Epidemiology and Molecular Pathology of Gallbladder Cancer

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ABSTRACT Gallbladder cancer is usually associated with gallstone disease, late diagnosis, unsatisfactory treatment, and poor prognosis. We report here the worldwide geographical distribution of gallbladder cancer, review the main etiologic hypotheses, and provide some comments on perspectives for prevention. The highest incidence rate of gallbladder cancer is found among populations of the Andean area, North American Indians, and Mexican Americans. Gallbladder cancer is up to three times higher among women than men in all populations. The highest incidence rates in Europe are found in Poland, the Czech Republic, and Slovakia. Incidence rates in other regions of the world are relatively low. The highest mortality rates are also reported from South America, 3.5-15.5 per 100,000 among Chilean Mapuche Indians, Bolivians, and Chilean Hispanics. Intermediate rates, 3.7 to 9.1 per 100,000, are reported from Peru, Ecuador, Colombia, and Brazil. Mortality rates are low in North America, with the exception of high rates among American Indians in New Mexico (11.3 per 100,000) and among Mexican Americans.

The main associated risk factors identified so far include cholelithiasis (especially untreated chronic symptomatic gallstones), obesity, reproductive factors, chronic infections of the gallbladder, and environmental exposure to specific chemicals. These suspected factors likely represent promoters of carcinogenesis. The main limitations of epidemiologic studies on gallbladder cancer are the small sample sizes and specific problems in quantifying exposure to putative risk factors. The natural history of gallbladder disease should be characterized to support the allocation of more resources for early treatment of symptomatic gallbladder disease in high-risk populations. Secondary prevention of gallbladder cancer could be effective if supported by cost-effective studies of prophylactic cholecystectomy among asymptomatic gallstone patients in high-risk areas.

INTRODUCTION

Gallbladder cancer was first described in 1777. More than 200 years later, late diagnosis and absence of effective treatment for many patients remain typical features of this disease. The prognosis is poor—only about a 32 percent five-year survival rate for lesions confined to the gallbladder mucosa and a 10 percent one-year survival rate for more advanced stages. The highest frequency of the disease is found among females over the age of 65. There is a marked regional and ethnic
variation in the incidence of gallbladder cancer. The highest mortality rates have been reported among Chilean Mapuche Indians and Hispanics, among Bolivians, North American Indians, and Mexican Americans. Incidence rates are much lower in Europe and India.

There is a clear worldwide association between chronic cholelithiasis and gallbladder cancer. Aside from gallstones and female gender, a number of associated risk factors appear to favor the development of gallbladder cancer either as neoplastic initiators, such as unknown endo- and exobiotic mutagens, or as neoplastic promoters, including chronic inflammation and infection. Among the latter factors, a link has been specifically proposed between chronic bacterial infection of the gallbladder with Salmonella typhi. In this review, we analyze the worldwide geographical distribution of gallbladder cancer and the main etiologic hypotheses. We also provide comments on molecular pathology, associated risk factors, and potential for prevention—particularly in high-risk populations.

DESCRIPTIVE EPIDEMIOLOGY

Source of Data for the Estimation of Incidence Rates

Specific age-adjusted incidence rates (standardized for the world population) were obtained from some countries from Volume VII of Cancer Incidence in Five Continents and from selected reports where pertinent. According to the Ninth Revision of the International Classification of Diseases (ICD-9), cancers of the biliary tract include tumors of the gallbladder (156.0), extrahepatic bile ducts (156.1), the ampulla of Vater (156.2), other and biliary tract, part unspecified (156.8-9). There is little geographical variation in the incidence of tumors of the extrahepatic bile ducts and the ampulla of Vater. Greater geographical variation is observed for gallbladder cancer and for other tumors as well as biliary tract cancer not otherwise specified (NOS), which are classified together.

In autopsy studies throughout the world, gallbladder cancer represents 80 to 95 percent of cancers of the biliary tree. We combine rates for tumors of the biliary tract NOS (156.8-156.9) with those of gallbladder cancer (156.0), since it is likely that a significant fraction of NOS cases were indeed gallbladder cancer.

The cancer registries fulfilling the following criteria were included in the present review: 1) availability of incidence rates in the period under study (1988 to 1992); 2) a minimum of 10 cases reported; and 3) representation of the highest rates for a given country. The selected registries as were listed in rank order for gallbladder cancer in various regions of the world based on the incidence among females.

A total of approximately 30 areas from five continents were assessed. Unpublished data provided by the Cancer Registries in La Paz, Bolivia, and Asuncion, Paraguay were included with the permission of the corresponding registries, as were data published from Northern India. There were no incidence rates of gallbladder cancer registered in Chile—the country with the highest mortality rate of gallbladder cancer in the world. Pooled age-specific incidence rates for Latin America were calculated using data from six cancer registries: Trujillo, Peru; Quito, Ecuador; Cali, Colombia; Porto Alegre, Brazil; Costa Rica; and Montevideo, Uruguay. Data from 10 registries from the SEER program in the US and from 12 Canadian Registries included in Volume VII of Cancer Incidence in Five Continents were included for North America.

There is substantial geographic variability of gallbladder cancer incidence as shown in Figure 1. The rates for gallbladder cancer are higher among women than men in all
The rates are highest in women from La Paz, Bolivia (15.5 per 100,000). Intermediate rates (from 3.7 to 9.1 per 100,000) are reported in Trujillo, Peru; Quito, Ecuador; Cali, Colombia; Porto Alegre, Brazil; and Montevideo, Uruguay. In North America, low rates predominate, with the exception of high rates reported among Indians in New Mexico (11.3 per 100,000) and intermediate rates among female immigrants from Latin America.

In Europe, the highest incidence is found in the countries of Eastern Europe: Poland (Kracow), the Czech Republic, and Slovakia. Likewise, population-based data indicate that the incidence of gallbladder cancer is relatively high in northern Indian cities (Uttar Pradesh, Bihar, Orissa, West Bengal, and Assam). Except for some high-risk areas such as Nagasaki, Japan, the rates in other regions of the world are relatively low.

In males, the highest incidence rates of gallbladder cancer are reported from La Paz, Bolivia (7.5 per 100,000) and Quito, Ecuador.
The highest female/male ratios (≥ 3) were seen in Porto Alegre, Brazil (4.69), in Israel (3.6), and among Hispanics in Central California (3.0). The female predominance was less marked in Nagasaki, Japan, the UK (England and Wales), and among black people in the US. The incidence increases progressively with age, both in males and females, in all populations included in the survey. The increase in males is more marked after 50 years of age in Latin America.

In summary, there is prominent geographic variability of gallbladder cancer incidence, which correlates with the prevalence of gallstones. The populations with the highest incidence are Chileans, Bolivians, North American Indians, Mexican-Americans, and Central Europeans, all of whom also have high prevalence of cholelithiasis. The high rates observed in Latin America are primarily in populations with high levels of Indian mixture, supporting the concept that increased susceptibility depends on genetic factors that predispose people to gallbladder cancer either as primary factors, or secondarily as promoters by favoring the development of cholesterol gallstones.

**TRENDS IN AGE-ADJUSTED MORTALITY RATES**

Mortality trends in the age-adjusted rates (standardized using the world population) over a 16-year period (1980 to 1995) were evaluated in twelve countries included in the World Health Organization (WHO) database (US, Canada, Hungary, United Kingdom, Italy, Austria, France, Spain, Japan, and Australia), and over a 10-year-period in Chile (1982 to 1991, standardized by the Chilean population of 1985). In Trujillo, Peru; Quito, Ecuador; Cali, Colombia; Porto Alegre, Brazil; Latina, Italy; and Costa Rica, approximately 20 percent of gallbladder cancer and biliary tract tumors in women were classified as NOS. In contrast, in Nagasaki, Japan; among Hispanics of Central California; in Haut Rhin, France; Graubunden, Switzerland; Croatia; Shanghai, China; and in the UK (England and Wales) this proportion was less than five percent (Table 1). In Chile, 80 percent of adenocarcinoma of the biliary tree originates in the gallbladder, as assessed by pathological criteria.

These gross differences probably reflect variations in the clinical or pathological criteria used to identify the exact origin of the adenocarcinoma of the biliary tree, which in the majority of the cases can only be diagnosed through laparotomy or necropsy. In spite of these difficulties, there is general agreement in the literature that the majority of adenocarcinoma of the biliary tree originates in the gallbladder mucosa.

The highest mortality rate of gallbladder cancer in the world, 35 per 100,000 inhabitants, is found in Southern Chile (the region of the Araucania), which is inhabited by Chilean Hispanics and Mapuche Indians. This high mortality rate contrasts with the mean of the whole country, which had an age-adjusted mortality rate of 16.2 for women and 5.4 per 100,000 for men in 1991. These rates have been inversely correlated with the rates of cholecystectomy during the 1980s and early 1990s. Whereas the cholecystectomy rate was 44 percent among Chilean Hispanics with gallbladder disease (including cholelithiasis and cholecystectomy); the cholecystectomy rate for Mapuche Indians from Southern Chile with gallstones was only 8.6 percent.

Analysis of mortality trends of a series of nine countries is shown in Figures 2 and 3. In the US, Canada, and Australia, mortality rates for both men and women decreased over the 16-year period under study; while in areas with high risk—such as in Japan—an increase is observed. Trends in mortality from gallbladder cancer for four European countries are...
### Table 1

| Cancer Registry                      | Gallbladder Cancer | Other and not specified | Total          | Female/Male Ratio |
|--------------------------------------|-------------------|-------------------------|----------------|------------------|
|                                      |       |                        | 156.8-9        | 156.0 + 156.8-9 |
|                                      | Males | Females                | Males | Females | Males | Females |
| New Mexico, US, American Indians     | 3.8   | 10.3                    | 0     | 1.0     | 3.8   | 11.3     | 2.97 |
| Setif, Algeria                       | 3.6   | 9.8                     | 0.3   | 0.8     | 3.9   | 10.6     | 2.71 |
| Trujillo, Peru                        | 2.3   | 6.9                     | 1.0   | 2.2     | 3.3   | 9.1      | 2.75 |
| Quito, Ecuador                       | 2.1   | 5.5                     | 2.0   | 2.2     | 4.1   | 7.7      | 1.80 |
| Cali, Colombia                       | 1.2   | 4.9                     | 1.6   | 2.6     | 2.8   | 7.5      | 2.67 |
| Krakow, Poland                       | 3.1   | 5.9                     | 0.6   | 0.6     | 3.7   | 6.5      | 1.75 |
| Porto Alegre, Brazil                 | 0.7   | 3.9                     | 0.6   | 2.2     | 1.3   | 6.1      | 4.69 |
| Czech Republic                       | 2.6   | 5.1                     | 0.4   | 0.6     | 3.0   | 5.7      | 1.90 |
| Slovakia                             | 1.8   | 5.0                     | 0.3   | 0.3     | 2.1   | 5.3      | 2.52 |
| Latina, Italy                        | 1.8   | 3.9                     | 0.6   | 1.0     | 2.4   | 4.9      | 2.04 |
| Nagasaki, Japan                      | 2.7   | 4.4                     | 0.3   | 0.2     | 3.0   | 4.6      | 1.53 |
| Costa Rica                           | 1.0   | 3.2                     | 1.0   | 1.3     | 2.0   | 4.5      | 2.25 |
| Granada, Spain                       | 1.7   | 3.5                     | 0.5   | 0.6     | 2.2   | 4.1      | 1.86 |
| Northwest Territories, Canada        | 2.1   | 4.0                     | 0     | 0       | 2.1   | 4.0      | 1.90 |
| Montevideo, Uruguay                  | 1.2   | 3.0                     | 0.6   | 0.7     | 1.8   | 3.7      | 2.05 |
| Central California, US, Hispanics    | 1.0   | 3.5                     | 0.2   | 0.1     | 1.2   | 3.6      | 3.00 |
| Sweden                               | 1.2   | 2.9                     | 0.3   | 0.4     | 1.5   | 3.3      | 2.20 |
| Haut Rhin, France                    | 1.4   | 2.9                     | 0.1   | 0       | 1.5   | 2.9      | 1.93 |
| Graubunden, Switzerland              | 0.9   | 2.5                     | 0.1   | 0       | 1.0   | 2.5      | 2.50 |
| Croatia                              | 1.0   | 2.3                     | 0     | 0.1     | 1.0   | 2.4      | 2.40 |
| Shanghai, China                      | 1.2   | 2.2                     | 0.1   | 0.1     | 1.3   | 2.3      | 1.77 |
| South Australia                      | 0.7   | 2.0                     | 0.2   | 0.2     | 0.9   | 2.2      | 2.44 |
| Israel, All Jews                     | 0.4   | 1.7                     | 0.1   | 0.1     | 0.5   | 1.8      | 3.60 |
| Canada                               | 0.7   | 1.2                     | 0.1   | 0.1     | 0.8   | 1.3      | 1.62 |
| US SEER, Whites                      | 0.4   | 1.0                     | 0.1   | 0.1     | 0.5   | 1.1      | 2.20 |
| US SEER, Blacks                      | 0.6   | 0.8                     | 0.1   | 0.1     | 0.7   | 0.9      | 1.28 |
| UK (England, Wales)                  | 0.4   | 0.6                     | 0.1   | 0       | 0.5   | 0.6      | 1.20 |

Source: Parkin DM, Whelan SL, Ferlay J, Raymond L, and Young J. Eds. Cancer Incidence in Five Continents, Vol. VII. (IARC Scientific Publications No. 143). International Agency for Research on Cancer, Lyon, France, 1997.
summarized in Figure 2. In countries with the highest rates (Hungary) and with the lowest rates (United Kingdom), a marked decline (especially in females) is observed. In Italy and Spain, two other countries with low rates, little variation is observed.

On the other hand, one of the higher mortality rates in the world was observed in Chile (10.8 per 100,000 in 1991) with a marked increase during the period from 1982 to 1991 as shown in Figure 3, a trend concurrent with declining cholecystectomy rates during the same period.20 This last factor was probably a consequence of the health policy that reallocated resources from the treatment of adult chronic diseases to pediatric and obstetrical health care.17

MORPHOLOGY AND PATHOGENESIS

Although it has been established that dysplasia and carcinoma in situ21 precede most gallbladder carcinomas, relatively little is known about the natural history of these precursor lesions. Most dysplasias and carcinomas in situ are diagnosed after cholecystectomy when the entire lesion is removed.22 However, there is indirect evidence that progression could occur from precursor lesions to infiltrating carcinoma. Dysplasia and carcinoma in situ are found in the mucosa adjacent to most carcinomas of the gallbladder, sometimes separated by histologically normal epithelium.22,23

*Age-standardized rate per 100,000.
Patients with dysplasia and carcinoma in situ are 15 and 5 years younger, respectively, than those with invasive carcinoma. If multiple sections of gallbladders removed for cholelithiasis are examined, dysplasia and carcinoma in situ are detected in 13.5 percent and 3.5 percent of the cases, respectively. 

There is consensus that if dysplasia and carcinoma in situ are found, multiple additional sections of the gallbladder should be examined to rule out invasive cancer.

Over 90 percent of gallbladder carcinomas are adenocarcinoma. On gross examination, approximately 10 to 37 percent of the gallbladder carcinomas cannot be identified with certainty, and their macroscopic findings are similar to those of chronic cholecystitis. Gallstones are found in almost all cases of gallbladder cancer (78 percent to 85 percent). Most carcinomas (60 percent) originate in the fundus of the gallbladder, 30 percent in the body, and 10 percent in the neck. The prevalence of gallbladder cancer associated with diffuse calcification of the gallbladder (so-called porcelain gallbladder) is 12 to 21 percent. Most gallbladder cancers are well-to-moderately differentiated adenocarcinomas. Some of these are papillary lesions that grow predominantly into the lumen of the gallbladder.

The main prognostic factor for gallbladder carcinoma is the clinical or pathologic stage. Two staging systems for gallbladder carcinoma have been widely used. In 1976, Nevin et al. proposed a staging system in which Stage I cancer is limited to the mucosa; Stage II to the...
muscular layer; and Stage III to the peri-muscular layer. Stage IV shows metastases in the lymph nodes; and Stage V has hepatic or other distant metastases. There is a correlation between level of tumor invasion in the gallbladder wall and the presence of lymph node metastases.24,27

In a large series of patients with gallbladder carcinoma,24 no lymph node metastases were detected in gallbladder tumors invading the muscularis only, whereas 62 percent of the tumors invading the serosa showed regional lymph node metastasis. Patients with localized stage disease have much better survival rates (Stages I to III, five-year survival rate of 41.9 percent) than those with regional (Stage IV, 3.8 percent) or distant (Stage V, 0.7 percent) metastasis.27,28 Similar results have been obtained using the TNM staging system of the International Union Against Cancer (UICC) and American Joint Committee on Cancer (AJCC), which has proven to be a good system for comparison of surgical results and prediction of patient outcome.29,30

Briefly, under the TNM classification: Stage I is a tumor limited to the mucosa or muscular layers; Stage II tumors invade the peri-muscular tissue; Stage III tumors invade serosa, liver less than two centimeters, or have regional (hepato-duodenal ligament) lymph node metastasis; and Stage IV shows liver invasion greater than two centimeters (Stage IVA), or metastasis to nonregional lymph nodes and/or distant organs (Stage IVB).

The gallbladder’s very thin wall and the discontinuous muscular layer are believed to facilitate tumor invasion and contribute to the advanced local and regional disease usually present at the time of diagnosis.35

Tsukada et al.31 reported that the five-year survival rate in patients with TNM Stage I tumors was 91 percent; 85 percent in patients with Stage II tumors; 40 percent in patients with Stage III tumors; and 19 percent in patients with Stage IV tumors. In the same study, in patients with TNM Stage III and Stage IV tumors the five-year survival rate was 52 percent after curative resection. This was significantly better than the five percent five-year survival rate after a noncurative resection.

Molecular Pathology

While considerable progress has been made in understanding the molecular pathogenesis of several human neoplasms, information about the genetic changes involved in gallbladder carcinogenesis is more limited.32 Most of these studies have focused on ras,33 TP53, and p16^ink4/CDKN2 gene abnormalities and deletions (“loss of heterozygosity”) at several chromosomal regions harboring known or putative tumor suppressor genes.32,34-35

The reported prevalence rates of ras gene mutations in series of gallbladder carcinomas is quite variable.3,34,36-37 While ras mutations were not detected in some small series,3 two other groups reported greater of K-ras mutations in 39 to 59 percent of patients.34,38 All the mutations occurred at codon 12 of the K-ras gene.34,36-37 However, this site has been the most intensively analyzed ras gene region. A greater frequency (50 to 83 percent) of K-ras gene mutations has been reported in gallbladder carcinomas from patients having anomalous junction of the pancreatico-biliary duct39-42 suggesting that reflux of pancreatic juice might contribute to the carcinogenic process.

TP53 gene abnormalities are frequent in gallbladder cancers.33 Although the frequency of p53 immunostaining in gallbladder carcinoma varies widely (ranging from 35 to 92 percent), two thirds of the studies show a frequency greater than 50 percent.39,44-60 Most of the TP53 mutation studies on gallbladder carcinoma have confirmed the immunohistochemical findings.39,50,56,59,60 Analyses of exon 5 to 8 of TP53 have demonstrated point mutations in 31 to 70 percent of gallbladder
carcinomas, and no particular “hot spot” has been identified.

In addition, deletions at the TP53 locus (17p13) have been frequently (58 to 92 percent) reported in gallbladder carcinomas, indicating that the inactivation of the TP53 plays an important role in the pathogenesis of this neoplasm. Yokoyama et al. compared the TP53 mutations in gallbladder carcinomas from two high-prevalence areas, Japan and Chile. No differences in the frequencies of TP53 mutations between both groups were detected. However, mutations from Japanese cases comprised transversions in 31 percent of cases with 46 percent of all mutations taking place at the A:T pair.

Whereas, in contrast, the TP53 mutation spectrum for Chilean cases demonstrated a very high incidence of transitions (100 percent) and of mutations at G:C pairs. While no Japanese cases showed G:C to A:T transitions at CpG sites, these were relatively frequent (33 percent) in Chilean cases. Frequent transitions, especially at CpG sites, are features of mutational spectra found in cancers not strongly linked to specific exogenous carcinogens. There are a few studies that suggest that CDKN2 gene, also known as MTS1 or p16INK4, may play a role in gallbladder carcinogenesis. Deletions at the CDKN2 region (9p21) have been reported in half of gallbladder cancers. However, there are no comprehensive studies at present on the CDKN2 gene status and the mechanism of inactivation in gallbladder carcinoma, including deletion, mutation, methylation, homozygous deletions, and protein expression analysis.

Although no comprehensive genome-wide allelotyping of gallbladder carcinoma has been published, two reports suggest that several chromosomal regions harboring known or putative tumor suppressor genes may undergo deletion in this neoplasm. These chromosomal regions, other than TP53 and CDKN2 gene loci, are 3p (20 to 52 percent); 5q21 (APC-MCC genes, 6 to 66 percent); 8p22-24 (22 to 44 percent); 13q14 (RB gene, 20 to 30 percent); and 18q22 (DCC gene, 18 to 31 percent).

The exact sequence of molecular changes that lead to neoplastic transformation in the gallbladder epithelium remains uncertain. More detailed understanding of the earliest molecular abnormalities may eventually provide methods for risk assessment and early detection of gallbladder carcinoma. The excess accumulation of p53 protein in gallbladder dysplasia (0 to 32 percent) and carcinoma in situ lesions (45 to 86 percent) suggests that TP53 abnormality is an early event. The presence of deletions at the TP53 locus in histologically normal epithelium near gallbladder carcinoma, and of TP53 mutations in precursor lesions, indicate an early and important role of TP53 inactivation.

Other early genetic changes include loss of heterozygosity at 9p21 (CDKN2 gene) and 18q21 (DCC gene) regions. Data regarding K-ras gene mutations in precursor lesions are controversial. While some studies fail to demonstrate K-ras mutation in precursor gallbladder lesions, others report a relatively high prevalence (22 to 44 percent) in precursor lesions accompanying invasive tumors. Interestingly, no differences in the frequency and spectrum of K-ras gene mutations were detected in nonmalignant gallbladder epithelium of patients with anomalous junction of the pancreato-biliary duct, with and without gallbladder carcinoma in Japan. The role of adenomas as precursors of gallbladder carcinoma is still unclear and controversial. Molecular analyses of gallbladder adenomas failed to detect the genetic changes frequently found in gallbladder carcinomas and their known precursor lesions, suggesting that adenomas might not be precursors of this neoplasm.
Among other risk factors, a number of genetic, dietary factors, endo- and exobiotics, and chronic gallbladder infections, have been associated with the development of gallbladder cancer. However, the primary risk factor of gallbladder cancer as reported in all studies is gallstone disease.\(^1\) Gallbladder cancer has also been associated with multiple familial polyposis/Gardner syndrome,\(^64\) Peutz-Jeghers syndrome,\(^65\) “porcelain” gallbladder,\(^66\) and anomalous pancreato-biliary ductal union.\(^67\) There has also been a consistently higher risk in women than in men, independent of cholelithiasis.\(^3\)\(^,\)\(^4\)\(^,\)\(^8\)\(^,\)\(^12\)

Gallbladder Cancer and Gallstones

The association between cholelithiasis and gallbladder cancer has been known since 1861\(^68\)\(^-\)\(^69\) and is supported by autopsy studies, screening surveys, and hospital-based case-control studies (Table 2).\(^1\)\(^-\)\(^6\)\(^,\)\(^8\)\(^-\)\(^14\)\(^,\)\(^50\)\(^-\)\(^70\) Cholelithiasis is more frequent in gallbladder cancer than in extrahepatic bile ducts cancer. In a recent case-control study performed in Australia, Canada, Holland, and Poland, a history of gallbladder symptoms requiring medical attention was identified as one of the major risk factors for gallbladder cancer.\(^73\)

This association was described in patients who had shown clinically symptomatic gallbladder disease for at least 20 years prior to gallbladder cancer diagnosis.\(^70\)\(^,\)\(^71\)\(^,\)\(^73\) The theoretical basis for this phenomenon is that the inflammation, chronic trauma, and infection in approximately one third of gallstone patients promotes epithelial dysplasia and adenocarcinoma formation.\(^79\) For this reason, it has been suggested that larger stones have a greater impact on the risk of developing gallbladder cancer, possibly reflecting greater duration and intensity of epithelial irritation. Diehl reported that in subjects with gallstones larger than three centimeters, the risk of gallbladder cancer is 10 times greater than in subjects with gallstones smaller than one centimeter.\(^80\) Warren et al.\(^81\) reported a larger mean stone diameter among 19 subjects with gallbladder cancer (20.3 mm) when compared with 883 subjects undergoing surgery for gallstones (11.9 mm). In contrast, Moerman et al.\(^82\) found no association between stone size and gallbladder cancer.

Cholesterol gallstones represent approximately 80 to 90 percent of all gallstone cases in the Western world and are considered to be a promoting factor. There is little available information as to whether pigment or cholesterol stones have a different activity as promoters of gallbladder cancer development.

Some constituents of bile might be endogenous carcinogens.\(^83\) It has been postulated that mutagenic endobiotic derivatives, presumably from sterol bacterial metabolites, might be the putative initiators. Mutagenic factors were isolated from stone extracts of a patient with a symptomatic choledochal cyst (a high-risk precancerous condition) with chronic bacterial infection of the biliary tree.\(^84\)

Only a small fraction (less than one percent) of patients with cholelithiasis develop gallbladder cancer,\(^85\) and approximately 20 percent of gallbladder cancer patients show no evidence of previous cholelithiasis.\(^86\) However in high-risk areas, this figure is certainly higher and increases markedly with age.\(^8\)\(^-\)\(^12\)

Gallstone Predisposition

The etiology of cholesterol cholelithiasis, like any other chronic disease, is thought to involve the interaction of genetic and environmental factors.\(^17\)\(^,\)\(^20\)\(^,\)\(^87\) The risk factors for cholesterol gallstones have been mainly associated with hypersecretion of biliary cholesterol, gallbladder hypomotility, and stasis.\(^88\) These conditions are positively correlated with age, female sex, genetic
factors, obesity, multiple pregnancies, a family history of gallstones, and low levels of physical activity.\textsuperscript{89-101} Obesity and high-energy intake have been positively associated with the risk of gallstones in cohort\textsuperscript{102} and case-control\textsuperscript{103} studies. Conversely, coffee\textsuperscript{99} and alcohol\textsuperscript{91,97,101} were associated with a decreased risk of gallstones in some studies. There is evidence that endogenous\textsuperscript{104} and exogenous estrogens\textsuperscript{105} and pregnancy increase the risk of cholelithiasis.\textsuperscript{106}

Most environmental factors, including diet, have been considered a consequence of the westernization of modern societies.\textsuperscript{87,99} The association of diet and gallbladder cancer remains unclear. Most likely, dietary factors might influence the production of gallbladder

| Site and Author | Population | Risk Factors | OR (CI 95%) |
|-----------------|------------|--------------|-------------|
| **Cohort Studies** |            |              |             |
| Denmark | 60,176 | Gallstones | Years of follow-up |
| Chow et al. 1999\textsuperscript{85} | Danish Cancer Registry | | 1-4 years 4.6 (3.0-6.7) |
| | | | > 4 years 2.7 (1.5-4.4) |
| **Case-Control Studies** |            |              |             |
| New York, US | 69 cases | Cholelithiasis and biliary tract cancer | 19.5 (6.4-59.4) |
| Khan et al. 1999\textsuperscript{75} | 138 controls | | |
| Australia, Canada, Netherlands, and Poland | 196 cases | History of gallbladder symptoms | 4.4 (2.6-7.5) |
| Zatonski et al. 1997\textsuperscript{76} | 1515 controls | Symptoms 20 years earlier | 6.2 (2.8-13.4) |
| Bolivia and Mexico | 84 cases | Family history of gallstones | 3.6 (1.3-11.4) |
| Strom et al. 1995\textsuperscript{7} | 126 controls without gallstones | | |
| WHO Collaborative Study 1989\textsuperscript{92} | 58 cases | Gallstones | 2.3 (1.2-4.4) |
| | 355 controls | | |
| **Cross-Sectional Studies** |            |              |             |
| Kofu, Japan | 194,767 | Gallstones | Increased risk |
| Okamoto et al. 1999\textsuperscript{74} | Screening surveys | | p < .01 |
| Tokyo, Japan | 4,482 autopsy surveys | Gallstones | Increased risk |
| Kimura et al. 1989\textsuperscript{68} | | | p < .01 |
| Chile | 14,768 autopsy surveys | Gallstones | 7 |
| Nervi et al.\textsuperscript{a} 1988 | | | p < .05 |
| Rochester, Minnesota | 2,583 screening surveys | Gallstones | Increased risk in men |
| Maringhini et al. 1987\textsuperscript{n} | | | p < .05 |
cancer through potential effects on cholesterol gallstone formation. The principal independent factors associated with gallstone disease in several studies were female sex, greater age, high BMI, slightly higher serum glucose, increasing parity, and low plasma HDL cholesterol.\textsuperscript{17,18,107-109}

**Genetic Epidemiology of Gallbladder Diseases**

There are some reports of familial tendencies toward gallbladder cancer, although they are based on a very small number of patients.\textsuperscript{110} A potential relationship between genetic predisposition and gallbladder cancer incidence has been suggested by population studies of gallstone prevalence in different latitudes.\textsuperscript{108-110} A recent study including three ethnic groups (Mapuche Indians and Hispanics from Chile and Maoris from Easter Island)\textsuperscript{17} concluded that cholesterol lithogenic genes are highly prevalent among Chilean Indians and Hispanics—populations with the highest mortality rates of gallbladder cancer.\textsuperscript{9}

These studies strongly supported the thesis that genetic factors play a major role in some specific populations by favoring the production of lithogenic bile.\textsuperscript{88} The genetic hypothesis of cholesterol gallstone formation has also been supported by family studies.\textsuperscript{111} Specific polymorphisms of apoproteins and plasma cholesterol ester transfer proteins seem to be correlated with cholesterol cholelithiasis.\textsuperscript{112}

**Chronic Gallbladder Infection**

During the last two decades, epidemiological evidence has linked chronic infections with several cancers; examples include hepatitis B and hepatitis C viruses with liver cancer; H. pylori and gastric cancer; and liver flukes with cholangiocarcinoma.\textsuperscript{113,114} Chronic infection by Salmonella typhi has been associated with an increased risk of gallbladder cancer. Caygill et al.\textsuperscript{115,116} hypothesized that typhoid and paratyphoid chronic carriage with concomitant gallstone formation favors mixed bacterial infection and higher risks of gallbladder carcinogenesis. Singh et al.\textsuperscript{117} proposed that the Salmonella carrier state has a significant association with gallbladder cancer independent of gallstones.

However, the possible carcinogenic mechanism involved has not yet been clarified. It is important to note that Salmonella sp. have β-glucuronidase activity, therefore hepatic inactivation of exogenous carcinogenic xenobiotics by glucuronidation could be reversed after bacterial hydrolysis of β-glucuronides.\textsuperscript{118} Strom et al.\textsuperscript{10} reported from Bolivia and Mexico a 12-fold increase in risk of gallbladder cancer in subjects with a history of typhoid fever (CI 95 percent 1.5-598), which unfortunately could not be corroborated with serological assays. Additionally, Nath et al.,\textsuperscript{119} in cultures of bile samples, found Salmonella typhi more frequently in patients with gallbladder cancer than in subjects with gallstones and free of biliary neoplasia.

**Occupation**

Increased risk of gallbladder cancer has been related among workers in the oil, paper, chemical, shoe, textile, and cellulose acetate fiber manufacturing industries suggesting occupational exposure to carcinogens.\textsuperscript{120,121} A higher risk of gallbladder cancer was also found among miners exposed to radon.\textsuperscript{122}

**PREVENTION AND FUTURE TRENDS**

Primary prevention of gallbladder cancer is unlikely in the near future, since many etiologic factors remain unknown. However, prevention of cholesterol gallstone formation is an important intervention that would certainly have a great impact in decreasing the incidence
of gallbladder cancer, particularly in high-risk areas. Availability of adequate and prompt laparoscopic cholecystectomy for symptomatic gallstone patients should be effective for secondary prevention.

Relationship between Cholecystectomy Rates and Gallbladder Cancer Incidence Rates

Cholecystectomy is the most frequent of all intra-abdominal operations. It is estimated that in the US more than 550,000 cholecystectomies are performed yearly within an estimated population of 20,000,000 at-risk patients with asymptomatic gallstones.123 The US cholecystectomy rate was 47.6 per 1000 inhabitants in 1993, whereas the rate was only 25.1 per 1000 inhabitants in 1992 in Chile, in spite of a three to four times higher prevalence of cholelithiasis.9,20,124 The incidence of gallbladder cancer has diminished considerably, in an inverse correlation to the increase in cholecystectomies in developed countries.125-127

In Chile, while the reported cholecystectomy rates decreased in the 1980s, time trends revealed an increase in the incidence of gallbladder cancer.9,20,124 In addition, the extremely low frequency of cholecystectomies found among Mapuche Indians with gallstones compared with Chilean Hispanics and Maoris17 might explain the high mortality rates of gallbladder cancer in Southern Chile in areas inhabited by Mapuche Indians. A descriptive survey published in 19959 showed that gallbladder cancer mortality rates were over four times higher in towns with a high proportion of Mapuche Indians (35 per 100,000 inhabitants in the region of the Araucania, Southern Chile), compared with towns with a predominantly Hispanic population (8 per 100,000 inhabitants in the municipality of La Florida, Santiago).9

Elective Laparoscopic Cholecystectomy for Secondary Prevention of Gallbladder Cancer

Three studies have analyzed the potential benefits of prophylactic cholecystectomy for low-risk populations. Based on decision analysis models, it was concluded that the benefits of prophylactic cholecystectomy were irrelevant when compared with expectant management of gallstone disease.128-130 The role of prophylactic cholecystectomy in high-risk populations, including North American Indians, Mexican Americans, Chileans, and Bolivians, has yet to be reported. A recent cost-effectiveness analysis of screening and treatment among Chilean women under 40 years old with asymptomatic cholelithiasis, showed that prophylactic laparoscopic cholecystectomy can significantly benefit the population at a very low incremental cost (Puschel et al., personal communication, Santiago, 2000).

CONCLUSIONS AND PERSPECTIVES

Little is still known about the etiology of gallbladder cancer. This overview of gallbladder cancer and the annual incidence of gallbladder cancer in various countries offers a comparative picture of the descriptive epidemiology of the disease, a summary of etiologic studies, and a discussion of associated risk factors, especially cholesterol gallstone disease—the major risk factor of gallbladder cancer. It is important that several remaining points be elucidated. Further investigation for this extremely lethal cancer is urgently needed. What do we really know for sure and what needs to be done?

1) The process of gallbladder carcinogenesis is usually related to a history of cholelithiasis, which is frequently present for at least 20 years before the tumor appears.

2) Although several risk factors have been
identified for cholesterol stones (obesity, multiple pregnancies, low plasma HDL, female hormones, and insulin resistance), they do not explain the full picture. Genetic susceptibility is likely to play an important role.

3) Because only a small fraction of patients with cholesterol gallstones develop gallbladder cancer, it is important to identify the factors that induce progression from cholelithiasis to gallbladder cancer. This may allow the identification and early treatment of gallstones of susceptible individuals within high-risk populations.

4) The role of chronic infection in the development of gallbladder cancer deserves further research. It is likely that aside from chronic Salmonella carriers, a number of other bacterial species chronically inhabiting the gallbladder might be important etiologic factors.

5) The limitations of epidemiologic gallbladder cancer studies have included the small size of populations studied and problems in quantifying exposure to putative exogenous risk factors, particularly potential carcinogenic xenobiotics. Multicenter case-control studies in high-risk populations in which accurate biomarkers of exposure to various risk factors are used and the genetic factors are assessed will be of great value in answering several of the questions raised in this review.

6) Primary prevention of gallbladder cancer is not expected in the near future. However, secondary prevention, primarily oriented to treatment of symptomatic gallstones, must be emphasized in endemic areas where cholelithiasis is highly prevalent. Prophylactic laparoscopic cholecystectomy might be cost effective. It is apparent that interventional programs are urgently needed to decrease the number of gallbladders at risk for gallbladder cancer development in high-risk areas, particularly in the Andean region.

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