RESEARCH PAPER | Medical Science | Volume : 5 | Issue : 4 | April 2015 | ISSN - 2249-555X

Gallbladder Carcinoma: Clinical Profile and its Management

KEYWORDS: Gallbladder carcinoma, Epidemiology, path biology, molecular biology

Dr. Arpit Bansal
Department of Surgery, Jeevan Jyoti Hospital, Allahabad, U.P. India

Dr. Ashwani Kumar Bansal
Department of Surgery, Jeevan Jyoti Hospital, Allahabad, U.P. India

Dr. Vandana Bansal
Guru Gobind Singh Memorial Vanadana Women's Hospital, Allahabad, U.P. India.

Dr. Ajay Kumar
Jeevan Jyoti Institute of Medical Sciences, Allahabad, U.P. India

ABSTRACT *Gallbladder carcinoma (GBC) is the most common malignant tumor of the biliary tract. High incidence is observed in Chile, Japan, and northern India. The etiology of this tumor is complex, but there is a strong association with gallstones. It affects women two to six times more frequently than men. The incidence of GBC increases steadily with increase in age. Carcinoma of gall bladder is the fifth most mortality causing cancer and its cases are alarmingly increasing in south-east Asia. It can arise from either a pathway involving metaplasia or dysplasia or one in which there is a pre-existing adenoma. The former is the more common and, because it is often not associated with a macroscopically recognizable lesion, leads to the recommendation that all gallbladders need to be examined microscopically. Accurate staging of invasive cancers is essential to determine prognosis and treatment, and this requires extensive tumor sampling. In this article we review the epidemiology, pathology, molecular biology, clinical phase of GBC and the management of patients with gallbladder cancer.*

Introduction:

Gallbladder is a small pear shaped hollow organ lies just beneath the right lobe of liver, where bile is stored and concentrated before it is released into the small intestine through a tube called common bile duct. Gallbladder is divided into three region: fundus, body and neck [1]. The wall of the gallbladder has 3 main layers of tissue. Mucosal (innermost) layer, Muscularis (middle, muscle) layer and Serosal (outer) layer. Primary gallbladder cancer starts in the innermost layer and spreads through the outer layers as it grows. The poor prognosis associated with GBC is thought to be related to advanced stage at diagnosis, which is due both to the anatomic position of the gallbladder, and the vagueness and non-specificity of symptoms. Gallbladder cancer is a cancer that starts in the gallbladder. Carcinoma of the gallbladder is the most common malignant tumor of the biliary tract and a particularly high incidence is observed in China, Japan, and northern India.

Carcinoma of the gallbladder is a highly fatal disease with poor prognosis. It is the most common malignant lesion of the biliary tract and the fifth most common among malignant neoplasms of the digestive tract. Even with the numerous diagnostic tests available, gallbladder cancer is frequently first diagnosed during laparotomy or laparoscopy procedures, which were expected to confirm the presence of benign gallbladder disease [2,3]. The poor prognosis of this disease is due to the anatomical position of the gallbladder and the high proportion of tumors that are advanced at the time of presentation. Since the symptoms and signs of gallbladder carcinoma are vague and nonspecific, it is difficult to diagnose clinically. However, with the recent improvements in preoperative imaging, early gallbladder carcinomas are now being diagnosed more frequently and the use of radical aggressive surgery promises an improvement in survival. Carcinoma of the gallbladder is an aggressive malignancy occurring predominantly in the elderly with mean age of 65.2 year. It constitutes nearly two third of the biliary tract cancers [4,5].

Epidemiology:

Cancer is big threat for our society after development of advance diagnostic method. Cancer is the second most common disease after cardiovascular disease in world [6]. Gallbladder carcinoma (GBC) has an unusual geographic distribution. It is uncommon in Europe and the United States, it is more frequent in Chile, Bolivia and Israel [7].

High rates of GBC are seen in South American countries, particularly Chile, Bolivia, and Ecuador, as well as some areas of India, Pakistan, Japan and Korea [8]. In Chile, mortality rates from GBC are the highest in the world. These populations all share a high prevalence of gallstones and/or salmonella infection, both recognized risk factors for GBC [9].

The incidence of GBC is high in many parts of the world including Chile, Peru, Bolivia, Korea, Japan, Czech Republic, Slovakia, Spain and India [10]. The incidence of GBC among women in northern India is one of the highest in the world, and the incidence of GBC is steadily increasing from 10.1/100,000 population in women in 1993 to 19.6/100,000 population in 2006 [11, 12].

Molecular Biology:

The genetic studies in cancer have provided important clues in our understanding of the molecular mechanisms of tumour development. Genetic mutations accumulate in a sequential manner during tumour progression. Cytogenetic and molecular genetic studies including high resolution mapping using sequence tagged repeat polymorphic markers have revealed multiple genetic alterations in GBC. Non-random losses at 3p, 4q, 5q, 9p, 10p, 10q, 11p, 14p, 14q, 15p, 17p and 21p were reported by Goruneova et al [13]. Loss of heterozygosity (LOH) using sequence tagged repeat polymorphic markers have been demonstrated on chromosomal arms 1p, 3p, 5p, 5q, 6q, 8p, 9p, 9q, 13q, 16q, and 17p in gallbladder carcinoma [14]. while LOH on 13q and 18q is frequent in higher grade (stage III and IV)

Volume : 5 | Issue : 4 | April 2015 | ISSN - 2249-555X

gallbladder carcinomas [15]. LOH in dysplasias on chromosomal arms 3p, 5q, 9p, 13q, 13q, 16q and 17p suggest that these are early changes in the pathogenesis of GBC [14]. Several of the LOH loci correspond to the sites of tumour suppressor genes viz., 1p34-36 (p73, p53-related gene), 3p25 (VHL), 3p24 (RARβ), 3p21.3 (RASSF1A), 3p14.2 (FHIT), 5q21 (APC), 8p21- 23(PRLTS, FEZ), 9p21 (p15 and p16), 9q (DBCCR1), 13q14 (RB), 16q24 (WWOX, FRA16D) and 17p13 (p53) [14].

In a limited panel of tumours Yoshida et al. (2000) have demonstrated LOH at p53, DCC, APC, RB and NM23-H1 tumour suppressors. LOH at 17p increase with the stage of tumour and correlates with the metastasis, it is more frequent in GBC with metastasis than without metastasis [16].

In a study of a panel of 169 microsatellite markers spanning all non acrocentric autosomes and X demonstrated LOH at 21 chromosomal arms including 7q, 11q, 12q, 18q, 19p, 22q and Xq in addition to the above mentioned chromosomal arms [17]. In a relatively recent study, homozygous deletion of the exon 2 of p16 with frequent LOH at 9p21-22, promoter hypermethylation of p16 and corresponding down regulation of p16 was demonstrated while no p16 mutation was detected suggesting deletion and promoter methylation as major mechanism of inactivation of the tumour suppressor p16 [18].

Role of activating/inactivating mutations of oncogenes/ tumour suppressor genes have been demonstrated in the oncogenic transformation of GBC, however, few of these are shown in dysplasias or adenomas. K-ras, p53 and p16 mutations were shown in gallbladder carcinoma but not in dysplasias or adenomas [19]. Rashid et al. demonstrated high expression of K-ras and p53 with high MSI and alteration of TGFβRII gene [20]. Also, overexpression of p53 with low expression of p16 and RB has been shown by immunohistochemistry [21]. RAS/RAF/MEK/ERK kinase cascade is activated in GBC by either K-ras mutation or B-raf mutation along with p53 mutations leading to aberrant Nrf2 transcription factor activity. However, K-ras and B-raf mutations were not observed together but p53 mutation could be observed with either of the two gene mutations. B-raf mutations were mostly at the hot spot codon 599 of exon 15 [22].

Family histories of cancer and genetic susceptibility to developing them are a topic of great interest. In this regard, several groups have studied genetic polymorphisms and have found that CA242 and CA125, when used together, achieved best sensitivity and specificity for discriminating patients with gallbladder carcinoma from those with cholelithiasis [23]. Heparanase and hypoxia-inducible factor-1a are frequently expressed in gallbladder carcinoma and are associated with decreased survival [24]. It appears that 'D' allele of lipoprotein receptor associated protein (LRPAP1) insertion / deletion polymorphism, the NAT2 slow acetylator phenotype and the X(+), D haplotype of apolipoprotein B may modulate the susceptibility to GBC, with the risk being independent of the presence of gallstones [25].

Path Biology:

Cholelithiasis (gallstone) is one of the major risk factor of GBC. Association of gallstones with gallbladder carcinoma has been well established [26]. It is not clear whether the association represents a causal link or the presence of common risk factor. Evidence in favour of a link between these diseases is substantial: gallstones are found in 65–90% of patients with gallbladder carcinoma [27]. However, several other factors may be important in the development of GBC because about 10–25% of patients with this disease do not have associated cholelithiasis and only a small proportion (1–3%) of patients that do have gallstones actually develop cancer. Carcinoma of the gallbladder progresses from dysplasia, to carcinoma in situ (CIS), to invasive carcinoma over about 15 years [28]. Dysplasia and CIS can be found in more than 90% of patients with gallbladder carcinoma although adenomas are thought not to be precancerous because molecular analysis of these lesions does not show the genetic changes associated with gallbladder carcinoma or its precursor lesions [29].

Gallbladder carcinoma usually produces asymmetric thickening of the gallbladder wall with infiltration to surrounding structures. Most cancers originate in the gallbladder fundus. As the tumor progresses, the gallbladder may fill with tumor or may contain pus, mucus, or stones. Early carcinomas can appear as a mucosal plaque, a polypoid or papillary outgrowth, or discrete thickening of the wall [30]. Macroscopically, gallbladder carcinomas can be divided into papillary, tubular, and nodular forms [31]. Papillary tumors are less likely to invade the liver directly and have lower incidence of lymph-node metastasis. Most carcinomas of the gallbladder are adenocarcinomas (80–95%), and can be papillary, tubular, mucinous, or signet cell type. Less common types include: undifferentiated or anaplastic carcinoma (2–7%), squamouscell carcinoma (1–6%), and adenosquamous carcinoma (1–4%) [2, 31]. Carcinoid tumours, small-cell carcinomas, malignant melanomas, lymphomas, and sarcomas are particularly rare.

The following modes of spread for gallbladder carcinoma: direct, lymphatic, vascular, neural, intraperitoneal, and intraductal. The gallbladder drains by a lymphatic plexus to lymph nodes along the biliary ductal system—the first level of lymph nodes. Subsequent drainage occurs to the superior, anterior, and posterior pancreaticoduodenal lymph nodes and lymph nodes posterior to the portal vein and around the common hepatic artery—the second level of lymph nodes. Metastasis may involve lymph nodes posterior to the head of pancreas and posterior to the portal vein. Further drainage occurs to the coeliac, superior, mesenteric, and para-aortic lymph nodes— the third level of lymph nodes. Nodes at the hilum of the liver are affected only in a retrograde direction. The overall rate of lymph-node metastasis in gallbladder carcinoma ranges from 54–64% and is strongly correlated with the depth of invasion [32, 33].

The most frequent histological type is adenocarcinoma, accounting for >98% of GBCs, among which almost two-thirds are moderately or poorly differentiated. The tubular and micropapillary subtypes are the most frequent. Some studies have shown that micropapillary carcinoma is rarer in Western countries than in Japan and is characterized by longer survival after surgical resection [34, 35]. Other less frequent histological types have been described (as in the World Health Organization classification), such as squamous, adenosquamous carcinomas [36, 37], carcinoids [38], malignant gastrointestinal stromal tumors [39]. and small cell carcinomas [40]. Tumor dissemination is mainly via the lymphatic pathway, but also by direct invasion into the liver [41]. This explains why the liver wedge resection plus lymphadenectomy with adjuvant chemotherapy is the best treatment currently known for PT2 (subserosal tumours)[42].

Clinical Presentation:

GBC does not have any specific symptoms but the general warning signs are pain in the right hypochondrium, weight

Volume : 5 | Issue : 4 | April 2015 | ISSN - 2249-555X

loss, anorexia, nausea and vomiting, lump in the right hypochondrium, jaundice, abdominal distention, pruritis, haematemesis and malena.

These nonspecific symptoms have been grouped into five clinical syndromes. The first of these is acute cholecystitis– about 1% of patients for acute cholecystitis have an earlier stage of carcinoma and they have improved survival. Second is chronic cholecystitis. The third syndrome is biliary-tract disease with symptoms of jaundice, weight loss, general weakness, and pain in the right upper quadrant. Patients with this clinical syndrome have extensive disease. The fourth category refers to malignant tumours outside the biliary tract, with symptoms of anorexia, weight loss, general weakness, and local complications of the tumour such as a fistula or invasion of adjacent organs. The last category is benign manifestations outside the biliary tract, the small group of patients with this syndrome present with gastrointestinal bleeding and upper gastrointestinal obstruction [43].

Clinical Phases:
It is the method to describing where the cancer is located, if or where it has spread, and whether it is affecting other parts of the body. There are two methods to describing GBC one is TMN staging and another is Nevin's staging.

TNM is an abbreviation for tumor (T), node (N), and metastasis (M). Doctors look at these three factors to determine the stage of cancer:

- How large is the primary tumor and where is it located? (Tumor, T)
- Has the tumor spread to the lymph nodes? (Node, N)
- Has the cancer metastasized to other parts of the body? (Metastasis, M)

There are five stages: stage 0 (zero) and stages I through IV (one through four). Table. 1.

In 1976 Navin proposed the staging system based upon depth of invasion and spread of disease and classified GBc into five stages. Table. 1

Nevin's stage	TNM Staging	Definition
Stage I - Intramucosal only	0	T1s - Carcinoma in situ N0 - No lymph node metastases M0 - No distant metastases
Stage II- Extends to the muscularis	I	T1a - Tumor invades mucosa T1b - Tumor invades muscularis N0 - No lymph node metastases M0 - No distant metastases
Stage III - extends through the liver	II	T2 - Tumor invades perimuscular connective tissue extension beyond serosa or into liver N0 - No lymph node metastases M0 - No distant metastases
Stage IV - transmural involvement	III	T3 - Tumor invades beyond serosa or into on adjacent organ or both (extension 2cm or liver) N1- regional lymph node metastates M0 - No distant metastases or T1N1M0 or T;
Stage V - Direct extension and/or distant metastases	IV	T4 - Tumor extends more than 2cm into liver and/.or into two or more adjacent organs (between stomach, duodenum, liver, omentum etc). N0 or N1 M0 or Any T1 any N1 M1 - distant metastases

Treatment:
The treatment depends primarily upon the stage of the disease at presentation. The only potentially curative therapy is surgical resection. Unfortunately the overall resection rates at presentation range from 10%-30% only. Broadly management guidelines can be divided into three clinical groups.

1. Incidentally discovered carcinoma gallbladder during laparotomy or after cholecystectomy for benign disease.

2. Carcinoma gallbladder suspected or confirmed preoperatively on diagnostic workup

3. Advanced carcinoma gallbladder diagnosed clinically or by preoperative investigations.

Incidentally discovered gallbladder carcinoma:
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. More commonly, however it is only after histopathological examination of the cholecystectomy specimen that the diagnosis is made. Further treatment of such cases remain controversial. While most authors feel that simple cholecystectomy is adequate if the tumour has not invaded beyond the muscle layer reporting 5 year survival rates of nearly 100% [44, 45], but some are against this [46].

At present there is no irrefutable evidence to recommend a uniform guideline for reexploration in all occult carcinoma gallbladder detected by the pathologist subsequently. The morbidity and mortality of reexploration has to be weighed against the lack of demonstrated efficacy of subsequent surgery. This underlines the importance of a preoperative or at least a peroperative diagnosis. In a resectable carcinoma recognized intraoperatively, extended resection including a wedge of liver and hepatoduodenal lymphatic tissue may improve the duration of survival for patients with gallbladder cancer invading beyond the mucosa (stage I & II) but not invading contiguous structure (Stage III & IV).

Preoperative diagnosed gallbladder carcinoma:
The surgical management of gallbladder carcinoma diagnosed preoperatively depends on the findings of clinical

Volume : 5 | Issue : 4 | April 2015 | ISSN - 2249-555X

investigations and subsequent laparotomy. Many patients with preoperatively diagnosed gallbladder carcinoma have locoregionally advanced disease; however, patients with organ-confined disease can be treated with radical cholecystectomy [47]. The surgical management of T3 and T4 gallbladder carcinoma is a challenging problem. Survival for these patients is generally poor compared with patients who have early-stage tumors, so the use of radical surgery is much debated. Support for radical resection of locally advanced disease has accumulated during the past decade, since it has been confirmed that long-term survival is achievable. Encouragingly, 5-year survivals of 15–63% and 7–25% have been reported for T3 and T4 tumours, respectively [48, 49]. Donohue and colleagues recorded 5-year survival of 29% after extended cholecystectomy in patients with transmural (T3, T4) tumor invasion and lymph-node involvement [43].

The criteria for respectability 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. Direct involvement of colon, duodenum or liver however, are not absolute contraindications for resectional surgery. 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 [50]. Patients not fit for such major resections or found unresectable are managed as patients with advanced disease.

Advanced gallbladder carcinoma:
Patients with advanced unresectable carcinoma of the gallbladder and obstructive jaundice may need palliation by a biliary enteric bypass or endoscopic/percutaneous stunting to relieve jaundice. Surgical biliary bypass is not straightforward, as the level of tumor obstruction is usually at the common hepatic duct or above. The round ligament approach to the segment III duct followed by biliary enteric anastomosis may produce excellent palliation for carcinoma of the gallbladder because of the distance between the anastomosis and the primary site of tumor. Tumor invasion of the umbilical fissure of the liver precludes this method of palliation and in this situation an alternative technique such as Longmire procedure or surgical intubation may be applicable [51]. 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 stunting 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 gallbladder.

External radiotherapy as an adjuvant treatment has shown some benefit in survival [52]. Todoroki and colleagues have used intraoperative radiotherapy for stage IV gallbladder carcinoma after complete tumor resection. 3-year cumulative survival was 10·1% for patients who were treated with surgical resection and radiotherapy compared with nil for a similar group of patients undergoing resection only [53]. Recently, a favorable survival has been reported in completely resected gallbladder carcinoma followed by adjuvant external-beam radiotherapy and chemotherapy with fluorouracil [54]. These results are encouraging and the role of radiotherapy as an adjuvant to surgery must be further studied before being recommended as standard therapy for gallbladder carcinoma.

Chemotherapy has not been widely studied in the treatment of gallbladder carcinoma [55]. 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. Hepatic arterial infusion of mitomycin c plus fluorouracil has also been used. Misra et. al. treated 26 patients with hepatic metastases from gallbladder carcinoma by intraarterial infusion of fluorouracil and mitomycin c [56]. The overall response rate was 34% and median duration of response was 7 months (range 3–16 months). The median survival of responders was 7·2 months compared with 2 months in non-responders. Adjuvant chemotherapy with intravenous fluorouracil and mitomycin c in stage I, II, and III disease did not cause any improvement in disease-free survival. Further studies are required before routine use of adjuvant chemotherapy for carcinoma of the gallbladder can be recommended. Gemcitabine has shown promise for treatment of advanced gallbladder carcinoma but, as with other new drugs, its effectiveness must be throughout assessed in trial settings before it can be used for the management of this tumor.

Conclusion:
Gallbladder carcinoma has a limited epidemiology with diverse geographical locations suggesting a larger role of environment including lifestyle tightly linked to genetic factors. For epidemiological purposes, GBCs are usually classified together with all other extrahepatic bile duct cancers. It would be valuable if they could be split off to ensure comparability of incidence data between different countries, and this would be especially useful in looking for temporal changes in the incidence of gallbladder tumors and in identifying risk factors at the population level. Although we know that the metaplasia–dysplasia pathway is the main one by which GBC develops, and a number of the genetic alterations important for this progression have been identified, much work remains to be carried out in this area. Questions remain as to whether the natural history and range of pathology are the same in high and low-prevalence areas. The difficulty in diagnosing these tumors, short of removing the gallbladder, highlights the need for more work in identifying serum markers. It can be seen, therefore, that all stages in the development and progression of these tumors provide challenges that will best be met by a collaborative, international approach.

Volume : 5 | Issue : 4 | April 2015 | ISSN - 2249-555X

REFERENCE

1. Drake, Richard L.; Vogl, Wayne; Tibbitts, Adam W.M. Mitchell; illustrations by Richard; Richardson, Paul (2005). Gray's anatomy for students. Philadelphia: Elsevier/Churchill Livingstone. p. 287. | 2. Misra NC, Misra S, Chaturvedi A. Carcinoma gallbladder. In: Johnson CD, Taylor I (Eds). Recent advances in surgery, volume 20. London: Churchill Livingstone, 1997: 69–87. | 3. Nagorney DM, McPherson GA. Carcinoma of the gallbladder and extrahepatic bile ducts. Semin Oncol 1988; 15: 106–15. | 4. Jones R. Carcinoma of the gallbladder. Surg Clin North Am. 1990;70:1419-28. | 5. Carriagea MT, Henson DE. Liver, gallbladder, extrahepatic bileducts and pancreas. Cancer, 1995;75:171-90. | 6. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 57, 43-66. | 7. Orth K, Berger HG. Gallbladder carcinoma and surgical treatment. Langenbecks Arch Surg 2000, 382: 501-508. | 8. Hamrick RE Jr, Liner FJ, Hastings PR, Cohn I Jr. Primary carcinoma of the gallbladder. Ann Surg 1982; 195:270. | 9. Yamaguchi K, Chijiiwa K, Ichimiya H, et al. Gallbladder carcinoma in the era of laparoscopic cholecystectomy. Arch Surg 1996; 131:981. | 10. Randi G, Franceschi S, La Vecchia C. Gallbladder cancer worldwide: geographical distribution and risk factors. Int J Cancer 2006;118:1592–1602. | 11. Cancer incidence and mortality in Delhi UT urban—2001. New Delhi: Delhi Cancer Registry,2006. | 12. Raina V, Tyagi BB, Manoharan N, et al. Cancer incidence and mortality in Delhi UT Urban, 2006. New Delhi: Delhi Cancer Registry, 2010. | 13. Gorunova, I., Parada, I., Johansson, B. et al. Nonrandom chromosome aberrations and cytogenetic heterogeneity in gallbladder carcinoma. Cancer.1999, 26: pp. 312–321. | 14. Kuroki, T., Tajima, Y., Matsuo, K., Kanematsu, T. Genetic Alterations in Gallbladder Carcinoma. Surg. Today. 2005, 35:101–105. | 15. Chang, H. J., Kim, S. W., Kim, Y. T., Kim, W. H.: Loss of heterozygosity in dysplasia and carcinoma of the gallbladder. Mod. Pathol.199;12: 763–769. | 16. Yoshida, T., Sugai, T., Habano, W., Nakamura, S., Uesugi, N., Funato, O., Saito, K. Microsatellite instability in gallbladder carcinoma: two independent genetic pathways of gallbladder carcinogenesis. J. Gastroenterol.2000;35:768-774. | 17. Wistuba, II., Tang, M., Maitra, A., Alvarez, H., Troncoso, P., Pimentel, F., Gazdar, A. F. Genome-wide alleletyping analysis reveals multiple sites of allelic loss in gallbladder carcinoma. Cancer Res.2001;61: 3795-3800. | 18. Tadokoro, H., Shigihara, T., Ikeda, T., Takase, M., Suyama, M. Two distinct pathways of p16 gene inactivation in gallbladder Cancer. World J. Gastroenterol.2007;13:6396-6403. | 19. Kim, Y. T., Kim, J., Jang, Y. H., Lee, W. J., Ryu, J. K., Park, Y. K., Kim, S. W., Kim, W. H., Yoon, Y. B., Kim, C. Y. Genetic alterations in gallbladder adenoma, dysplasia and carcinoma. Cancer Lett.2001;169:59–68. | 20. Rashid, A., Ueki, T., Gao, Y. T., Houlihan, P. S., Wallace, C., Wang, B. S., Shen, M. C., Deng, J., Hsing, A. W. K-ras mutation, p53 overexpression, and microsatellite instability in biliary tract cancers: A population-based study in China. Clin Cancer Res.2002; 8: 3156–3163. | 21. Parwani, A. V., Geradts, J., Caspers, E., Offerhaus, G. J., Yeo, C. J., Cameron, J. L., Klimstra, D. S., Maitra, A., Hruban, R. H., Argani, P. Immunohistochemical and Genetic Analysis of Non–Small Cell and Small Cell Gallbladder Carcinoma and Their Precursor Lesions. Mod. Pathol.2003;16: 299-308. | 22. Saetta, A. A., Papanastasiou, P., Michalopoulos, N. V., Gigelou, F., Korkolopoulou, P., Bei, T., Patsouris, E. Mutational analysis of BRAF in gallbladder carcinomas in association with K-ras and p53 mutations and microsatellite instability. Virchows Arch. 2004; 445:179–182. | 23. Shukla VK, Singh G, Sharma D, Dixit VK, Singh U. Diagnostic value of serum CA242, CA 19-9, CA 15-3 and CA 125 in patients with carcinoma of the gallbladder. Trop. Gastroenterol. 2006; 27; 160–165. | 24. Wu W, Pan C, Yu H, Gong H, Wang Y. Heparanase expression in gallbladder carcinoma and its correlation to prognosis. J. Gastroenterol. Hepatol. 2008; 23; 491–497. | 25. 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: 1008–1015. | 26. Zatonski, W. A., Lowenfels, A. B., Boyle, P., Maisonneuve, P., Bueno de Mesquita, H. B., Ghadirian, P., Jain, M., Przewozniak, K., Baghurst, P., Moerman, C. J., Simard, A., Howe, G. R., McMichael, A. J., Hsieh, C. C., Walker, A. M. Epidemiologic aspects of gallbladder cancer: a case-control study of the SEARCH program of the International Agency for Research on Cancer. J. Natl. Cancer Inst.1997;89:1132–38. | 27. Misra, N. C., et al. Epidemiology, aetiology and chemotherapy of cancer gallbladder with special reference to intrahepatic arterial infusion with mitomycin-c (MMC) and 5- fluorouracil (5 FU). Proceedings of the 5th International Congress on Anti-Cancer Treatment, Pari. 1995. 0–737, p. 162. | 28. Roa I, Araya JC, Villaseca M, et al. Preneoplastic lesions and gallbladder cancer: an estimate of the period required for progression. Gastroenterology. 1996; 111: 232–36. | 29. Wistuba II, Miquel JF, Gazdar AF, Albores-Saavedra J. Gallbladder adenomas have molecular abnormalities different from those present in gallbladder carcinomas. Hum Pathol 1999; 30: 21–25. | 30. Sumiyoshi K, Nagai E, Chijiiwa K, Nakayama F. Pathology of carcinoma of the gallbladder. World J Surg 1991; 15: 315–21. | 31. Albores- Saavedra J, Henson DE, Sobin LH. The WHO histological classification of tumours of the gallbladder and extrahepatic bile duct. A commentary on the second edition. Cancer 1992; 70: 410–14. | 32. Fahim RB, McDonald JR, Richards JC, Ferris DO. Carcinoma of the gallbladder: a study of its modes of spread. Ann Surg 1962; 156:114–24. | 33. Muratore A, Polastri R, Capussotti L. Radical surgery for gallbladder cancer: current options. Eur J Surg Oncol 2000; 26: 438–43. | 34. Nuzzo G, Clemente G, Cadeddu F, Ardito F, Ricci R, Vecchio FM. Papillary carcinoma of the gallbladder and anomalous pancreatico-biliary junction. Report of three cases and review of the literature. Hepatogastroenterology 2005; 52; 1034–1038. | 35. Cangemi V, Fiori E, Picchi C et al. Early gallbladder carcinoma: a single-center experience. Tumori 2006; 92; 487–490. | 36. Lada PE, Taborda B, Sanchez M et al. [Adenosquamous and squamous carcinoma of the gallbladder]. Cir. Esp. 2007; 81; 202–206. | 37. Mingoli A, Brachini G, Petroni R et al. Squamous and adenosquamous cell carcinomas of the gallbladder. J. Exp. Clin. Cancer Res. 2005; 24; 143–150. | 38. Anjaneyulu V, Shankar-Swarnalatha G, Rao SC. Carcinoid tumor of the gall bladder. Ann. Diagn. Pathol. 2007; 11; 113–116. | 39. Park JK, Choi SH, Lee S, Min KO, Yun SS, Jeon HM. Malignant gastrointestinal stromal tumor of the gallbladder.J.Korean.MedSci.2004;19;763–767. | 40. Fujii H, Aotake T, Horiuchi T, Chiba Y, Imamura Y, Tanaka K. Small cell carcinoma of the gallbladder: a case report and review of 53 cases in the literature. Hepatogastroenterology. 2001; 48; 1588–1593. | 41. Roa I, de Aretxabala X, Araya JC et al. [Findings in surgicalreinterventions for cancer of the gallbladder in patients with and without preoperative chemotherapy and radiotherapy]. Rev. Med. Chil. 2001; 129; 1013–1020. | 42. De Aretxabala X, Losada H, Mora J et al. [Neoadjuvant chemoradiotherapy in gallbladder cancer]. Rev. Med.Chil.2004;132;51–57. | 43. Piehler JM, Crichlow RW. Primary carcinoma of the gallbladder. Surg Gynaecol Obstet 1978; 146: 929–42. | 44. Donohue JM, Nagorney DM, Grant CS et al. Carcinoma of the gallbladder - does radical resection improve outcome Arch Surg 1990, 125: 237-241. | 45. Cubertafond P, Gainant A, Cucchiaro G. Surgical treatment of 724 carcinomas of the gallbladder: results of the French Surgical Association Survey. Ann Surg 1994, 219: 275-280. | 46. Ouchi K, Suzuki M, Tominaga T, Saijo S, Matsumo S. Survival after surgery for cancer of the gallbladder. Br J Surg 1994, 81: 1655-1657. | 47. Gall FP, Kockerling F, Scheels J, et al. Radical operations for carcinoma of the gallbladder: present in Germany. World J Surg 1991; 15: 328–36. | 48. Shirai Y, Yoshida K, Tsukada K, Muto T. Inapparent carcinoma of the gallbladder. An appraisal of a radical second operation after simple cholecystectomy. Ann Surg 1992; 215: 326–31. | 49. Bartlett DL, Fong Y, Fortner JG, et al. Long-term results after resection for gallbladder cancer. Implications for staging and management. Ann Surg 1996; 224: 639–46. | 50. Baxter I, Garden OJ. Surgical palliation of carcinoma of the gallbladder. Hepatogastroenterology 1999, 46: 1572-1577. | 51. Blumgart LH, Thompson JN. The management of malignant strictures of the bile duct. Curr Probl Surg 1987; 24: 69–127. | 52. Houry S, Haccart V, Huguier M, Schlienger M. Gallbladder cancer: role of radiation therapy. Hepatogastroenterology 1999; 46: 1578–84. | 53. Todoroki T, Iwaski Y, Orii K, et al. Resection combined with intraoperative radiation therapy (IORT) for stage IV (TNM) gallbladder carcinoma. World J Surg 1991; 15: 357–66. | 54. Kresl JJ, Schild SE, Henning GT, et al. Adjuvant external beam radiation therapy with concurrent chemotherapy in the management of gallbladder carcinoma. Int J Radiat Oncol Biol Phys 2002; 52: 167–75. | 55. Misra NC, Misra S, Chaturvedi A, et al. Epidemiology, aetiology and multimodal therapy in cancer gallbladder with special reference to radical surgery and newer chemotherapy protocols: A prospective study of 631 patients. Proceedings of the American Society of Clinical Oncology Pan Asia Cancer conference, New Delhi, India. 2002: 85–87. | 56. Misra NC, Chaturvedi A, Jaiswal MSD, et al. Intrahepatic arterial infusion with combination of mitomycin c (MMC) and 5- fluorouracil (5-FU) for treatment of primary and metastatic carcinoma liver. Reg Cancer Treat 1992; 5: 12–16. |