Infection as a Risk Factor for Gallbladder Cancer

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Gallbladder cancer is a common hepato-biliary malignancy with poor prognosis. The main associated risk factors identified so far include cholelithiasis (especially mixed gall stone), chronic infections of the gall bladder, obesity, reproductive factors, diet, hepato-biliary anomalies, and environmental exposure to specific chemicals. Genetic and molecular predisposing factors have also been described. This article reviews the association of chronic infection and gall bladder cancer. Most of the studies have shown a good association of mixed bacterial and Salmonella infections in the carcinogenesis of cancer gall bladder especially in the area of high endemicity of typhoid. Bacterial degradation of bile and chronic inflammation may also play some role in the carcinogenic process. Mutations in multiple tumor suppressor gene and oncogenes (P53 and K-ras) have also been found in a few studies. This review seeks to bring out many hidden infective etiological aspects of the pathogenesis of gall bladder cancer. Review of the entire published literature suggests a need for further studies for better understanding of the disease.

KEY WORDS: infection; enteric fever—Salmonella; Helicobacter; gallbladder cancer

Gallbladder cancer is a common hepato-biliary malignancy with poor prognosis. The main associated risk factors identified so far include cholelithiasis (especially mixed gall stone), chronic infections of the gall bladder, obesity, reproductive factors, diet, hepato-biliary anomalies, and environmental exposure to specific chemicals. Genetic and molecular predisposing factors have also been described. This article reviews the association of chronic infection and gall bladder cancer.

Most of the studies have shown a good association of mixed bacterial and Salmonella infections in the carcinogenesis of cancer gall bladder especially in the area of high endemicity of typhoid. Bacterial degradation of bile and chronic inflammation may also play some role in the carcinogenic process. Mutations in multiple tumor suppressor gene and oncogenes (P53 and K-ras) have also been found in a few studies. This review seeks to bring out many hidden infective etiological aspects of the pathogenesis of gall bladder cancer. Review of the entire published literature suggests a need for further studies for better understanding of the disease.

The etiology of gall bladder cancer is not well known. The putative risk factors described are gall stones [1], diet, [2–4], and congenital hepato-biliary anomalies [5–7] besides the genetic and the molecular predisposition factors [8]. Several studies have shown high risk of gall bladder cancer associated with chronic infection and inflammation [9]. Several studies are discrete utilizing various biological evidences and study designs with no combinability of data. Hence, a meta-analysis has not been performed. Nevertheless, not a single study has refuted increased association of chronic infection with gall bladder carcinoma. The present review provides a brief description of studies correlating different chronic infections leading to gallbladder cancer.

Various infections are associated with cancers of different organs. For example Helicobacter species with gastric cancer [10], bilharziasis with urinary bladder cancer [11,12], Papilloma virus with uterine cervical cancer [13–15], Hepatitis ‘B’ with hepatocellular carcinoma [16–19], liver fluke with cholangiocarcinoma [20], etc. Chronic infection, inflammation, and irritation play a possible role in carcinomatous change, which may

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lead to gallbladder carcinoma. The evidence to this effect may come from experimental carcinogenesis models, ecological studies, cohorts, and case control studies. So far, there have been only two studies on animal models of gall bladder cancer in which diet has been implicated in its etio-pathogenesis [2,3]. However, no experimental carcinogenesis study has been conducted to show the pathogenesis of chronic infection.

There is substantial geographical variability of incidence of gall bladder cancer. Population-based data indicate that the incidence of gall bladder cancer was relatively high in northern Indian states [21,22] (Uttar Pradesh, Bihar, Orissa, West Bengal, and Assam). The rates of gall bladder cancer were higher among women than men in all populations. The incidence rates were higher in the women from Bolivia (15.5 per 100,000) and lower in Peru, Ecuador, Columbia, Brazil, Paraguay, and Uruguay (from 3.7 to 9.1 per 100,000). The incidence rates of carcinoma of gall bladder were low in North America (<2 per 100,000) with the exception of high rates among Indians in New Mexico (11.3 per 100,000) and among female immigrants from Latin America. In Europe, the highest incidence was found in the countries of Eastern Europe; Poland, the Czech Republic, and Slovakia. Except for some high-risk areas such as Nagasaki, Japan, the rates in other regions of Japan and the developed world were relatively low (<2 per 100,000) [23]. Ecologically, such geographical distribution of high incidence of gall bladder cancer has been reported from the areas where typhoid infection is more likely to occur such as Chile [24] and northern India [25,26]. In addition, gall bladder cancer occurs more frequently in the areas where proportion of mixed gall stone disease is higher as compared to pure cholesterol stones. The mixed varieties of gall stones are likely to originate from a nidus of infection [25].

**EVIDENCE FROM COHORT STUDY**

The most convincing evidence, however, came from a long-term cohort study by Cargill et al. [27] based on Aberdeen (Scotland) typhoid outbreak of 1964 (Table I). A total of 507 typhoid and para-typhoid cases were reported. Of the traceable patients, 386 (76%) were cured and were non-carriers, 83 (16%) were reported as chronic carriers. After excluding those dead (139), non-traceable (121) and still carrying the disease (6) the cancers of pancreas, biliary tract, lung, and all other neoplasm, were analyzed as observed/expected cases (O/E). The carriers of typhoid and paratyphoid showed greater probability of gall bladder cancer (167; 95% CI 54.1–389), cancer of the pancreas (8.1; 95% CI 1.67–23.7), colo-rectum cancer (3; 95% CI 0.62–8.77), lung cancer (2.5; 95% CI 0.82–5.89), and all other neoplasm (2.6; 95% CI 1.57–3.96). This study provides strong evidence of high risk for gall bladder cancer (167 times O/E) in chronic typhoid carriers as opposed to acute typhoid patients. It may be noted, however, that the risk is not only for gall bladder cancer but to some extent to other cancers also. The authors suggested that some of the non-carriers in their study were those who must have had a cholecystectomy and therefore the calculated excess risk is an underestimate. The authors did not comment on gall stone formation in this cohort. Gall stones associated with typhoid carrier status were more common in females in other studies which were mostly case control studies only [9,28]. Another multicentric case control study reported a 12-fold increase in risk of gallbladder cancer in subjects with history of typhoid fever (12.7; 95% CI 1.5–598), which unfortunately could not be correlated with serological assays [29].

**EVIDENCE FROM CASE CONTROL STUDY**

The possible role of bacterial degradation of primary bile acids in gall bladder carcinogenesis was examined [30]. High performance liquid chromatography (HPLC) analysis for biliary bile acids, aerobic and anaerobic cultures were carried out in 10 patients, each with carcinoma gallbladder and cholelithiasis. Bacteria was seen in the bile of 4/10 (40%) patients with carcinoma gallbladder and 3/10 (30%) with cholelithiasis. Among the carcinoma patients, culture showed two aerobic, one anaerobic, and one mixed infection. Patients with carcinoma of gallbladder significantly had high level of secondary bile acids (lithocholate and deoxycholate) compared to cholelithiasis. Among the cancer patients, those having positive bile culture had a significantly higher level of secondary bile acids compared to culture negative patients (\(P < 0.001\)). Carcinoma gallbladder patients with negative bile culture too, had significantly high secondary bile acids compared to culture positive cholelithiasis (\(P < 0.0052\)) and culture negative cholelithiasis patients (\(P < 0.00056\)). The study suggested that bacterial degradation of primary bile acids in the gallbladder may be responsible for gall bladder carcinogenesis [30].

A case-control study from northern India was performed on patients with biliary diseases and healthy controls to detect typhoid carrier state [31]. Typhoid carrier state was detected in patients with cholelithiasis, carcinoma of gall bladder, and control. This was indeed the first study of its kind in an area of high endemicity for both typhoid infection and carcinoma of gall bladder. An indirect hemagglutination assay measuring antibodies against highly purified \(S.\) *typhi* Vi polysaccharide antigen was used. Vi polysaccharide positivity was significantly higher in patients with gall bladder cancer (29.4%)...
compared to controls (5%) ($\chi^2 = 6.325, P < 0.004, \text{OR} = 7.19$) and patients with cholelithiasis (10.7%) ($\chi^2 = 5.066, P < 0.01, \text{OR} = 3.86$). There was 8.47 times greater risk of developing carcinoma of gallbladder in culture positive typhoid carriers than in non-carriers. The study concluded that typhoid carrier state was a risk factor for gall bladder cancer development.

The role of chronic bacterial infection in gallbladder cancer was evaluated in a study where the evidence of Salmonella infection was found in serum, bile, and surgically removed gallbladder tissue specimens [32]. The methods applied included detection of Vi antibodies from serum, microbial culture from bile, and tissue detection of Salmonella DNA by PCR. A total of 395 samples from all the three components of study were obtained (gallbladder cancer—107, gall stone—118, and normal subjects—170). Vi antibodies were found to be significantly higher ($P < 0.001$) in gall bladder cancer patients (68%) compared to gallstone patients (44%) and normal subjects (28%). Microbial culture was performed on samples obtained from patients of gall bladder cancer (bile—31, tissue—23), gall stone (bile—11, tissue—47), and normal subjects (bile—0, tissue—7). Growth of Salmonella was not obtained in cultures of the tissue and bile owing probably to the use of antibiotics. Hence there was no demonstrable direct evidence. Presence of Salmonella DNA was tested by PCR in gall bladder cancer patients (bile—6, tissue—11) that were positive in two cases. It may be noted, however, that the low detection rate of Salmonella DNA by PCR could be due to inhibitory presence of bile in the tissue. Overall, the study concluded that chronic Salmonella carrier state is strongly associated with gall bladder cancer as well as gall bladder stones [32].

The bacteriological features were analyzed in the bile of controls, gall stones and carcinoma gall bladder patients [33]. Bile cultures were obtained from a total of 372 patients (controls—36, symptomatic gall stones—165, acute cholecystitis—46, common bile duct stone—67, cancer gall bladder—58). No pathogenic bacteria were grown from the bile of controls but positive bile culture was grown from the bile of symptomatic patients of gall stones 52/165 (32%), acute cholecystitis 19/46 (41%), common bile duct stones 39/67 (58%), and cancer gall bladder 47/58 (81%). The most commonly associated bacteria were Escherichia coli, Streptococcus faecalis, Klebsiella, and Enterobacter but Salmonella was uncommon in cancer gall bladder 4/58 (8.5%). However, more sensitive tests like scanning electron microscopy and molecular genetic techniques for chronic infection, were not used. Patients over the age of 60 years tended to be more likely to have organisms in their bile than patients aged 60 or less, and the difference was significant for symptomatic gall stone disease ($P < 0.003$). Significant differences were also found between patients with symptomatic gall stone disease and those with carcinoma gall bladder in both age groups ($P < 0.002$ in each case). The advanced age associated with positive bacterial culture signifies chronicity of infection in the process of carcinogenesis [33].

In yet another significant prospective study [34] conducted from northern India, cultures were obtained from the core of gall stones after breaking a freshly removed stone on a culture plate. The stone was sterilized at surface in order to remove the bile and culture the bacteria inside the stone. Of the 100 patients studied (chronic cholecystitis—85, cancer gall bladder—14, periampullary carcinoma—1) who underwent cholecystectomy, the results demonstrated positive culture in 81% of cholelithiasis and 77% of cancer gall bladder patients of which 51.5% were enteric and 20% non-enteric bacteria. Only enteric organisms were cultured from 11 patients of cancer gall bladder. The mean age of patients with cholelithiasis was lower than that of patients with cancer gall bladder (43.48 ± 14.07 vs. 57.45 ± 12.51, $P = 0.005$). The mean age of patients harboring enteric bacteria in their calculi was higher than that of patients harboring non-enteric bacteria (47.52 ± 12.8 vs. 36.57 ± 12.63, $P = 0.0069$). The Salmonella organism, however, was not isolated from inside the stone contrary to popular belief that bacteria inside gall stones are dead (gall stones as tombs). The gall stones were shown to harbor live bacteria from inside (gall stones as wombs). The authors did not simultaneously culture the bile or the surface of the gall stone. Therefore, the study, though important for the pathogenesis of gall stones, fails to throw light on its impact on the etiopathogenesis of cancer of the gall bladder. Also, the authors have not done a sub-group analysis of the bacteria grown depending upon the type of stone whether mixed or cholesterol [34].

**STUDIES ON HELICOBACTER SPECIES**

To see the association of Helicobacter species, the bile and gall bladder tissue of 46 patients with chronic cholecystitis who underwent cholecystectomy, was studied. [35] These specimens were cultured for Helicobacter species and subjected to PCR analysis taking Helicobacter-specific 16s ribosomal RNA primer. Recovery of Helicobacter species from frozen specimens was unsuccessful. However, using PCR analysis, 13/23 bile samples and 9/23 gall bladder tissues were found positive for Helicobacter. Eight of the Helicobacter-specific PCR amplicons were sequenced and subjected to phylogenetic analysis. Five sequences represented strains of H. bilis, two strains of Floxispira rappini, and one of H. pullorum. This study also supported an association of bile
### TABLE I. Studies on Infections as a Cause of Gallbladder Cancer: Methodology, Results, and Level of Evidence

<table>
<thead>
<tr>
<th>S No.</th>
<th>Authors</th>
<th>Methodology</th>
<th>Interpretation</th>
<th>Outcome level of evidence</th>
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<tr>
<td><strong>TABLE IA.</strong></td>
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<tr>
<td>1</td>
<td>Caygill et al., 1994 [27]</td>
<td>Risk of cancer biliary tract, lung, pancreas, colorectal, and others were studied in typhoid carriers</td>
<td>↑↑ Risk in GBC, colorectal, lung, and others</td>
<td>+++++</td>
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<tr>
<td>2</td>
<td>Strom et al., 1995 [29]</td>
<td>Risk of GBC in subjects with history of typhoid fever</td>
<td>↑↑ Risk of GBC in subjects with history of typhoid fever</td>
<td>++</td>
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<tr>
<td><strong>TABLE IB.</strong></td>
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<td>3</td>
<td>Pandey et al., 1995 [30]</td>
<td>Analysis of secondary bile acids, aerobic and anaerobic cultures was carried in 10 patients each with GBC and GS</td>
<td>+ Bile culture GS 3/10(30%) GBC ↑↑ level of sec. bile acids GBC (+ve culture) versus GS (–ve culture) → ↑↑ level of sec. bile acids in GBC (P &lt; 0.001) GBC (–ve culture) versus GS (+ve and –ve culture) → ↑↑ level of sec. bile acids in GBC patients (P &lt; 0.0052) and (0.000056) Bacterial degradation of primary bile acids in the gall bladder may be responsible for gall bladder carcinogenesis</td>
<td>+ +</td>
</tr>
<tr>
<td>4</td>
<td>Shukla et al., 2000 [31]</td>
<td>Case control study. Typhoid carrier state in patients of GS, GBC, and normal subjects using indirect hemagglutination assay measuring antibodies against highly purified S. typhi Vi polysaccharide antigen</td>
<td>Vi polysaccharide → significantly high in GBC (29.4%), Controls (5%) (χ² = 6.325, OR = 3.86, P &lt; 0.004) GS (10.7%) (χ² = 5.066, OR = 3.86, P &lt; 0.001) 8.47 times ↑ risk of GBC in carriers</td>
<td>++</td>
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<td>5</td>
<td>Singh et al., 2001 [32]</td>
<td>Vi antibodies, bile culture, and Salmonella DNA by PCR were detected in 395 patients (GBC—107, GS—118, Controls—170)</td>
<td>Vi antigen significantly high in GBC (68% P &lt; 0.001) versus GS (44%) and Controls (28%) —ve microbial culture, may be due to use of antibiotics. Low DNA detection rate probably due to inhibitory effect of bile.</td>
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<tr>
<td>6</td>
<td>Csendes et al., 1994 [33]</td>
<td>A case control study, bile culture were obtained from 372 subjects (Controls—36, acute cholecystitis—46, GS—165, CBD stone—67, GBC—58)</td>
<td>Microbial culture — Salmonella in 2 No culture were seen in controls Positive culture in Ac cholecystitis 19/46 (41%) CBD stone—39/67 (58%) GS 52/165 (32%) GBC 47/58 (81%) (P &lt; 0.001) Bacteria may have role in development of carcinoma gallbladder but S. typhi cultures were uncommon.</td>
<td>+ +</td>
</tr>
<tr>
<td>7</td>
<td>Hazrath et al., 2004 [34]</td>
<td>Prospective study in which bacterial cultures were obtained from the core of gall stones in 100 patients (GS and CBD stone—85, GBC—14, Periampullary cancer 1) who had cholecystectomy.</td>
<td>+ve cultures GS 81% GBC 77% Enteric 57% Non-enteric 20% No Salmonella was grown although this has long been considered a causative agent.</td>
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The association between Helicobacter species in bile and biliary tract malignancies in Japan and Thailand, was shown to have high incidence of bile duct carcinoma and gall stones [36]. Bile samples from 45 Japanese and 40 Thai patients were subjected to PCR analysis using *H. bilis*-specific primers and six of the *H. bilis* amplicons were sequenced. Japanese 13/15 (87%) and Thai 11/14 (79%) patients with bile duct or gall bladder cancer were positive for *H. bilis* in their bile. Of the Japanese 8/16 (50%) and Thai 10/26 (38%) patients with gall stone and/or cholecystitis tested positive for *H. bilis*. The comparative analysis of Japanese patients having bile duct and gall bladder cancer with non biliary disease demonstrated significantly high positive rates (*P* < 0.01) of *H. bilis*. The odds ratio for bile duct or gall bladder cancer with *H. bilis* in comparison with gall stone and/or cholecystitis were 6.5 (95% CI 1.09–38.63) in Japanese and 5.86 (95% CI 1.31–26.33) in the Thai patients. The study supports an association of *H. bilis* infection of bile with biliary tract and gall bladder cancer.

Some investigators in Osaka, Japan, studied archival gall bladder specimens from 34 patients (gall bladder cancer 11, bile duct cancer 3, cholelithiasis 16, and pancreatic cancer 4). DNA was extracted by PCR using 16S rRNA of *H. bilis* as primer. Amplification was observed in gall bladder cancer cases (3/11, 27.2%) and bile duct cancer (1/3, 33.3%). A total of 4 of 14 (28.6%) were positive for *H. bilis* in bile duct cancer. This was indeed suggestive of the possible role of *H. bilis* in biliary tract diseases, particularly biliary tract cancer.

In a study of 46 patients, the relationship between infection of different bacteria and gall bladder cancer was studied [38]. The bacterial gene fragments were detected in 28 patients (tissue from resected GBC—28, bile—18) by PCR. The positive rate of bacterial DNA in gall bladder cancer tissue was 36/46 (78.3%). Bacterial gene fragments of multiple kinds included *collibacillus* sp., *B. fragilis*, *klebsiella*, *C. perfringens*, and *Clostridium*. This study also indicates that gall bladder mucosa stimulated by anaerobic and aerobie bacteria might be a cause of the development of gall bladder cancer. The classical geographical distribution supports the hypothesis that areas with high endemicity of typhoid carriers and mixed gall stones have also reported higher prevalence of carcinoma of gall bladder. However, there

### Table IC

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Sample</th>
<th>PCR Analysis</th>
<th>Positive Rate</th>
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<tr>
<td>Fox et al., 1998 [35]</td>
<td>1998</td>
<td>Bile and gall bladder tissue</td>
<td>Of 46 patient of chronic cholecystitis was cultured for helicobacter and subjected to PCR analysis.</td>
<td>+ve Helicobacter amplicon Gallbladder 9/23 (39.1%)</td>
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<tr>
<td>Matsakura et al., 2002 [36]</td>
<td>2002</td>
<td>Bile samples from 45 Japanese and 40 Thais subjected to PCR analysis using <em>H. bilis</em> specific primers</td>
<td>Bile 13/23 (56.5%) Bile duct and GBC Japanese 13/15 (87%) → +ve for <em>H. bilis</em> Thai 11/14 (79%) → +ve for <em>H. bilis</em> GS/cholecystitis Japanese 8/16 (50%) Thai 10/26 (38%) tested +ve GBC/cholecystitis Japanese 8/16 (50%) → +ve <em>H. bilis</em> GBC (3/11, 27.2%), Biliary duct cancer (1/3, 33.3%) Biliary tract cancer (4/14, 28.6%)</td>
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<td>Muarta et al., 2004 [37]</td>
<td>2004</td>
<td>DNA extraction of <em>H. bilis</em> by PCR from 34 subjects (GBC—11, Bile duct cancer—3, GS—16, and Pancreatic cancer—4) for <em>H. bilis</em></td>
<td>+ve <em>H. bilis</em> GBC (3/11, 27.2%), Biliary duct cancer (1/3, 33.3%) Biliary tract cancer (4/14, 28.6%)</td>
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<tr>
<td>Lu Y et al., 2004 [38]</td>
<td>2004</td>
<td>The bacterial gene fragment was detected in 46 patients (tissue from resected GBC—28, bile—18) by PCR</td>
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</table>

**CONCLUSION**

The association between *Helicobacter* species and gall bladder disease including cancer was shown to have high incidence of bile duct carcinoma and gall stones [36]. Bile samples from 45 Japanese and 40 Thai patients were subjected to PCR analysis using *H. bilis*-specific primers and six of the *H. bilis* amplicons were sequenced. Japanese 13/15 (87%) and Thai 11/14 (79%) patients with bile duct or gall bladder cancer were positive for *H. bilis*. The odds ratio for bile duct or gall bladder cancer with *H. bilis* in comparison with gall stone and/or cholecystitis were 6.5 (95% CI 1.09–38.63) in Japanese and 5.86 (95% CI 1.31–26.33) in the Thai patients. The study supports an association of *H. bilis* infection of bile with biliary tract and gall bladder cancer.
may be areas of high prevalence of communicable diseases like salmonella and helicobacter infectivity where cancer prevalence rates have not been studied. The best evidence so far has come from a long-term cohort study reported from an otherwise low prevalence area. The case control studies utilizing bacterial cultures, HPLC, detection of carrier state by anti-bodies measurements, PCR, etc. have shown convincing evidence to prove the hypothesis. What remains to be shown are the time trend changes in the occurrence of cancer gall bladder? Control over communicable diseases should result in reducing the rates of incidence of gall bladder cancer. Bacterial degradation of bile and chronic inflammation, may be responsible in the pathogenesis of cancer gall bladder. The possible carcinogenic mechanism involved on account of chronic infection has not yet been clarified. It is important to note that salmonella species have β glucuronidase activity. The hepatic inactivation of exogenous carcinogenic xenobiotics by glucoronidation could be reversed after bacterial hydrolysis of β glucuronides. Like the paradigm shift in gastric cancer, now considered to be caused by H pylori infection, the recognition of biliary tract infections in the etio-pathogenesis of gall bladder cancer, may help in re-shaping the present knowledge. Nevertheless, the jury of medical experts in the world community will allow cancere-en-bacteria theory, only after a few more convincing studies in this area. The studies on genes and viruses in hepatobiliary neoplasia were described and multiple tumor suppressor genes and oncogenes including P53 and K-ras were shown to be altered in biliary tract cancers. Furthermore, molecular characterization of hepatobiliary cancers in case control models, in patients with chronic gall bladder infections, may lead to better understanding of the role of infection in the pathogenesis of gall bladder cancer. The bio-medical technologies of genomics and transcriptomics, which examine genetic complement and gene expression, respectively; the technique of proteomics, which involves the analysis of protein synthesis and metabolomics that provides the global determinations of bio-chemical profiles in body fluids and tissues, are yet to be utilized seriously in the understanding of physio and morpho pathology of carcinoma gall-bladder.

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