

REVIEW

Carcinoma Gall Bladder: Past, Present, and Future

Ghosh Y*, Thakurdas B.

*Punjab Institute of Medical Sciences
Jalandhar, India.*

Abstract

Carcinoma gall bladder is a very aggressive disease with poor outcomes. Despite achievements in the field of advanced imaging techniques, there is a very high mortality rate of the disease. Cancer is the second most common disease in India responsible for maximum mortality with about 0.3 million deaths per year. The magnitude of cancer problem in the Indian Sub-continent (sheer numbers) is increasing due to poor to moderate living standards and inadequate medical facilities. Women are more commonly affected than men. The peak incidence occurs in people in their 60s, but the disease age range is from 29 to 90 years of age and there is great geographic and ethnic variation. Carcinoma gall bladder, a disease of old age, is now found in the younger age group and presents with greater ferocity.

Keywords: *gall bladder; age; carcinoma.*

Introduction

The incidence of gall bladder is less as compared to other cancers at different sites. The prevalence of cancer in India is estimated to be around 2.5 million, with about 8,00,000 new cases and 5,50,000 deaths per annum [1]. It has been reported that during 2008, the cases of gallbladder cancer at the global level were 145,662 with an age-standardized rate (ASR) of 2.0 per 105 person years [2]. Gall bladder cancer is the most common abdominal malignancy in the northern part of India [3]. An incidence rate of 4.5 and 10.1% per 100 000 population of males and females, respectively, has been reported by the Indian Council of Medical Research Cancer Registry in some northern parts of India [4]. Incidence varies geographically with higher rates in certain areas of Latin America (Colombia, Peru, and Ecuador), Japan, and Eastern Europe (Poland, the Czech Republic, Slovakia, Hungary, and the former East Germany). In North America, high rates of gallbladder cancer have been noted in Hispanic and American Indian populations [5].

Gallbladder carcinomas are associated with gallstones (80%), porcelain gallbladder (10-20%), and abnormal choledochopancreatic duct junction. Anomalous pancreaticobiliary duct junction is a rare congenital

malformation of the biliary tract, in which the pancreatic duct drains into the biliary tract outside the duodenal wall [6]. More prevalent in Asians (particularly Japanese patients), this anomaly carries a heightened risk of developing biliary tract cancer; 3–18% develop gallbladder cancer [6-8]. Size of the gallstones may also be a risk factor, as patients with stones larger than 3 cm have a significantly greater risk of developing carcinoma [9]. Other factors that increase the risk for gallbladder cancer include obesity, a high-carbohydrate diet, smoking, and alcohol use [10].

Clearly, the most significant risk factor for gallbladder cancer is the presence of chronic inflammatory state of the gallbladder, usually as a result of gallstones. Most of the variance in geographic and racial gallbladder cancer rates can be explained by the varying incidence of gallstones in the populations. But only 0.3 to 3.0 percent of patients with gallstones develop gallbladder cancer [11]. Gall bladder polyps are associated with a risk of malignancy [12]. Choledochal cyst has been implicated as a risk for malignancy [13]. Obesity has shown to be an important risk factor for development of gallbladder cancer while adequate intake of fruits and vegetables have been shown to be a protective factor. Findings from various studies on the adequate consumption of vegetables indicate an inverse association with gallbladder cancer risk [14-15]. Several large population based and observational epidemiological studies have highlighted the importance of vegetables and fruits in reducing the risk of cancer in a variety of organs and tissues.

*Corresponding author: Yatin Ghosh. Punjab Institute of Medical Sciences. Jalandhar, India.
E-mail: yatinghosh@gmail.com

Discussion

There is a direct link between gall stones and Carcinoma gall bladder. The link appears to be contingent upon the length of time that the stones reside in the gallbladder. A long duration provides the necessary time for such chronic trauma to the mucosa to initiate a sequence of pathologic changes that culminate in cancer. This would explain the inverse correlation that exists between cholecystectomy rates and gallbladder cancer; socioeconomic issues can delay access to cholecystectomy for cholelithiasis, increasing gallbladder cancer rates [6,16]. In patients with Carcinoma gall bladder, the incidence of cholelithiasis ranges from 54% to 97%. Carcinoma gall bladder is more common in patients with Mirizzi's syndrome and typhoid carriers are a high-risk group. Moreover, porcelain (calcified) gall bladder has a high malignant potential and large, sessile polyps (more than 10 mm) are more likely to be malignant than multiple, small, pedunculated ones [17-18]. Bacterial colonization often accompanies chronic cholecystitis, it has been proposed that bacteria may play an important role in carcinogenesis [19]. There are many other factors which are associated with gall bladder cancer though their exact role in the pathogenesis of gall bladder cancer is not proven such as high concentrations of free radical oxidation products [20]. Some chemicals have been implicated in the gall bladder cancer including methyl dopa, oral contraceptives, isoniazid and occupational exposure in the rubber industry [21]. Gallbladder cancer appears to develop from dysplastic mucosa that progress to carcinoma in situ and then to invasive carcinoma [22]. Salmonella Typhi (~6% of carriers develop gallbladder cancer: a 12-fold risk increase) and Helicobacter bilis have been implicated in gallbladder cancer [23,24]. Gallbladder polyps are considered a risk factor. Polypoidal masses of the gallbladder affect 5% of adults (range, 0.3–7%), depending upon the population studied. Most gallbladder polyps (over two thirds) are composed of cholesterol esters, the common composition of those under 5 mm, yet they are not particularly associated with cholesterol gallstones. Other polypoidal lesions are adenomas, leiomyomas, or inflammatory polyps. The majority of these immobile hyperechoic shadows are incidental findings discovered on abdominal ultrasound performed for other purposes. Most polyps do not grow or change in size. Features predicting malignancy of polypoidal gallbladder masses include large polyps (>10 mm; one quarter are malignant); a solitary lesion; a sessile polyp; polyp growth; age over 50–60 years; or associated gallstones [6,25]. Features suggesting a malignant polyp, or when accompanied by gallbladder symptoms (biliary-type pain), warrant cholecystectomy. Polyps over 18 mm must be removed, as they are likely malignant [26].

Approximately 60% of tumors originate in the fundus of the gallbladder, 30 % originate in the body, and 10 % originate in the neck Gallbladder cancers can be categorized into infiltrative, nodular, combined nodular infiltrative, papillary, and combined papillary-infiltrative forms . Infiltrated tumors cause thickening and induration of the gallbladder wall. They spread easily in a subserosal plane, which is the same

plane used for routine cholecystectomy. Nodular types show early invasion through the gallbladder wall into the liver or neighboring structures and may be easier to surgically control than the infiltrative form. Papillary carcinomas have the best prognosis and exhibit a polypoid cauliflower-like appearance. These may completely fill the lumen of the gallbladder, with only minimal invasion of the gallbladder wall. Histologically, the most common type of gallbladder cancer is adenocarcinoma. Other types, such as adenosquamous carcinoma, oat cell carcinoma and sarcomas are also seen. In 92% of invasive carcinomas, 86% of carcinomas in situ, and 28% of dysplastic epithelia p53 protein is identifiable [27].

In 39% of gallbladder cancers K-Ras mutations are identified [28]. Allele-specific deletions of the p53, deleted in colon cancer (DCC), and 9p genes play an important role in the pathogenesis of gallbladder cancer [29]. In some studies p53 and p21 have been found to be abnormally expressed in the mucosa of gall bladders harboring chronic cholecystitis [29]. Other genetic abnormalities that have been documented include overexpression of c-erbB-2 gene [30] and decreased expression of the nm23 gene product [31].

Cholesterol stones, the factors best identified are the genes responsible for specific biliary lipid transporters in the canalicular membrane—the ATP-binding cassette (ABC) transporters. These transporters include ABCG5/ABCG8 for cholesterol secretion, ABCB11 as the bile salt export pump, and ABCB4 for phospholipids and lecithin [16]. Mutations in the gene ABCG5/G8, as the variant D19H, result in increased cholesterol secretion into bile, making it an important susceptibility factor. Defective ABCB4 leads to decreased lecithin secretion and stone formation. In gallbladder cancer, variants of the APOB gene is responsible for apolipoprotein B function which influences cholesterol handling by the liver, has been associated with an increased risk for gallbladder cancer [32]. One comprehensive explanation for the association of gallbladder cancer with cholesterol gallstones suggests an interdependent disposal pathway for cholesterol and environmental toxins exported into bile, linked by the activity of hepatic nuclear receptors and ABC transporter pumps [33]. Female sex hormones increase the secretion of cholesterol and xenobiotics into bile. Furthermore, prolonged gallbladder residence time (stasis due to impaired contractility) results from progesterone and the excessive cholesterol secreted in bile. Such protracted exposure allows environmental carcinogens such as aflatoxin B, possibly the culprit in some endemic areas [16]. TPN is a well-known risk factor for developing microlithiasis (biliary sludge) and gallstone disease, in addition to acute acalculous cholecystitis in critically ill patients [34].

Gallbladder cancer can spread by direct invasion through the gallbladder wall into the liver or peritoneal cavity. The gallbladder has a narrow wall consisting of a thin lamina propria and a single muscle layer. Once a gallbladder cancer penetrates this muscle layer, it has access to major lymphatic and vascular channels as well as the liver or peritoneal cavity by penetration through the wall [35]. Gall bladder cancer can also spread via lymphatic, hematogenously and along biopsy tracks or surgical wound tracks. Hematogenous spread

originates from the small veins extending directly from the gall bladder into the portal venous system of the gall bladder fossa leading to segments IV and V of the liver or via large veins to the portal venous branches of segments V and VIII [36]. Boerma and colleagues reviewed the literature and determined that only 10 percent of gallbladder cancers were confined to the gallbladder wall at presentation, 59 percent invaded the liver, 45 percent invaded regional lymph nodes, 34 percent had distant hepatic metastases, and 20 percent had extrahepatic hematogenous metastases [36-37].

The most common site of extra-abdominal metastases is the lung, but it is rare to have pulmonary metastases in the absence of advanced intra-abdominal disease. The stage of disease is the most reliable predictor of outcome and ultimately outweighs histology, grade, or other biologic parameters. The main staging systems over the past 5 years include the modified Nevin system [37] and Japanese Biliary Surgical Society system [38]. The American Joint Committee on Cancer has designated staging by the TNM classification to define gallbladder cancer [39].

The AJCC Staging System for Gallbladder Cancer (7th Edition) [39]

Primary Tumor (T)

- TX: Primary tumor cannot be assessed.
 TO: No evidence of primary tumor.
 Tis: Carcinoma in situ
 T1: Tumor invades lamina propria or muscular layer.
 (T1a): Tumor invades lamina propria.
 (T1b): Tumor invades muscle layer.
 T2: Tumor invades perimuscular connective tissue; no extension beyond serosa or into liver.
 T3: Tumor perforates the serosa (visceral peritoneum) and/or directly invades the liver and/or one other adjacent organ or structure, such as the stomach, duodenum, colon, pancreas, omentum, or extrahepatic bile ducts.
 T4: Tumor invades main portal vein or hepatic artery or invades at least two extrahepatic organs or structures.

Regional Lymph Nodes (N)

- NX: Regional lymph nodes cannot be assessed.
 N0: No regional nodal metastases
 N1: Metastases to nodes along cystic duct, hepatic artery, common bile duct, and/or portal vein
 N2: Metastases to pericaval, periaortic, superior mesenteric artery, and/or celiac artery nodes

Distant Metastasis (M)

- M0: No distant metastases
 M1: Distant metastases

Gallbladder cancer is either discovered early, an incidental finding when cholecystectomy is performed for symptomatic cholelithiasis, or late, when the tumor has invaded the bile ducts or has metastasized [40]. The clinical presentation of gallbladder cancer is difficult to separate from that of biliary colic. Advanced symptoms such as persistent pain, weight loss, and jaundice are often signs of

unresectability. Elderly patients with a history of biliary colic that changes to a persistent, unremitting, dull pain should be suspected of having gallbladder cancer, especially in the presence of weight loss or a right-upper quadrant mass. Any new right-upper-quadrant symptoms should prompt a work-up. The presence of jaundice is a particularly ominous finding. The median survival of patients with jaundice was 6 months as compared to patients without jaundice where the survival was 16 months [41,42]. Signs of malignant disease on ultrasound examination include discontinuous mucosa, echogenic mucosa, and submucosal echolucency [42]. Diffuse thickening of the gallbladder is also common in gallbladder cancer but is also found in benign condition.

Laboratory examination generally is not very helpful except for the typical signs of advanced disease such as anemia, hypoalbuminemia, leukocytosis and elevated alkaline phosphate or bilirubin. Tumor markers may be of help and should be considered if gallbladder cancer is suspected. Serum carcinoembryonic antigen (CEA) greater than 4 ng/mL is 93 percent specific and 50 percent sensitive for detecting gallbladder cancer in the presence of appropriate symptoms [43] and a CA 19-9 serum level greater than 20 U/mL is 79.4 percent sensitive and 79.2 percent specific [44]. Increased epidermal growth factor receptor (EGF receptor) expression has been noted in various cancers such as colon, squamous cell of the head and neck, non-small cell lung and breast cancers. Several small studies from Asia, Europe and Australia have examined the expression of EGFR in gallbladder cancer. The improved understanding of EGFR's role in oncogenesis has made it an attractive target for therapeutic intervention in several cancers. In a study by Matthew Kaufman they found that EGFR was over expressed in their patients of carcinoma gall bladder, they found that 3+ EGFR correlated with poorly differentiated carcinoma and patients with 1+ EGFR correlated with well differentiated carcinoma [45].

Early carcinoma gallbladder may be detected on abdominal USG as a fixed polypoidal mass projecting in to the lumen of the gallbladder with absence of acoustic shadowing or as an asymmetric thickening of the gallbladder wall. The diagnostic accuracy of USG is over 80% in detecting carcinoma gallbladder (Chijiwa et al 1991) [46]. A helical computed tomography (CT) scan with fine cuts through the liver may provide improved imaging over ultrasonography and should be examined carefully for evidence of liver metastases and enlarged celiac, perihepatic, and interaortocaval lymph nodes. A magnetic resonance (MR) scan with MR cholangiography is an ideal study.

Carcinoma gallbladder is incidentally discovered during cholecystectomy for benign diseases in 12-36% of patients (Bergdahl 1980) [47]. If carcinoma gallbladder is discovered intraoperatively the surgeon has to decide whether curative surgery is possible after determining the extent of disease. If the disease is so extensive as to preclude curative resection then a biopsy along with the appropriate palliative procedure may be carried out. Sometimes the probability of carcinoma gallbladder becomes evident only after the gallbladder is opened up after removal hence it is important to examine the opened gallbladder carefully before closing the abdomen. A

difficult gallbladder at surgery usually raises the suspicion of cancer. Unusual findings at surgery such as a gallbladder mass, dense adhesion of the organs which are adjacent to the gallbladder and a difficult dissection of the gallbladder from the liver-bed are all pointers to the presence of a possible malignancy. Surgery is the only curative form of treatment with results depending upon the stage of the disease. Patients with disease confined to the gallbladder are treated by extended or radical cholecystectomy (Gall et al, 1991) [48]. Definitive resection for gallbladder cancer depends on the stage and location of the tumor as well as whether it is a repeat resection after a previous simple cholecystectomy. T1 (stage IA) tumors can be treated with simple cholecystectomy. Stage IB, II, and selected stage III (T4NO) tumors should be treated with en bloc resection of the gallbladder, segments IVb and V of the liver and regional lymph node dissection [35]. Donohue et al (1990) reported a 5 year survival of 29% after extended cholecystectomy in patients with transmural (T3, T4) tumour invasion and lymph node involvement [49]. Nakamura et al (1989), Ogura et al (1991) and Ouchi et al (1994) advocate more extensive surgery such as excision of bile ducts, more extensive liver resections and even pancreaticoduodenectomy to further increase survival rates [50,51]. The criteria for resectability can vary but presence of multiple peritoneal or liver metastases, distant metastases, extensive involvement of hepatoduodenal ligament, encasement or occlusion of major vessels and poor performance status are contraindications for surgical resection. Recommendations for liver resection for gallbladder cancer have ranged from a limited wedge excision of 2 cm of liver around the gallbladder bed to routine extended right hepatic lobectomy. The goal is to achieve a negative margin on the tumor, encompassing cells that have directly infiltrated the liver [35].

In patients not fit for tumour resection, some form of palliative procedure such as a surgical bilioenteric bypass or endoscopic/percutaneous stenting in patients with obstructive jaundice may be done (Baxter&Garden, 1999) [52]. Advances during the past decade in both endoscopic and radiologically guided percutaneous stenting of the biliary tract have made operative bypass, in cases of unresectable cancers, largely unnecessary. Non-operative stenting is the preferred approach. Duodenal or intestinal bypass may be done as a palliative procedure if gastric outlet or intestinal obstruction is present. In addition, patients may require palliation of pain, which is a major problem in advanced carcinoma of the gall bladder [53]. The incidence of gallbladder cancer is low compared with the incidence of gallstones in the population, so prophylactic cholecystectomy for asymptomatic cholelithiasis to prevent the development of carcinoma is not indicated. Studies have suggested that the prognosis is different for pT1 a and pT1 b tumors after simple cholecystectomy [54]. T1 a staging, no extended cholecystectomy is indicated, and simple cholecystectomy should result in a 100% 5-year survival. These tumors are recognized incidentally at the time of pathologic review, and as long as the cystic duct margin is negative, no further surgery is indicated. T1b staged tumors, an extended cholecystectomy is indicated, as these tumors have been reported to recur after simple cholecystectomy. Patients

with stage II disease (T2NO) are best treated with an extended cholecystectomy. When an extended cholecystectomy is performed for T2 disease, the 5-year survival has been reported to be as high as 100% , but probably falls in the range of 70% to 90%. Simple cholecystectomy alone is associated with a 5-year survival rate of 20% to 40.5 % [35,55]. For patients with stage III disease (T3 N1), an extended cholecystectomy is the recommended treatment approach. This may include en bloc resection of the common bile duct for grossly positive periportal lymph nodes in order to improve periportal lymph node clearance. The 5-year survival ranges from 45% to 63% for patients having metastatic disease to N1 nodes. The 3-year survival has ranged from 38% to 80% in various trials. Stage III gallbladder cancer represents an advanced malignancy that is generally beyond surgical treatment. However, patients with T4NO disease, representing a mass-forming gallbladder cancer, may achieve long-term survival after an extended resection. Patients with nodal metastases beyond the hepatoduodenal ligament have a poor prognosis, and in general for these cases the authors would advocate palliative care [35,56].

Chemotherapy has not been widely studied in the treatment of gallbladder carcinoma. Fluorouracil is the most extensively used drug and fluorouracil-based combinations such as FAM (fluorouracil, adriamycin, and mitomycin c) have been used without much success. The best responses were obtained by use of combined gemcitabine and cisplatin—survival was significantly improved. Ben-David et al reported on 14 patients with gallbladder cancer treated at the University of Michigan with resection followed by radiotherapy or chemoradiotherapy. The median radiation dose was 54 Gy and approximately half the patients received concurrent chemotherapy [57]. The median survival was 23 months, interestingly there was no difference in survival between R0 and R1 resection patients. Wang et al analyzed the SEER data and identified 4,180 patients who underwent resection between 1988 and 2003. The authors constructed a multivariate Cox proportional hazards model for overall survival. Age, gender, papillary histology, stage, and adjuvant radiotherapy were significant predictors of survival. The model predicts that adjuvant radiotherapy provides a survival benefit in node positive or T2 or higher disease. The unadjusted median overall survival in patients who received radiotherapy was 15 months compared to 8 months in those who did not ($P<0.0001$) [58]. Balachandran et al described 117 patients with gallbladder cancer, of whom 80 underwent simple cholecystectomy and 37 underwent extended resections. Seventy-three patients received adjuvant chemoradiotherapy and 44 did not. No details were provided regarding this therapy or the selection criteria for adjuvant therapy. The median survival of all 117 patients was 16 months. On multivariate analysis, T stage and the use of adjuvant therapy were the only statistically significant independent predictors of survival. Median survival was 24 months, 11 months in patients with or without adjuvant chemoradiotherapy ($P=0.001$), and this difference was most pronounced for patients with T3, node-positive disease or after a simple cholecystectomy [58,35]. The high risk of systemic spread and locoregional failure associated

with gallbladder cancer that extends beyond the mucosa has led most cancer centers to recommend consideration of adjuvant chemotherapy and radiotherapy. The American Joint Committee on Cancer has designated staging by the TNM classification to define gallbladder cancer [59].

Conclusion

Carcinoma gall bladder is a devastating disease with dismal results. There is an increase in the number of cases of carcinoma gall bladder, may be due to better imaging techniques and more awareness among the patients due to better facilities. The progression of the disease is rapid with 5 year survival quite low. Key to survival is early detection of the pathology and aggressive treatment strategy. Surgery is the only curative form of treatment which can achieve its intended goal if done at an early stage (T1) otherwise with locoregional spread and jaundice survival is barely 6 months. Resection of hematogenous metastasis is not justified nor is resection of distant nodal disease. Radiotherapy and chemotherapy are the adjuvants to treatment but still the gold standard treatment is surgical resection (Ro) at an early stage.

References

1. Nanda Kumar A. Consolidated report of the population based cancer registries. National cancer Registry Programme. Indian Council of Medical research (1990-96); New Delhi, India.
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. GLOBOCAN 2008 v2.0, Cancer Incidence and Mortality Worldwide. Lyon, France: International Agency for Research on Cancer; 2010. IARC Cancer Base No 10.
3. Singh M K, Chetri K, Pandey U B, Mittal B, Kapoor VK, Choudhuri G. Multinational spectrum of K-RASs oncogene among Indian patients with gall bladder cancer. *J Gastroenterol Hepatol* 2004; 19:916-2.
4. Indian council of Medical Research (1996). Annual report of population based cancer registries of the National programme (1993), ICMR, New Delhi.
5. Curado MP, Edwards B, Shin HR, Storm H, Ferlay J, Heanue M and Boyle P, editors. Cancer Incidence in Five Continents. Vol IX. Lyon, France: International Agency for Research on Cancer; 2007. IARC Scientific Publication No 160.
6. Shaffer EA. Gallbladder Cancer. The Basics. *Gastroenterol Hepatol* (NY) 2008; 4(10): 737-741.
7. Chijiwa K, Kimura H, Tanaka M. Malignant potential of the gallbladder in patients with anomalous pancreaticobiliary ductal junction. *Int Surg* 1995; 80: 61-64.
8. Kang CM, Kim KS, Choi JS, Lee WJ, Kim BR. Gallbladder carcinoma associated with anomalous pancreaticobiliary duct junction. *Can J Gastroenterol* 2007; 21(6): 383-387.
9. Tran H Giang, Tran TB Ngoc, Lewis A Hassell. Carcinoma involving the gallbladder: a retrospective review of 23 cases - pitfalls in diagnosis of gallbladder carcinoma. *Diagn Pathol* 2012, 7:10.
10. Moerman CJ, Bueno-de-Mesquita HB. The epidemiology of gallbladder cancer: lifestyle related risk factors and limited surgical possibilities for prevention. *Hepatogastroenterology* 1999; 46: 1533-9.
11. David L. Bartlett, Yuman Fong. *Hepatobiliary Cancer*. In Leslie H. Blumgart, Yuman Fong, William R. Jarnagin, editors. American Cancer Society. Atlas of Clinical Oncology. Hepatobiliary Cancer. B. C. Decker Inc. Hamilton•London; 2001:211-224.
12. Kwon W, Jang JY, Lee SE, Hwang DW, Kim SW. Clinicopathologic features of polypoid lesions of the gall bladder and risk factors of gall bladder cancer. *J Korean Med Sci* 2009; 24:481-7.
13. Lee SE, Jang JY, Lee JY, Choi DW, Cho BH, et al. Choledochal cyst and associated malignant tumors in adults: A multicentric survey in South Korea. *Arch Surg* 2011;146:1143-7.
14. Tyagi BB, Manoharan N, Raina V. Risk factors for gallbladder cancer: a population based case-control study in Delhi. *Indian J Med Paediatr Oncol* 2008; 29(1):16-26.
15. Pandey M, Shukla VK. Diet and gallbladder cancer: a case control study. *Eur J Cancer Prev* 2002; 11(4):365-368.
16. Gupta SK, Shukla VK. Gall bladder cancer. Etiopathology and Treatment. *Health Administrator* 2005; 17(1):145-142.
17. Redaelli C A, Buchler M W, Schilling MK, Krahenbuhl L, Ruchti C, Blumgart LH, et al. High coincidence of Mirizzi syndrome and gall bladder carcinoma. *Surgery* 1997; 121: 58-63.
18. Aldridge M C, Bismuth H. Gall Bladder cancer: the polyp cancer sequence. *Br J Surg* 1990; 77: 363-4.
19. D'Angelica M, Jarnagin WR. Tumors of the Gall Bladder. In L.H. Blumgart, editor. *Surgery of the Liver, Biliary Tract, and Pancreas*. L.H. Blumgart. 4th edition, Saunders, Philadelphia; 2007:764-781.
20. Shukla VK, Khandelwal C, Roy SK, Vaidya MP. Lipid peroxidation product in the bile from patients with carcinoma of the gall bladder: a preliminary study. *J Surg Oncol* 1994; 56:258-262.
21. Mancuso TF, Brennan MJ. Epidemiological considerations of cancer of the gall bladder, bile ducts and salivary glands in the rubber industry. *J Occup Med* 1970; 12:333-341.
22. Albores-Saavedra J, Henson DE. Atlas of tumor pathology, second series fascicle 22: tumors of the gallbladder and extrahepatic bile ducts. 2nd ed. Bethesda (MD): Armed Forces Institute of Pathology; 1986: 28-123.
23. Kumar S, Kumar S, Kumar S. Infection as a risk factor for gallbladder cancer. *J Surg Oncol*. 2006; 93: 633-639.
24. Dutta U, Garg PK, Kumar R, Tandon RK. Typhoid carriers among patients with gallstones are at increased risk for carcinoma of the gallbladder. *Am J Gastroenterol* 2000; 95:784-7.
25. Myers RP, Shaffer EA, Beck PL. Gallbladder polyps: epidemiology, natural history and management. *Can J Gastroenterol* 2002;16:187-194.
26. Kubota K, Bandai Y, Noie T, Ishizaki Y, Teruya M, Makuuchi M. How should polypoid lesions of the gallbladder be treated in the era of laparoscopic cholecystectomy? *Surgery* 1995; 117:481-487.
27. Wee A, Teh M, Raju GC. Clinical importance of p53 protein in gall bladder carcinoma and its precursor lesions. *J Clin Pathol* 1994; 47: 453-6.
28. Imai M, Hoshi T, Ogawa K. K-Ras codon 12 mutations in biliary tract tumors detected by polymerase chain reaction denaturing gradient gel electrophoresis. *Cancer* 1994; 73: 2727-33.
29. Yanagisawa N, Mikami T, Koike M, Okayasu I. Enhanced cell kinetics, p53 accumulation and high p21 WAF1 expression in chronic cholecystitis: comparison with background mucosa of gall bladder carcinoma. *Histopathology* 2000; 36(1): 54-61.

30. Huang SM, Chan SH, Mo LR, Hwang MH, Su WC. Significance of c-erbB-2 expression in normal and neoplastic epithelium of biliary tract. *Anticancer Res* 1995;15(3):1055-1059.
31. Fujii K, Yasui W, Shimamoto F, Yokozaki H, Nakayama H, Kajiyama G, Tahara E. Immunohistochemical analysis of nm23 gene product in human gall bladder carcinomas. *Virchows Arch* 1995, 426(4): 355-9.
32. Pandey SN, Srivastava A, Dixit M, Choudhuri G, Mittal B. Haplotype analysis of signal peptide (insertion/deletion) and XbaI polymorphisms of the APOB gene in gallbladder cancer. *Liver Int* 2007; 27(7):1008-15.
33. Venniyoor A. Cholesterol gallstones and cancer of the gallbladder (CABG): molecular links. *Med Hypotheses* 2008; 70(3):646-653.
34. Roslyn JJ, Pitt HA, Mann LL, Ament ME, Den Besten L. Gallbladder disease in patients on long-term parenteral nutrition. *Gastroenterology* 1983; 84(1):148-54.
35. David L, Bartlett, Ramesh K, Ramanathan and EdgerBen, Josef. Cancer of the biliary tree. In *DeVita, Hellman, and Rosenberg's Cancer: Principles & Practice of Oncology, 9th Edition*. Philadelphia: Lippincott Williams & Wilkins; 2011: 1019-1047.
36. Boerma EJ. Towards an oncological resection of gall bladder cancer. *Eur J Surg Oncol* 1994.20(5):537-544.
37. Nevin JE, Moran TJ, Kay S, King R. Carcinoma of the gallbladder: staging, treatment, and prognosis. *Cancer* 1976; 37(1):141-8.
38. Onoyama H, Yamamoto M, Tseng A, Ajiki T, Saitoh Y. Extended cholecystectomy for carcinoma of the gallbladder. *World J Surg* 1995; 19(5):758-63.
39. Gallbladder. In: Edge SB, Byrd DR, Compton CC, editors. *AJCC Cancer Staging Manual*. 7th ed. New York, NY: Springer; 2010:211-7.
40. de Aretxabala X, Roa I, Burgos L, Losada H, Roa JC, Mora J, et al. Gallbladder cancer: an analysis of a series of 139 patients with invasion restricted to the subserosal layer. *J Gastrointest Surg* 2006; 10(2):186-92.
41. Wibbenmeyer LA, Sharafuddin MJ, Wolverson MK, Heiberg EV, Wade TP, Shields JB. et al. Sonographic diagnosis of unsuspected gallbladder cancer: imaging findings in comparison with benign gallbladder conditions. *AJR Am J Roentgenol* 1995; 165(5):1169-74.
42. Misra S, Chaturvedi A, Misra NC, Sharma ID. Sharma. Carcinoma of the gallbladder. *Lancet Oncol* 2003; 4(3):167-76.
43. Strom BL, Maislin G, West SL, Atkinson B, Herlyn M, Saul S, et al. Serum CEA and CA 19-9: potential future diagnostic or screening tests for gallbladder cancer? *Int J Cancer* 1990; 45(5):821-4.
44. Ritts RE, Jr, Nagorney DM, Jacobsen DJ, Talbot RW, Zurawski VR Jr. Comparison of preoperative serum CA19-9 levels with results of diagnostic imaging modalities in patients undergoing laparotomy for suspected pancreatic or gallbladder disease. *Pancreas* 1994; 9(6):707-16.
45. Kaufman M, Mehrotra B, Limaye S, White S, Fuchs A, Lebowicz Y, et al. EGFR Expression in gallbladder carcinoma in North America. *Int J Med Sci* 2008 5(5):285-91.
46. Chijiwa K, Sumiyoshi K, Nakayama F. Impact of recent advances in hepatobiliary imaging techniques on the preoperative diagnosis of carcinoma of the gallbladder. *World J Surg* 1991, 15(3):332-337.
47. Bergdahl I. Gallbladder carcinoma first diagnosed at microscopic examination of gallbladders removed for presumed benign disease. *Ann Surg* 1980; 191(1):19-22.
48. Gall FP, Kockerling F, Scheele J, Schneider C, Hobenberger W. Radical operations for carcinoma of the gallbladder: present status in Germany. *World J Surg* 1991; 15(3): 328-36.
49. Donohue JM, Nagorney DM, Grant CS, Tsushima K, Ilstrup DM, Adson MA. Carcinoma of the gallbladder. Does radical resection improve outcome *Arch Surg* 1990; 125(2):237-41.
50. Nakamura S, Sakaguchi S, Suzuki S, Muro H. Aggressive surgery for carcinoma of gallbladder. *Surgery* 1989; 106(3):467-73.
51. Ogura Y, Mizumoto R, Isoji S, Kusuda T, Matsuda S, Tabata M. Radical operations for carcinoma of the gallbladder: present status in Japan. *World J Surg* 1991; 15(3):337-43.
52. Baxter I, Garden OJ. Surgical palliation of carcinoma of the gallbladder. *Hepatogastroenterology* 1999; 46(27):1572-7.
53. Ouchi K, Owada Y, Matsumo S, Sato T. Prognostic factors in the surgical treatment of gallbladder carcinoma. *Surgery* 1987; 101(6): 731-7.
54. Otero JC, Proske A, Vallilengua C, Luján M, Poletto L, Pezzotto SM, et al. Gallbladder cancer: surgical results after cholecystectomy in 25 patients with lamina propria invasion and 26 patients with muscular layer invasion. *J Hepatobiliary Pancreat Surg* 2006; 13(6):562-6.
55. Pawlik TM, Gleisner AL, Vigano L, Kooby DA, Bauer TW, Frilling A, et al. Incidence of finding residual disease for incidental gallbladder carcinoma: implications for re-resection. *J Gastrointest Surg* 2007; 11(11): 1478-86.
56. D'Angelica M, Dalal KM, DeMatteo RP, Fong Y, Blumgart LH, Jarnagin WR. Analysis of the extent of resection for adenocarcinoma of the gallbladder. *Ann Surg Oncol* 2009; 16 (4):806-16.
57. Ben-David MA, Griffith KA, Abu-Isa E, Lawrence TS, Knol J, Zalupski M, et al. External-beam radiotherapy for localized extrahepatic cholangiocarcinoma. *Int J Radiat Oncol Bio Phys* 2006; 66(3):772-9.
58. Wang SJ, Fuller CD, Kim JS, Sittig DF, Thomas CR Jr, Ravdin PM. Prediction model for estimating the survival benefit of adjuvant radiotherapy for gallbladder cancer. *J Clin Oncol* 2008; 26 (13): 2112-7.