and *S. typhi*. An association of chronic typhoid carriage and carcinoma of the gallbladder was first reported by Axelrod *et al*[77]. Welton *et al*[78] observed increased incidence of cancer of the hepatobiliary system in typhoid carriers; this was later confirmed by other studies[79,80]. Caygill *et al*[81] studied cancer mortality in people infected during the Aberdeen typhoid outbreak in 1964; their results suggested a lifetime risk of developing gallbladder cancer in 6% of the carriers. Strom *et al*[82] from Bolivia and Mexico have reported a 12-fold increase in CaGB in subjects with a history of typhoid fever. However, they could not prove the same by serology. Moreover, Shukla *et al*[83] from Northern India, using Vi serology, showed a 7.9 times increased risk for CaGB in chronic typhoid carriers. Earlier also from North India, Nath *et al*[84] demonstrated significantly higher isolation rates of *Salmonella typhi* and *paratyphi*-A from bile, gallbladder tissue, and stones from patients with CaGB as compared to those suffering from benign gallbladder diseases. The relative risk of developing CaGB was reported to be 9.2. Based on serology, Dutta *et al*[85], from North India, reported a 14-fold increased risk of CaGB in a case-control study. In Japan, an area with an extremely low prevalence of typhoid fever, in a large cohort of 113 394, the relative risk of developing CaGB was reported to be 2.1[86]. Recently, Nath *et al*[87] reported the prevalence of chronic typhoid carriers in CaGB patients using a very sensitive and specific nested PCR technique, in hepatobiliary specimens, to exclude the limitations of serology based detection and culture isolation (low sensitivity of culture and variable individual immune response, depending on the stage of the disease). They showed that 67.3% of the CaGB patients were typhoid carriers, as compared to 8.3% of the healthy population (hepatobiliary specimens from dead bodies; victims of unnatural deaths) in the typhoid endemic area of North India (Odds ratio 22.8). In the same study, the authors tried to locate the niche of the *S. typhi* bacterium in chronic typhoid carriers, and found that the bacterium was most prevalent in the liver[88]. Therefore, it could be proposed that *S. typhi* lives in the liver and is excreted into the gallbladder intermittently. Metabolites (mutagens and inflammation inducers) and toxins produced by the multiplying bacteria are further concentrated about 10 times in the gallbladder, which thereby bears the major brunt of the mutational changes. Various carcinogens produced by *S. typhi* have been suggested: Bacterial glucuronidase, yielding some high energy intermediates after acting on bile[89],

bacterial enzymes acting upon primary bile acids and producing carcinogenic secondary bile acids at very high concentrations[90], and the production of nitroso compounds from nitrates by the action of bacterial enzymes[91]. Chronic bacterial infection leads to obstruction and persistent chemical and mechanical injuries[92].

Cytolethal distending toxin (CDT), the first bacterial genotoxin described, is also produced by *S. typhi*[58] in addition to *Escherichia coli*[93] and other causative agents of chronic infection, such as *Campylobacter jejuni*[94], *Haemophilus ducreyi*[95], *Shigella dysenteriae*[96], *Actinobacillus actinomycetemcomitans*[97], *Helicobacter hepaticus*[98], and other species[99,100]. The holotoxin is a tripartite complex, where the CdtB subunit, a structural and functional homolog of mammalian DNAse I, is the active subunit, while CdtA and CdtC mediate the binding of the holotoxin to the plasma membrane of the target cells. In a cell culture study (Cos2 and Henle-407 cell lines), Haghjoo *et al*[58] found that *S. typhi* produced a unique CdtB-dependent CDT that required bacterial internalization into host cells. When Cos-2 cells were transfected with *S. typhi*, the effects of the CdtB subunit were severe fragmentation of chromatin, a typical characteristic of the CdtB subunit of CDT expressed by other species. The authors proposed that *S. typhi* subsequent to internalization deviated from the usual endocytic pathway that leads to lysosomes, reaching an unusual membrane-bound compartment where it can survive and replicate due to its ability to produce abundant antiphagocytic Vi capsule. What is the role of CDT in *S. typhi* pathogenesis? It is worth mentioning that *S. typhi* is the only serovar of Salmonella that encodes CdtB. Furthermore, *S. typhi* is a human-restricted pathogen, causing chronic persistent infections. CDT might facilitate the persistence of infection, because this toxin is known for its immunomodulatory activity[97]. CdtB, after being delivered to the cytosol, reaches the nucleus of the target cell where it causes DNA damage[101]. Therefore, in typhoid endemic areas, *S. typhi* might be one of the important etiologic factors for CaGB.

Gallbladder cancer and Helicobacter species

Helicobacter pylori (*H. pylori*) infection is a well-established cause of stomach cancer[102]. Since the discovery of *H. pylori* in 1982, thirty other *Helicobacter* species have been identified from the stomach, intestinal tract, and liver of mammals and birds. A few species found in human bile and biliary tract tissue biopsies (*H. bilis, H. pullorum, H. hepaticus, H. pylori etc.*) have been suspected to cause biliary tract diseases. As discussed earlier, any bacteria, *Helicobacter* spp. in particular, causing persistent infection in the biliary tract might induce chronic inflammation and gallstone formation, especially due to urease production[103,104]. Gallstones further aggravate chronic inflammation and can induce transformation, which is further amplified many fold by several toxins and metabolites of known carcinogenic potentials produced by the Helicobacters[105,106]. *H. hepaticus* is a known agent causing chronic active infection of biliary canaliculi progressing to liver cancer[2]. PCR-based detection rates of different species of *Helicobacter* spp. in biliary tract cancer vary from 0%-82.8%[107]. Using species-specific primers, *H. bilis* was found in 35 out of 67 specimens (52.2%) from four different studies, whereas *H. hepaticus* was searched for in two studies, but only in one study were four out of 19 specimens (21.1%) found to be positive for the bacterium. In contrast, Pradhan *et al*[108] from Nepal have shown *Helicobacter hepaticus* infection in 82% of non-malignant gallbladders and in 87.5% of malignant cases. Whether *Helicobacter hepaticus* is the number one cause of the type of gallstone formation that ultimately leads to malignancy, or is itself a risk factor for the pathogenesis of carcinoma gallbladder, is yet to be determined. Murata *et al*[109] showed that *H. bilis* specific sequences could be amplified in three of 11 (27.2%) gallbladder cancer cases and in one of three (33.3%) cases with biliary duct cancer. One study conducted in Japanese and Thai populations showed that patients positive for *H. bilis* had a 6-fold higher risk of biliary tract carcinoma. However, it is premature to make conclusion about the role of *Helicobacter species* in causing CaGB. *H. pylori* infection was also identified as a risk factor for biliary tract cancer and the corresponding relative risk (RR) was 9.9 (95% CI: 1.4-70.5) after adjustment for age and sex. *H. bilis* and *H. pylori* have been identified in bile specimens and associated with risk of biliary tract cancer. Another study[109] found a positive association between *H. bilis* and CaGB, with a crude RR of 2.6 (95% CI: 0.6-4.6). Larger epidemiological studies are required before Helicobacters can be in gallbladder cancer, but only after the development and validation of specific serological tests and direct detection of these bacterial species in the gallbladder itself.

Gallbladder cancer and Escherichia coli

E. coli is the normal inhabitant of the human intestine and can become highly pathogenic following the acquisition of virulence factors, usually by horizontal gene transfer. Cytotoxic necrotizing factor 1 is one of the important protein toxins acquired in this way. *Escherichia coli* is the commonest species isolated from gallbladder specimens and CDT is present in many isolates of *E. coli*. Lax[37] reported a novel genotoxin, named as colibactin. The mechanism of action of this toxin is yet to be explored; however, it causes double stranded DNA breaks. It is likely that some of these acquired gene(s) enable these strains cause persistent infection, and facultative or obligate intracellular invasion, leading in turn to more chances of transformation of the host cells. In support of above speculation, Yamamoto *et al*[110] have shown that there was marked enhancement of rat urinary bladder carcinogenesis by heat killed *E. coli*. Furthermore, the occurrence of chronic urinary tract infections leading to carcinogenesis lends support to the above suggestion[111].

Gallbladder cancer and other bacteria

Several bacteria and their products display potentially carcinogenic characteristics. There is ample evidence to support the view that some bacteria can establish chronic infection, often without overt sign of the disease. In fact,

many bacteria form the chronic carrier state, usually in viable but non-cultivable (VBNC) states or in a cell wall deficient form. They are relatively dormant but retain their virulence[112,113]. The poor yield of bacterial isolation as compared to detection by PCR affirms this possibility[87]. Therefore, any bacteria (aerobic or anaerobic) acquiring genes that enable them to cause persistent infection, and are capable of producing carcinogenic toxin, secondary metabolites, and most importantly chronically released inflammatory mediators, might be able to transform the host cells. It must be stressed that the isolates from affected sites with chronic infection or cancer must be characterized in terms of their ability to colonize, and for the production of metabolites with carcinogenic potential.

CONCLUSION

Carcinoma of the gallbladder is one of the commonest malignancies of the biliary tract. The main associated risk factors identified to date include chronic cholelithiasis, chronic infection, obesity, hormonal factors, environmental exposure to specific mutagens, and genetic predisposition. Tumorigenesis is a long and complex process, and the gap between initiation and development of cancer might hide the role of microbial infection. Therefore, a direct link between bacterial infection and cancer is often not detectable, and the etiological role of the former in causation of cancer is mostly underestimated. However, chronic infections lead to the persistent release of mediators of inflammation, toxins, and metabolites and these factors may be potentially mutagenic and/or cell cycle modulators. A strong association between chronic Salmonella carriage and cancer of the gallbladder has now been proposed. However, it is likely that other bacteria in addition to *S. typhi*, which persistently inhabit the gallbladder, might be important etiological factors. Attempts have been made to conquer cancer over many decades, but the conventional strategies like chemotherapy and radiotherapy, often cannot prevent or cure cancer. Eradication of causative microbes by antibiotic therapy, and immunological potentiation by active, as well as passive, methods, will definitely lead to reduction in the incidence of bacteria-induced cancers. In addition to this approach, numerous anti-inflammatory agents of natural and synthetic origin are reported to have inhibitory effects on inflammation-induced carcinogenesis. Cellular miRNAs might also have the potential to control and prevent carcinogenesis. Knowing the genetic susceptibility for persistence of a specific bacterial agent will help in the choice of prophylactic measures in such individuals. Thus, limiting the reservoir and transmission of such potentially pathogenic microorganisms will help in decreasing the incidence of chronic and acute diseases.

FUTURE PERSPECTIVE

There is a strong need for in-depth studies looking into the role of persistent bacterial infections and carcinogenesis of the same or related parts of the body. Further study is required into the mechanism of chronic inflammatory mediators and bacterial toxins in cell transformations. Detailed study needs to be carried out to delineate whether it is the infection or the disease that occurs first in the case of gallbladder pathology. Effort should be made to substantiate the exact role of bacteria like *Salmonella typhi*, *Escherichia coli*, and *Helicobacter* species in the causation of biliary tract cancer in suitable animal models. Once the role of chronic bacterial infection in carcinogenesis is established, ways could be found to cure or eradication such agents from the community by chemotherapy, immunotherapy, and hygienic practices. Anti-inflammatory therapeutic approaches to cancer development, which can block/modify carcinogenic mechanism/s, such as anti-inflammatory agents or miRNA, should be explored.

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