Risk Factors

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Introduction

While generally considered rare, gallbladder cancer is the most common malignant tumor of the biliary tract worldwide. It is an extremely lethal disease, with an overall mean survival of six months [1]. The reason for poor prognosis is partly due to its aggressive biologic behavior and a *lack* of sensitive screening tests for early detection. Epidemiological studies have shown a wide range of geographical and ethnic variations, with higher rates in certain areas and remarkable rarity in others [2]. This difference suggests that the combination of local environmental factors and genetic predisposition is associated with its carcinogenesis. This review provides a comprehensive overview of risk factors associated with the development of gallbladder cancer.

Risk Factors

Multifactorial factors contribute to the development of gallbladder cancer. The risk factors for gallbladder cancer can be divided into three broad categories as follows.

(1) Demographic factors

① Age
② Female sex and parity
③ Obesity
④ Geography and ethnicity
⑤ Genetic factors and family history

(2) Diseases of the gallbladder and bile duct

① Gallstones
② Gallbladder polyps
③ Gallbladder adenomyomatosis
④ Xanthogranulomatous cholecystitis
⑤ Porcelain gallbladder
⑥ Pancreaticobiliary maljunction
⑦ Primary sclerosing cholangitis

(3) Environmental/lifestyle factors

① Chronic infection: *Salmonella, Helicobactor*
② Exposures and medications
③ Lifestyle factors

Demographic Factors

Age

The incidence of gallbladder cancer tends to increase continuously with age. More than two-thirds of patients diagnosed with gallbladder cancer are older than 65 years, and the average age at diagnosis in the US is

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J. B. Chung and K. Okazaki (eds.), *Diseases of the Gallbladder*,
https://doi.org/10.1007/978-981-15-6010-1_15

72 years. According to US data from 2015, the age-adjusted incidence rates (per 100,000 people) increased from 0.2 among those 20–49 years of age to 1.6 in those 50–64 years of age, to 4.3 among those 65–74 years of age, and to 8.1 for individuals over the age of 75 years [3].

Female Sex and Parity

Gallbladder cancer occurs two to six times more often in women than men in most countries [4]; therefore, sex is a recognized risk factor for gallbladder cancer. The female predominance of gallbladder cancer suggests a role of female sex hormones. Estrogen increases biliary cholesterol saturation and decreases bile salt secretion, while progesterone impairs gallbladder emptying to promote the development of gallstones, which is the most important risk factor for gallbladder cancer. Prolonged lifetime estrogen exposure is associated with an increased risk of gallbladder cancer through early menarches, late menopause, multiple pregnancies, and estrogen replacement therapy. Serum estrogen levels rise about 100-fold during pregnancy; thus, higher parity is associated with increased lifetime exposure to estrogen. The female-to-male ratio of gallbladder cancer is above 2 in most countries but is only about 1.3 in Korea and Japan [5]. One possible explanation for the relatively high incidence of gallbladder cancer in men than women in these countries may be the difference in the prevalence of *Clonorchis sinensis*.

Obesity

Obese individuals have an increased risk of developing gallbladder cancer. Overweight and obesity were associated with 14 and 56% higher risk of gallbladder cancer, respectively. For each five-point increase in Body Mass Index (BMI), the Relative Risks (RRs) of developing gallbladder cancer increase by 1.59 for women and 1.09 for men [6]. The association between obesity and risk of gallbladder cancer is stronger in women than in men, and overweight is only associated with gallbladder cancer in women [7]. Other anthropometric factors such as waist circumference, hip circumference, and waist-to-hip ratio were all associated with gallbladder cancer. Diabetes mellitus is a risk factor for gallstone disease and gallbladder cancer. In a meta-analysis of 20 studies, diabetic individuals had a 1.56-fold increased risk of gallbladder cancer compared to that in nondiabetics [8]. Patients with diabetes have an increased risk of developing gallbladder cancer even in the absence of gallstones [9]. It is not clear whether obesity plays an important mediating role in the association between diabetes and gallbladder cancer.

The potential biological mechanisms for the carcinogenesis of gallbladder associated with obesity include increased concentrations of hormones such as insulin or estrogen, which increase the formation of gallstones. Obesity-related mediators such as Insulin-like Growth Factor (IGF)-1, adipokines, inflammatory factors, and pro-inflammatory cytokines may contribute to cancer-related processes. Leptin and adiponectin secreted by adipose tissue are also involved in carcinogenesis [6].

In conclusion, adiposity is associated with an increased risk of gallbladder cancer, suggesting that weight management can help to minimize the risk of gallbladder cancer [10].

Geography and Ethnicity

The incidence of gallbladder cancer shows marked geographical variability. The rates are highest in Chile (27/100,000), India (21.5/100,000), Poland (14/100,000), south Pakistan (11.3/100,000), and Japan (7/100,000) [11]. The incidence in Korea from 1999 to 2013 was 2.96–3.12 per 100,000 in men and 2.79–3.03 in women [12]. In contrast, gallbladder cancer is rare in the western world including the US, UK, and Canada. Gallbladder cancer tends to particularly afflict indigenous populations, and ethnic rates can prevail even in different geographic locations. This suggests that gallbladder cancer may result from interactions between innate genetic predisposition and exposure to environmental risk factors.

Genetic Factors and Family History

A family history of gallbladder cancer can slightly increase the risk of gallbladder cancer [13]. A Swedish family-cancer database was the first to report the familial clustering of gallbladder cancer, in which the risk of gallbladder cancer was increased in female immigrants from Chile and the Indian subcontinent while some Northern European immigrants showed decreased risks compared to native Swedes [14]. Therefore, some persistent damage was inflicted before emigration, and racial factors were more important than environmental factors. A study from the Utah Cancer Registry estimated that 26% of all gallbladder cancers are familial [15].

Gallbladder cancer has also been associated with multiple familial polyposis/Gardner syndrome and Peutz–Jeghers syndrome. Multiple genetic mutations are likely involved in the pathogenesis of gallbladder cancer. The early molecular changes may include p53 mutation, cyclooxygenase-2 overexpression, mitochondrial DNA mutations, and abnormal hypermethylation of various tumor suppressor gene promoters [16].

Diseases of the Gallbladder and Bile Duct

Gallstones

Gallstones are the most important risk factor for the development of gallbladder cancer, with a Relative Risk (RR) of 4.9 [5]. Among patients with gallbladder cancer, 70–90% have a history of gallstones. Furthermore, the incidence of gallbladder cancers is well correlated with the prevalence of gallstone disease. However, compared to the high prevalence of gallstones, gallbladder cancer occurs in less than 1% of patients with gallstones; therefore, gallstones alone cannot be considered a single cause of gallbladder cancer [17]. The reasons why some individuals with gallstone disease develop cancer while most do not remain unknown.

Gallstone characteristics influence the risk of gallbladder cancer. Increasing gallstone size is associated with a greater risk of gallbladder cancer. The risk of developing gallbladder cancer increases by 10.1-fold and 2.4-fold for gallstones larger than 3 and 2.0–2.9 cm in diameter, respectively, compared to stones less than 1 cm [18]. In addition to size, gallstone weight and volume are also associated with gallbladder cancer. Average volumes of 6, 8, and 10 mL have relative cancer risks of 5, 7, and 11-fold, respectively [19]. As the duration of gallstone increases, so does the RR of gallbladder cancer, with an RR of 4.9 for gallstones with duration of 5–19 years and 6.2 for durations exceeding 20 years [20] American Indians who have a high incidence of gallbladder cancer also have a high prevalence of cholesterol gallstones, suggesting that those with cholesterol stones are at a higher risk than those with pigment stone [21, 22].

The exact mechanism by which gallstones predisposes individuals to gallbladder cancer remains debatable. During the secretion of cholesterol from the liver to gallbladder in gallstone formation, other toxic substances may be released simultaneously and cause malignant changes in the gallbladder. Two candidates are orphan nuclear receptors and the adenosine triphosphate-binding cassette transporter family, which may increase gallbladder epithelium exposure to carcinogenic compounds [23]. Chronic irritation due to gallstones and the local production of carcinogens such as secondary bile acid promote progressive morphological damage through a metaplasia–dysplasia–carcinoma sequence [24]. A Danish study reported that it takes approximately 15 years to progress from dysplasia to advanced cancer [21].

Although gallstones are an associated risk factor and there is an inverse correlation between cholecystectomy for gallstone and gallbladder cancer rate [25], studies of their natural history and decision analysis do not favor prophylactic cholecystectomy for clinically silent gallstones [26, 27]. Screening for gallbladder cancer in patients with gallstones is not currently recommended.

Gallbladder Polyps

Gallbladder polyps are incidentally found on Ultrasound (US) and appear as fixed, echogenic masses protruding into the gallbladder lumen without an acoustic shadow [28]. Gallbladder polyps are relatively common, with a prevalence of 3–7% in abdominal US and 2–12% in cholecystectomy specimens. The majority are pseudotumors with no neoplastic potential, including cholesterol polyps (60%), adenomyomatosis (25%), or inflammatory polyps (10%). The most common benign neoplastic lesion is adenoma. Benign adenomas, accounting for approximately 4% of all gallbladder polyps, have malignant potential, although the role of adenomas in the pathogenesis of carcinoma is controversial. Adenomas may play a role in some cases of gallbladder cancer; however, the absence of adenoma remnants in mucosa adjacent to adenocarcinoma suggests that these tumors may not universally play a role in carcinogenesis [1]. In the carcinogenesis of gallbladder cancer, the dysplasia-carcinoma sequence is considered the predominant mechanism over the adenoma-carcinoma sequence because malignant transformation of adenoma or concomitant presence of adenoma and carcinoma are not common findings [29, 30].

Although most gallbladder polyps are benign, malignant polyps are present in some cases. Polyp size is the most important risk factor for malignancy, with gallbladder polyps larger than 10 mm significant predictors of malignancy, while most polyps less than 10 mm are benign and remain static for long periods [31]. Other factors predicting malignancy include solitary sessile polyps, presence of gallstones, patient age over 50 years, and, most importantly, rapid polyp growth [32].

The management of incidentally detected gallbladder polyp is controversial. Cholecystectomy is recommended for gallbladder polyps $\geq$ 10 mm or symptomatic polyps irrespective of size. If the patient has risk factors for gallbladder malignancy and a polyp measuring 6–9 mm, cholecystectomy is suggested if the patient is fit and accepts surgery [33]. However, these suggestions are not firm evidence-based consensus guidelines.

Gallbladder polyps should be followed by serial US; however, there is no consensus regarding screening interval. Practical recommendations advise an initial review within 6 months and then annual follow-up or every 6 months for at least 2 years until stability is documented [34]. The most recent European Society of Gastrointestinal and Abdominal Radiology (ESGAR) guidelines recommended follow-up US of the gallbladder at 6 months and 1 year and then yearly up to 5 years in polyps measuring 6–9 mm. In polyps under 6 mm, follow-up is advised at 1, 3, and 5 years; however, patients with risk factors for malignancy should receive the more extensive follow-up recommended for polyps of 6–9 mm without risk factors [33]. The duration of follow-up in patients with apparently stable gallbladder polyps has not been established. Wiles et al. demonstrated that gallbladder polyps that do grow appear to do so slowly and concluded that a 5-year follow-up should be advised [28]. Another study suggested that a follow-up of at least 10 years were necessary [35] because it took about 7 years for the development of neoplasia in gallbladder polyps [36]. The decision to terminate follow-up is dependent on the judgment of the clinician, and it may be more efficient to develop flexible and tailored follow-up plans rather than follow fixed or inflexible guidelines.

Alternative imaging modalities may provide additional information for the diagnosis of gallbladder polyps. Compared to conventional US, Endoscopic Ultrasound (EUS) showed a greater accuracy to predict neoplastic gallbladder polyps by analyzing EUS features and scoring systems [37]. Utilization of other imaging modalities such as ContrastEnhanced Ultrasound (CEUS) and Contrast-Enhanced Harmonic EUS (CEH-EUS) increased the diagnostic accuracy for gallbladder polyps. CEUS features highly suggestive of malignancy include the destruction of the gallbladder wall, heterogeneously enhancing patterns, and rapid contrast washout [38]. The presence of irregular intratumoral vessels or perfusion defects on CEH-EUS may be sensitive and accurate predictors of malignant polyps [39].

As a new technique, real-time elastography showed that all benign gallbladder polyps appeared to have a high-strain elastographic pattern, which may be useful in the characterization of gallbladder polyps but should be used as an auxiliary means of diagnosis [40].

Gallbladder Adenomyomatosis

Gallbladder Adenomyomatosis (GBA) is characterized by epithelial proliferation and hypertrophy of the muscles of the gallbladder wall with the outpouching of the mucosa into the thickened muscular layer, known as Rokitansky-Aschoff sinuses (RAS). GBA is frequently observed in cholecystectomy specimens, with a prevalence of 1–9% [41]. GBA has three morphological types—fundal (localized), segmental, and diffuse—according to the localization in the gallbladder wall. The fundal type is the most common pattern and is characterized by a local wall thickening on the gallbladder fundus. The segmental type, located in the gallbladder body, is annular and separates the gallbladder into two communicating compartments. The diffuse type includes thickening of the entire GB wall [42]. Gallstones are present in more than 50% of patients with GBA and up to 90% with segmental type [43]. Most patients with GBA are asymptomatic but, if present, the symptoms may result from the presence of gallstones. US is the imaging modality of choice for GBA diagnosis. The features on US are focal or diffuse gallbladder wall thickening with small anechoic cystic spaces and/or echogenic foci and comet-tail artifacts. Small anechoic cystic spaces (1–10 mm) representing clear bile-filled RAS are pathognomonic for GBA.

GBA is a benign lesion as the hyperplastic epithelium of GBA has no higher neoplastic potential than that of a normal gallbladder. Some studies have reported an increased prevalence of gallbladder cancer in patients with segmental-type GBA (6.6%) compared to that in patients without GBA or with other GBA patterns (4.3%), with more marked differences in patients over 60 years of age [43, 44]. However, these results may have been influenced by the higher prevalence of gallstones in patients with segmental-type GBA, which is a well-known risk factor for gallbladder cancer. Thus, GBA itself is not a precancerous lesion but gallstones secondary to GBA may lead to dysplastic changes and cancer. GBA may increase in size over time; however, this change alone is not considered an index of malignancy [41, 45]. Although GBA is not generally considered a premalignant lesion, GBA-positive gallbladder cancer is more often diagnosed in advanced stages because preceding GBA may interfere with early gallbladder cancer detection [46, 47].

There are no universally accepted guidelines for GBA management. Given the lack of malignancy potential, asymptomatic GBA theoretically requires no specific treatment when imaging provides a definite diagnosis. Cholecystectomy is usually indicated for symptomatic patients or in cases with inconclusive imaging findings. However, some doctors may prefer surgical options in patients with segmental-type GBA, given its higher association with gallbladder cancer, and in patients with diffuse-type GBA given the possible difficulties in identifying coexisting malignancies [42, 48].

New methods have been proposed to improve diagnostic accuracy. CEUS can be performed if RAS cannot be clearly identified in baseline US. RAS appear avascular in every phase of the dynamic study in CEUS, independently from their content. Magnetic Resonance Imaging (MRI) should be reserved for cases that are unclear on US and CEUS. At MRI, RAS can be identified with extremely high sensitivity by visualizing intramural cystic images in a "pearl necklace" configuration, which is pathognomonic of GBA [41].

Xanthogranulomatous Cholecystitis

Xanthogranulomatous Cholecystitis (XGC) is an uncommon form of chronic cholecystitis characterized by abnormal wall thickening and severe proliferative fibrosis with multiple yellow-brown intramural nodules. The incidence of XGC was 1.3–5.2% in resected gallbladder specimens [49]. XGC is frequently misdiagnosed as gallbladder cancer because its clinical and radiological features often mimic those of gallbladder

cancer. The ultrasonographic characteristics of XGC include moderate-to-marked thickening of the gallbladder wall with oval hypoechoic nodules. As the frequency of coexisting XGC and gallbladder cancer is nearly 10% [49], XGC association with cancer is controversial. One study suggests the malignant potential of XGC for its upregulated oncogenes (BCL-2, c-Myc) [50], while another suggests the inflammatory nature of XGC through the expression of p53, proliferating cell nuclear antigen (PCNA), and beta-catenin [51]. Although XGC itself may not be the direct cause of gallbladder cancer, knowledge of clinicopathological features would help clinicians to manage gallbladder lesions associated with XCG because the association of XGC with gallbladder cancer makes treatment decisions difficult.

Porcelain Gallbladder

Porcelain Gallbladder (PGB) refers to gallbladder wall calcification. When calcium deposits become extensive, the gallbladder is called "porcelain" due to its bluish color and fragile, even brittle, consistency. The pathogenesis is associated with chronic gallbladder inflammation, and approximately 90% of patients have associated gallstones. PGB occurs in 0.8% of all cholecystectomies [22]. It has a female preponderance (5:1) and is usually diagnosed in the sixth decade. Patients are usually asymptomatic and PGB is found incidentally on plain abdominal radiographs, US, or Computed Tomography (CT) images showing the characteristic calcification of the gallbladder wall. In general, US findings are classified into three types based on the extent and pattern of wall calcification: type I is characterized by a hyperechoic semilunar structure with posterior acoustic shadowing; type II displays a curvilinear echogenic structure with acoustic shadowing; and type III is characterized by irregular clumps of echoes with posterior acoustic shadowing [22]. While type I corresponds to complete intramural calcification, types II and III reflect changes in selective mucosal calcification.

There is no definite consensus on the incidence rate of gallbladder cancer from PGB. Previous studies reported concomitant incidence of gallbladder cancer ranging from 12.5 to 61% [52, 53]. A recent systematic review of 340 patients with gallbladder calcifications reported an incidence of gallbladder malignancy of 21%. However, when studies with obvious selection bias were excluded, the rate of gallbladder malignancy fell to 6% in patients with gallbladder calcification compared to 1% in a matched cohort of patients without gallbladder calcification [54]. Khan et al. reported incidence of gallbladder cancer in PGB patients as low as 2–3% [55]. Stephen et al. found that the incidence of gallbladder cancer was related to the calcification pattern, with selective mucosal calcification causing a significant cancer risk compared to diffuse intramural calcification, which was less likely to develop malignancy [52]. Hence, gallbladders with partial, stippled, or multiple punctate calcifications in the mucosa may benefit from prophylactic cholecystectomy while an observational approach may be appropriate for those with a continuous band of calcification in the muscular layer [54, 56].

The management of PGB has been controversial for decades. Due to the high incidence of cancer in early studies, prophylactic cholecystectomy was previously routinely recommended. However, based on recent evidence suggesting a much lower incidence of cancer, prophylactic cholecystectomy appears appropriate for healthy patients, whereas a nonoperative approach should be considered in patients with significant comorbidities. Patients who are managed conservatively may need close follow-up to detect malignancy.

Pancreaticobiliary Maljunction

Pancreaticobiliary Maljunction (PBM), also known as anomalous pancreaticobiliary ductal junction or anomalous pancreaticobiliary ductal union, is an established risk factor for gallbladder cancer especially in relatively young female patients without gallbladder stones. PBM is particularly common in the Asian population and may explain the high incidence of gallbladder cancer in East Asia [57]. Approximately 10% of patients with gallbladder cancer have

this junction anomaly [1]. PBM is a congenital malformation in which the junction of the pancreatic and bile ducts is located outside the duodenal wall. PBM can be divided into two types: with biliary dilatation (congenital biliary dilatation) and without biliary dilatation. Most PBM cases detected in childhood are associated with biliary dilatation; however, one-third of PBM detected in adults do not show biliary dilatation. PBM patients with biliary dilatation often present symptoms of pancreatitis or cholangitis in childhood, whereas those without biliary dilatation rarely have symptoms and most patients are not diagnosed until the onset of advanced-stage gallbladder cancer [58]. PBM is diagnosed when an abnormally long common channel and/or an abnormal union between the pancreatic and bile ducts is evident on direct cholangiography such as Endoscopic Retrograde Cholangiopancreatography (ERCP) or Magnetic Resonance Cholangiopancreatography (MRCP).

The sphincter of Oddi, which is normally located at the distal end of the pancreatic and bile ducts, regulates the outflow of the pancreatic juice and bile. Because of the markedly long common channel in PBM, sphincter activity does not affect the pancreaticobiliary junction, which allows reciprocal reflux of pancreatic juice or bile. As the hydropressure in the pancreatic duct is usually greater than that in the bile duct, reflux of pancreatic juice into the biliary tract is frequent in PBM, which explains the higher rates of carcinogenesis of the biliary tract in patients with PBM [57, 59]. Carcinogenesis in PBM appears to be related to the stagnation of the pancreatic juice refluxed into the biliary tract. Refluxed proteolytic pancreatic enzymes and phospholipase A2 activation in the biliary tract produce strong cytotoxic substances such as lysolecithin. Exposure to harmful substances induces epithelial hyperplasia with increased cell proliferation. This leads to *K-ras* oncogene and/or *p53* tumor suppressor gene mutations in the epithelium and subsequent cancer development and progression. The reported incidence of epithelial hyperplasia of the gallbladder of PBM patients without biliary dilatation was 72–91% [58]. The Ki-67 labeling index, a marker of cell proliferation activity, was significantly higher in the gallbladder epithelium of PBM patients than in that of controls (8.1 vs. 1.4%) [60]. The predominant mechanism responsible for the development of biliary tract cancer in patients with PBM appears to be the hyperplasia-dysplasia-carcinoma sequence resulting from chronic inflammation induced by refluxed pancreatic juice, which is different from the adenoma-carcinoma sequence or de novo carcinogenesis seen in patients without PBM [57, 61].

A nationwide survey in Japan [62] reported prevalences of biliary tract cancers in adult PBM patients with and without biliary dilatation of 21.6 and 42.4%, respectively. Among PBM patients with biliary tract cancers, bile duct and gallbladder cancers were present in 32.1 and 62.3% of patients with biliary dilatation and 7.3 and 88.1% of those without biliary dilatation. Thus, bile duct and gallbladder cancer occurred in 6.9 and 13.4% of patients with congenital biliary dilatation and 3.1 and 37.4% of patients without biliary dilatation. Furthermore, biliary tract cancers developed at an early age in PBM patients (mean 50–60 years), about 15–20 years earlier than those without PBM [57].

Once PBM is diagnosed, prophylactic biliary surgery is recommended before the onset of malignant changes. Cholecystectomy and resection of the extrahepatic bile duct is an established standard for the surgical treatment in PBM patients with congenital biliary dilatation [63]. However, the treatment of PBM without biliary dilatation remains controversial. Only prophylactic cholecystectomy is performed in many institutes because most biliary cancers in PBM patients without biliary dilatation are gallbladder cancer [64]. However, some surgeons suggest excision of the extrahepatic bile duct with the gallbladder in PBM patients without biliary dilatation because such patients have a higher risk of bile duct cancer than the general population [65].

Compared to congenital biliary dilatation, PBM without biliary dilatation is difficult to diagnose early because they rarely evoke symptoms. In PBM without biliary dilatation, epithelial

hyperplasia of the gallbladder induced by long-standing continuous stasis of the bile intermingled with refluxed pancreatic juice is a characteristic pathological change [66]. To achieve early detection of PBM without biliary dilatation, MRCP is recommended for patients with gallbladder wall thickening on screening ultrasonography under suspicion of PBM [58, 61].

Primary Sclerosing Cholangitis

Primary Sclerosing Cholangitis (PSC) is a chronic liver disease characterized by chronic inflammation and fibrosis of the intra- and extrahepatic bile ducts. Patients with PSC have a significantly increased risk of cholangiocarcinoma, gallbladder cancer, and colorectal cancer. The risk of gallbladder cancer increases via a metaplasia-dysplasia-carcinoma sequence [22], and the lifetime incidence of gallbladder cancer in patients with PSC is estimated to be 3–14% [67]. On imaging, gallbladder cancer can manifest a mass (45–60%), thickened gallbladder wall (20–30%), or polypoid lesion (15–25%) [68]. The European Association for the Study of the Liver (EASL) reported that adenocarcinoma is found in more than 50% of patients of PSC with gallbladder mass lesions regardless of their size [69]. Therefore, cholecystectomy should be considered in all patients with PSC for gallbladder masses of any size or gallbladder polyps greater than 8 mm in size, while polyps less than 8 mm may be closely monitored because of the high surgical risk of PSC patients and the unlikelihood to be malignant [69–71]. The American Association for the Study of Liver Diseases (AASLD) recommends annual surveillance of gallbladder cancer with US screening to detect mass lesions in the gallbladder [72].

Environmental/Lifestyle Factors

Chronic Infection

Chronic infection by *Salmonella* (*S. typhi* and *S. paratyphi*) or *Helicobacter (H. pylori and H. bilis)* may predispose individuals to gallbladder cancer [73, 74]. Approximately 2–5% of patients with acute *S. Typhi* infection become chronic asymptomatic carriers, with the gallbladder being a site of persistence [75]. Chronic infection with *S. typhi* has been associated with an increased risk of gallbladder cancer, although the mechanism underlying this association is unknown. Epidemiological studies have shown that chronic *S. typhi* carriers have an 8.47-fold increased risk of gallbladder cancer compared to non-carriers [76]. In a meta-analysis of studies from South Asia, Nagaraja et al. reported a 4.28-fold increased risk of gallbladder cancer in chronic typhoid carriers [77]. Latin American countries such as Chile and Bolivia, where typhoid fever is endemic, have the highest rates of gallbladder cancer worldwide, with summary RRs of 4.6 and 4.7 for studies using Vi antibody serology and culture techniques, respectively [78]. Due to the risk of gallbladder cancer, management options for chronic typhoid carriers should include either elective cholecystectomy or careful monitoring by US [77].

Several *Helicobacter* species can colonize the hepatobiliary tract in humans, where they cause chronic inflammation. A study of Japanese and Thai populations found that the Odds Ratios (ORs) for bile duct or gallbladder cancer with *H. bilis* compared to gallstone and/or cholecystitis were 6.50 in Japanese and 5.86 in Thai patients [79]. *H. pylori* was detected in the bile 9.9 times more frequently in patients with biliary tract cancer compared to patients in the control group [80]. However, other studies failed to demonstrate an increased risk of gallbladder cancer in the presence of *H. bilis* [81] or *H. pylori* [82]. Therefore, the possible role of *Helicobacter* species in the development of gallbladder cancer requires verification. Other chronic infections with liver flukes, including *Clonorchis sinensis* and *Opisthorchis viverrini,* are highly associated with cholangiocarcinoma but have not been evaluated for the risk of gallbladder cancer.

Chronic gallbladder infection may predispose individuals to gallbladder cancer through several mechanisms. Beta-glucuronidase excreted by bacteria may deconjugate bile acids and metabolites, rendering them highly active intermediates

that bind to DNA [83]. Long-term chronic inflammation results in the sustained release of inflammatory mediators such as cytokines, chemokines, reactive oxygen species, and prostaglandins into the tissue microenvironment. Inflammation by cytokines induces oncogene activation and tumor suppressor gene inactivation, leading to cell transformation, mutated cell proliferation, and apoptosis inhibition, eventually leading to gallbladder cancer [84].

Exposures and Medications

Occupational and environmental exposures to carcinogens may increase the risk of gallbladder cancer. The presumed mechanism by which toxic substances contribute to gallbladder carcinogenesis is chronic exposure of the gallbladder epithelium to carcinogenic metabolites of toxins excreted from the liver into bile [85]. Prolonged exposure to some heavy metals may result in gallbladder cancer. Patients with gallbladder cancer have significantly lower levels of selenium and zinc and higher levels of copper, lead, cadmium, chromium, and nickel in serum and bile. These findings indicate that deficiencies in trace elements such as selenium and zinc may lead to a loss of their protective roles against cancer and that the remaining heavy metals have carcinogenic properties [86, 87]. Workers in rubber plants or textile factories or those exposed to nitrosamines have increased risks of gallbladder cancer [88]. An increased risk was also reported among miners exposed to radon [1]. Those living in the Gangetic belt in India, an industrial region with high levels of pollutants in untreated domestic sewage, industrial, and agricultural effluents containing aromatic hydrocarbons, nitrosamines, and chemicals such as nitrates and nitrites, have a nearly 10-fold increased risk of developing gallbladder cancer [89]. Fungal aflatoxin, ochratoxin A, and arsenic exposure may also be associated with increased risks of gallbladder cancer [90–92].

Increased risk of gallbladder cancer has also been associated with some medications. Hormone Replacement Therapy (HRT) and use of oral contraceptives have different impacts on the risk of gallbladder cancer. Postmenopausal women undergoing oral estrogen or estrogen-progesterone therapy are at increased risks of gallstones and gallbladder cancer [93]; however, the risk associated with oral contraceptive use remains controversial [94]. Large prospective cohort studies have suggested that HRT increases the risk of gallbladder disease in postmenopausal women, and transdermal estrogen replacement therapy has a lower risk for gallbladder disease than oral therapy (RR 1.17 *vs*. 1.74) [95]. Transdermal estrogens are absorbed through the skin and enter the systemic circulation directly, bypassing first-pass metabolism by the liver. Therefore, the concentrations of estrogens and their metabolites in the bile are low, which explains the lower risk of gallbladder disease. Drugs including methyldopa and isoniazid may increase the risk of gallbladder cancer; however, the associations are weak [96, 97].

Lifestyle Factors

Although the association between dietary intake and gallbladder cancer is unclear, dietary factors may influence the production of gallbladder cancer through potential effects on gallstone formation [31]. Higher intakes of energy and carbohydrate can increase the risk of gallbladder cancer because obesity plays an important role in the development of gallbladder cancer. However, adequate intake of fruits and vegetables may have a preventive effect against gallbladder cancer, which could be attributed to their high levels of vitamins, carotenes, and fiber [98]. High consumption of fish may also play an important role in gallbladder cancer prevention by increasing the inhibitory effect of biliary linolenic acid on mutagens in gallbladder bile [99]. A recent prospective cohort study of Swedish adults showed an association between coffee consumption and a reduced risk of gallbladder cancer. The protective effect of coffee consumption may be mediated via reduced gallstone formation. Coffee consumption stimulates cholecystokinin release, enhances gallbladder contraction, and decreases cholesterol crystallization in the bile [100]. Tea consumption also reduced the risk of gallbladder cancer in women but not in men [101].

Cigarettes can damage DNA and cause genetic mutations, which may increase the risk of gallbladder cancer. A recent study reported a 19% increased RR among current smokers and a dose-response relationship between smoking and gallbladder cancer [102]. The association between alcohol consumption and the risk of gallbladder cancer was also dose-dependent. A meta-analysis by Bagnardi et al. of eight studies showed that heavy drinking (>50 g of alcohol/day), but not moderate and light drinking, was associated with an RR of 2.64 for gallbladder cancer [103].

Conclusion

Gallbladder cancer is a deadly, aggressive, and multifactorial disease with complex interplays between genetic and environmental risk factors. In the carcinogenesis of gallbladder cancer, the dysplasia-carcinoma and metaplasia-dysplasia-carcinoma sequences induced by chronic inflammation are considered more important models than the adenoma-carcinoma sequence. In countries with high rates of cholecystectomy, the incidence of gallbladder cancer has decreased following the recommendation for prophylactic cholecystectomy in patients with risk factors. Therefore, investigation of risk factors is important. While gallstones are the most common risk factor for gallbladder cancer, less than 1% of patients with gallstones develop gallbladder cancer; thus, prophylactic cholecystectomy or screening for gallbladder cancer is not necessary. Patients with gallbladder polyps>10 mm have increased malignant potential and prophylactic cholecystectomy is recommended. However, for small gallbladder polyps, less concern seems appropriate since most do not become gallbladder cancer. Based on recent evidence suggesting a much lower incidence of cancer in patients with PGB, cholecystectomy is not routinely recommended for all patients and is limited to healthy patients. PBM, which is more common in young Asian women without gallstones, should be kept in mind as these patients may have a high risk of gallbladder cancer. Once PBM is diagnosed, prophylactic biliary surgery is recommended. Patients with PSC would benefit from annual surveillance of gallbladder cancer with US screening to detect mass lesions in the gallbladder. For the primary prevention of gallbladder cancer, further research is needed to identify the complex relationship between environmental and genetic risks and elucidate the multifactorial pathophysiology of gallbladder cancer. Cancer genomics and new molecular biology techniques may be helpful to reveal the overall molecular pathogenesis of gallbladder cancer, which will guide strategies for prevention and modern therapeutic approaches.

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