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Original Article

Clinicopathological features and survival of gallbladder squamous cell carcinoma: analysis of 121 cases

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Abstract: Purpose: The aim of the present study is to promote deeper pathological and clinical understanding of gallbladder squamous cell carcinoma (GBSCC) and provide new evidence for its diagnosis and treatment. Methods: Two cases of GBSCC from the First Affiliated Hospital of Guangxi Medical University were collected. A comprehensive analysis was conducted based upon these 2 cases and another 119 GBSCC cases from the literature. Survival analysis was performed using the Kaplan-Meier method. Results: Among all the patients, GBSCC was frequently diagnosed in older women with a mean age of 62.8 years old. Abdominal pain was the most common symptom. The majority of GBSCC cases were combined with cholelithiasis. Keratinization was frequently observed microscopically. Among 90 cases with histology data, most showed high or, high-moderate differentiation (60%, 54/90). More cases were diagnosed in advanced TNM stages (85.4%, 82/96). In 73 cases, the follow-up time was 0.5-125 months, with a mean survival time 47.3 months and a median survival time of 12 months. Survival analysis indicated that patients with polypoid lesions ($P = 0.047$) and receiving R0 radical operation ($P < 0.001$) had better prognoses. Conclusion: Given the scarcity and implicit clinical manifestations of GBSCC, early diagnosis is challenging. The key to better survival is a radical operation with no remaining lesion.

Keywords: Squamous cell carcinoma, gallbladder, survival analysis

Introduction

Gallbladder carcinoma (GBC) is the most common malignant tumor in the biliary tract, and adenocarcinoma (AC) is the main subtype according to its histology classification. GBC is highly malignant and metastasizes easily. However, the early stages of GBC seldom show symptoms and patients are often diagnosed at the advanced stages. As a result, the overall survival rate of GBC is 6 months, and the 5-year survival rate is only 5% [1]. Squamous cell carcinoma (SCC) often occurs in sites that are covered with squamous cells or cells differentiating into squamous cells via metaplasia, including the skin, trachea, esophagus, oral cavity, larynx, cervix, and perineum. Research demonstrates that gallbladder SCC (GBSCC) has a worse prognosis than AC in the gallbladder, mainly because the former is more malignant and challenging to diagnose in time as well as unresponsive to chemotherapy [2, 3]. Accounting for 1.1-3.7% of all GBC, primary GBSCC is rare [2, 4-13]. The limited literature studying GBSCC primarily comprises case reports. Little is known about the clinical features of GBSCC and the factors affecting its prognosis. The present study reports on two cases of primary GBSCC in our institute and reviews 121 cases of primary GBSCC from the literature to explore the pathogenesis, clinicopathological features, diagnosis, treatments, and prognosis of GBSCC. The current paper may help promote a deeper pathological and clinical understanding of GBSCC and provide new references and evidence for its clinical diagnosis and treatment.

Methods and materials

Introduction of clinical data

The present study reviewed 93 GBC cases confirmed by pathology at the First Affiliated Hospital of Guangxi Medical University from January 2008 to October 2017, including 87 AC

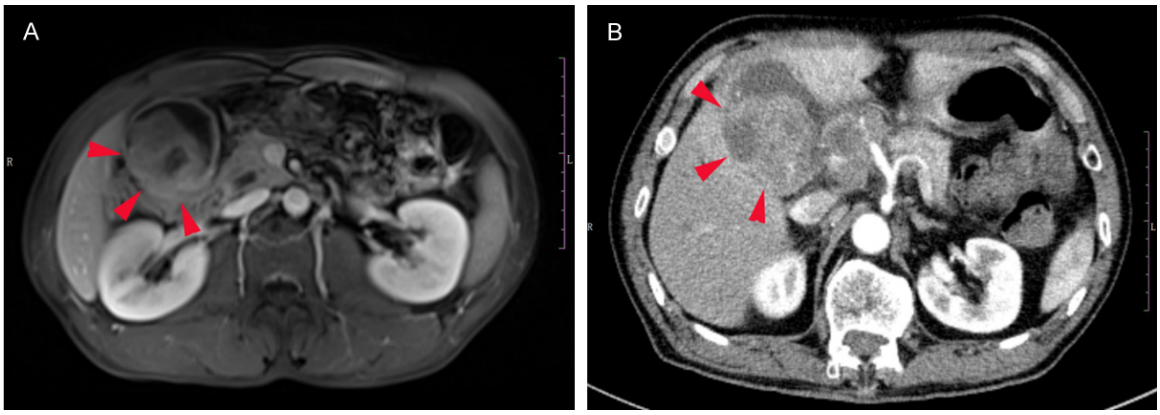

Figure 1. Imaging manifestations of 2 clinical cases. Imaging A shows an MRI scan of the hepatic portal system in Case 1, presenting an irregular mass in the gallbladder with soft tissue density. Imaging B shows an enhanced CT scan of the upper abdomen in Case 2, presenting a mass with soft tissue intensity in gallbladder bed.

cases (93.5%), 4 adenosquamous carcinoma cases (ASC, 4.3%) and 2 SCC cases (2.2%). Subsequently, the following details of 2 SCC cases were collected.

Case 1: Male, 45 years old, recurrent abdominal pain for 2 months. Physical examination: T: 36°C, P: 70 bpm, R: 20 bpm, BP: 97/61 mmHg. No swelling of the superficial lymph nodes. Abdominal wall was soft. Gallbladder was palpable under right costal margin with smooth surface, medium texture, slight tenderness, and negative Murphy sign. Laboratory tests: WBC 22.30 * 10^9/L, N% 85.3%; CA125 50.68 U/ml (H 0.00-35.00). AFP, CEA, CA199 were normal. Routine and enhanced CT scan performed in another hospital showed a mass in the gallbladder which was highly suspected of GBC. Both intrahepatic and extrahepatic bile ducts were dilated. Magnetic resonance cholangiopancreatography (MRCP) was suggested. Routine and enhanced MRI scans of the hepatic portal system showed that the gallbladder was apparently enlarged with an irregular mass with soft tissue density observed within. The size of the mass was around 5.5 × 3.7 cm (**Figure 1A**). The external wall of the gallbladder was still smooth. After enhancement, the lesion was obviously enhanced, but the inner necrosis area was not. The gallbladder wall was equally enhanced. No abnormal enhancement was found in gallbladder fossa or adjacent liver parenchyma. Without thickened walls and narrowed cavity, the common bile duct was unobstructed. The intrahepatic bile duct was not dilated. The primary impression was a gallbladder space-occupying lesion, with a higher possibility of being benign, yet with a possibility of being leiomyosarcoma. Therefore, the diagnosis before the operation was a gallbladder tumor. During the operation, no lesion was observed in the liver, stomach, and small intestine. With its wall thickened, the gallbladder was hard and enlarged to a size of 13 × 6.5 × 6.5 cm. The lesion was in the neck of the gallbladder, about 7 × 6 × 6 cm. It was cauliflower-like from the outside and was fish flesh-like after being sectioned. The tumor had broken through the serosa; however, it had not invaded the liver, bile duct, or intestines. The sentinel lymph node of the gallbladder was 2 × 1.5 cm and hard. A GBC radical operation was performed. After surgery, the general condition of the patient was favorable. He did not receive radiotherapy or chemotherapy, but instead was given Fufang Banmao, a patented Chinese medicine, by capsule. The patient received re-examination regularly for serum tumor markers and abdominal color-Doppler ultrasound. During the follow-up, which lasted 3 years 5 months, no relapse or metastasis was detected.

Case 2: Male, 64 years old, admitted for recurrent abdominal pain for 7 months and aggravated pain for 1 month. The patient complained of vague, upper-right abdominal pain from February 2016. The pain was mild, appearing 5-6 times per day and lasting a few minutes each time. Since the pain alleviated spontaneously, the patient did not pay attention to it or seek treatment initially. One month prior to

seeking treatment, the patient felt a tingling pain, mainly in the right upper abdomen, accompanied by a rather acute abdominal distension. There was no fever. He had suffered from cholecystitis in 1980, but it had improved with treatment. He drank and smoked for about 40 years. Physical examination: T: 36.7°C, P: 81 bpm, R: 20 bpm, BP: 151/84 mmHg. No jaundice or swelling of the superficial lymph nodes was found. The abdominal wall was soft. A slight tenderness was palpated under the xiphoid. No rebound tenderness or mass was palpated. Laboratory tests: CEA 16.55 ng/ml (H 0.00-5.00), CA125 39.20 U/ml (H 0.00-35.00), CA153 34.74 U/ml (H 0.00-31.30). AFP and CA199 were normal. Abdominal B-mode ultrasound showed a solid mass in the gallbladder (high probability of GBC, further examination suggested). Routine and enhanced CT scan of the upper abdomen showed a mass measured at 11 × 9 × 8.4 cm (**Figure 1B**), with soft tissue intensity in the gallbladder bed of the hepatic hilum area, in which heterogeneous intensity was observed. The enhancement was obviously uneven, and the margin was the main enhanced area. With irregular boundary and implicit margin, large scale necrosis was noticed. The multiple lesions were lobulated and surrounded the hepatic hilum, invading the common hepatic artery, the trunk of the portal vein, the common hepatic duct, and the common bile duct, whose cavity was rather narrow and whose wall was rigid. The adjacent liver, duodenal bulb, descending duodenum, head and neck of the pancreas, and hepatic flexure were compressed and displaced by the tumor, some parts of which had vague boundaries. The intrahepatic bile duct was dilated. The primary impression was a space-occupying lesion in hepatic hilum, suggesting a malignant lesion, with a high possibility of being GBC. Invasion of the hepatic hilum and dilation of the intrahepatic bile duct were also observed. An endoscopic, ultrasound-guided, fine-needle aspiration biopsy of the gallbladder was conducted on September 10, 2016, and the diagnosis was a gallbladder space-occupying lesion, suspected as carcinoma which had invaded the duodenum. After consultation with the oncologists, it was considered GBC accompanied by abdominal cavity metastasis, with no indication for surgery. The patient refused chemotherapy and requested discharge. He died after the 5-month follow-up.

Case collection based on Chinese and English literature

The Chinese keywords “gallbladder”, “biliary system”, “tumor”, “mass”, “carcinoma” and “squamous cell carcinoma” were searched in Chinese databases of published clinical case reports, including CNKI, Chong Qing VIP, Wan Fang, and China Biology Medicine disc. The search strategy for English databases was as follows: (malignan* OR cancer OR tumor OR tumour OR neoplas* OR carcinoma OR SCC OR SC OR “squamous cell carcinomas” OR “epidermoid carcinoma” OR “pavement cell cancer” OR “Squamous-celled Carcinoma”) AND (gallbladder OR “gall bladder” OR “gall-bladder” OR GBC). The English literature databases chosen were as follows: PubMed, Wiley Online Library, Embase, Cochrane, Web of Science, Google Scholar, and Baidu Scholar. Studies that met the following standards were included: 1) Basic information, clinical manifestations, pathological features, and the results of immunohistochemical analyses and serum marker tests of the patient were included. 2) The primary GBSCC cases had been confirmed by pathological diagnosis. 3) The studies included were published before October 31, 2017. The exclusion criteria were as follows: 1) The patient in the study was not confirmed to be primary GBSCC. 2) There were infiltrating AC tissues found in a histopathologic examination. 3) No basic information such as gender, age, etc. on the patient was provided. 4) Only the abstract of the study was available. 5) In the instance of multiple studies based on the same patient or based on data from the same research institution, only the most recent studies were included, the rest being excluded.

Information including gender, age, race, cholelithiasis, pathology, treatment options, surgery, and survival time of the included cases was extracted from the included studies. Features such as clinical manifestations, gross appearances of lesions, histopathological characteristics, immunohistochemical analyses, serum tumor markers, stage, treatment, prognosis, etc., were summarized and analyzed. Evaluation, screening, and data extraction of the

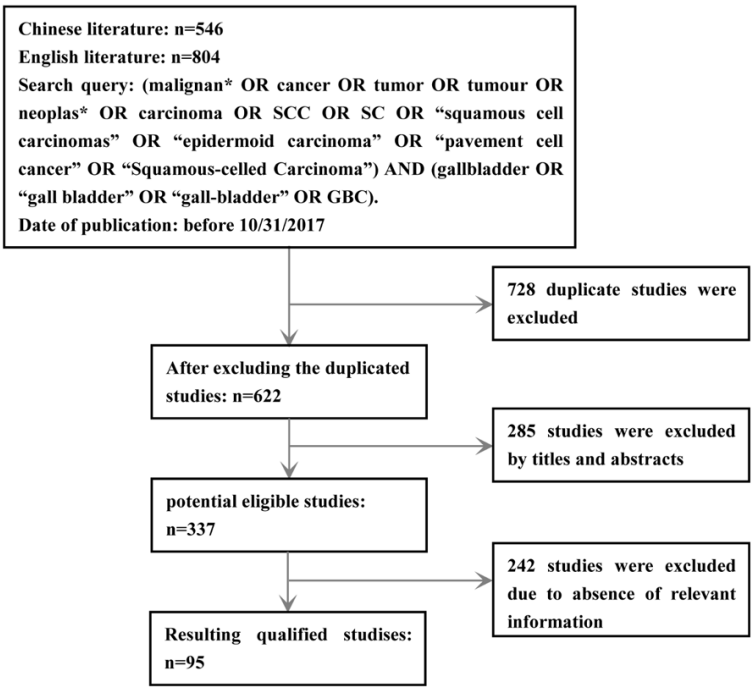

Figure 2. Flow chart of literature screening.

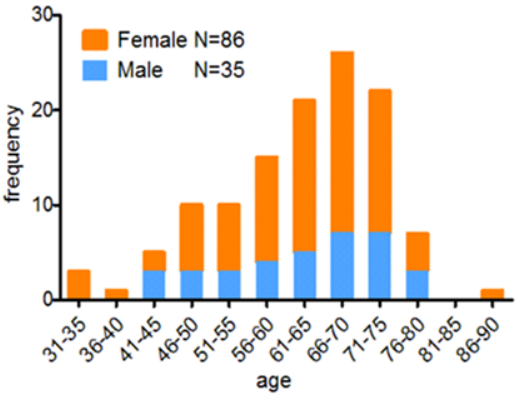

Figure 3. Age and sex ratios of the 121 cases of gallbladder squamous cell carcinoma (GBSCC) included in the study.

selected studies were independently performed by 2 researchers. In the instance of a divergence of views, this was settled by discussion or judged by a third researcher.

Statistical analysis

All data were analyzed applying SPSS 22.0 (Armonk, NY, USA). The survival analysis was performed using a Kaplan-Meier curve, and the survival rate utilized a log-rank test. A *P*-value < 0.05 was considered statistically significant.

Results

Literature acquired from databases

Originally, 248 studies were retrieved from PubMed, 26 studies from Wiley Online Library, 107 studies from Embase, 93 studies from Web of Science, 148 studies from Google Scholar, 182 studies from Baidu Scholar, 90 studies from CNKI, 168 studies from Wan Fang, 78 studies from Chong Qing VIP, and 210 studies from CBM. No article was attained from Cochrane. After being screened according to the criteria (**Figure 2**), 95 qualified case reports were collected, including 54 in Chinese and 65 in English, as some of the studies provided several cases. With the 2 cases from First Affiliated Hospital of Guangxi Medical University, 121 primary GBSCC cases were included in total in the current study.

Clinical parameters of included cases

Among the 121 included cases, 35 were male and 86 female, with a male-to-female ratio of 1:2.5 (**Figure 3**). The patient ages ranged from 35 to 88 years, with an average of 62.9 years and a median of 65 years. The age of male patients ranged from 44 to 80 years, averaging 63.2 and with a median of 65. Female patients ranged in age from 35 to 88 years, with an average of 62.8 and a median of 65. Only 101 cases had information about the patients' clinical symptoms. Abdominal pain was the most common symptom (81.2%, 82/101 cases). Other symptoms were fever (25.7%, 26/101), tenderness (24.8%, 25/101), abdominal mass (22.8%, 23/101), jaundice (17.8%, 18/101), Murphy sign positive (17.8%, 18/101), and abdominal discomfort (7.9%, 8/101). Accompanying symptoms included nausea (15.8%, 16/101 cases), vomiting (13.9%, 14/101), weight loss (14.9%, 15/101), asthenia (7.9%, 8/101), abdominal distension (6.9%, 7/101),

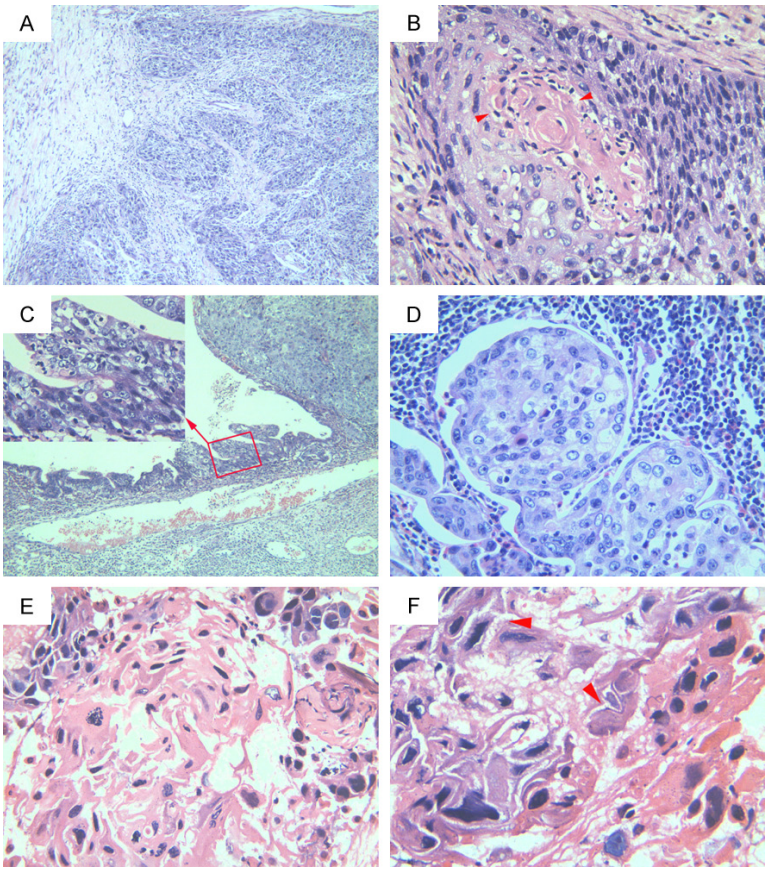

Figure 4. Histopathologic figures of 2 cases from the First Affiliated Hospital of Guangxi Medical University (A-D show Case 1, E, F show Case 2). Image A manifests the infiltrating growth pattern of the irregular cancer nests in the gallbladder wall. The interstitial area around the cancer nests promotes the proliferation of fibrous tissue. Image B shows the polygonal, highly eosinophilic cancer cells and keratin pearl at high magnification. The columnar epithelium of the gallbladder surrounding SCC tissues show moderate-severe dysplasia in image C. Image D shows metastatic SCC components in the lymph node. Significant keratinization of the punctured sample is visible in image E's microscopic image. Image F shows noticeable intercellular bridges.

anorexia (5.0%, 5/101), indigestion (4.0%, 4/101), and radiating pain (3.0%, 3/101). Only 84 cases provided gallstone test information. In 68 out of 84 cases (81.0%), gallstones were discovered. No aforementioned symptom was specifically linked to GBSCC. Laboratory testing was not uniform. Of those patients tested, the following presented elevated levels. In terms of laboratory tests, elevation was detected in the following markers: ALP (52.4%, 11/21 cases), CEA (18.8%, 3/16), CA125 (75%, 3/4), CA199 (13.3%, 2/15), CA153 (100%, 1/1), AFP (7.7%, 1/13), and SCCA (100%, 1/1).

As for imaging examinations, 65 patients were examined by B-ultrasound and 24 cases were misdiagnosed as benign lesions (36.9%, 24/65), 10 cases were suspected to be a tumor (15.4%), 23 cases were diagnosed as "gallbladder tumor" (35.4%), and only 8 cases were clearly identified as GBC (12.3%). Altogether, 43 cases were detected by CT, which resulted in 18 out of 43 GBSCC patients diagnosed as gallbladder tumor (41.9%) and 15 received definite diagnoses GBC (34.9%). A thickened gallbladder wall was reported without explicit diagnosis in 3 cases (7.0%). Benign lesions were misdiagnosed by CT in 7 cases (16.3%). For the 8 patients who had an MRI, 3 were diagnosed as having GBC (37.5%) and 2 were diagnosed as having a gallbladder tumor (25%). In 2 cases, the MRI merely reported "thickened gallbladder wall". Only 1 patient was misdiagnosed as having benign lesions by the MRI. Of the 3 patients who received MRCP examinations, a thickened gallbladder wall was reported without an explicit diagnosis in 2 cases. Benign lesion was diagnosed by MRCP in 1 case. As for other diagnostic approaches, an endoscopic retrograde colangiopancreatography (ERCP) discovered GBC in 1 patient. X-ray and endoscopic ultrasonography (EUS) diagnosed 1 gallbladder tumor each. Of the 93 cases, 79.6% (74/93) of the preoperative diagnoses were consistent with those made after surgery. In all, 19 cases were misdiagnosed as benign before surgery (20.4%), including 11 cases of cholelithiasis and cholecystitis (11.8%), 4 cases of acute cholecystitis (4.3%), 1 case of chronic cholecystitis (1.1%), 1 case of cholelithiasis combined with gallbladder atrophy (1.1%), 1 case of hepatic cyst (1.1%), and 1 case of gallbladder rupture and ascites (1.1%).

Gross appearance of lesions

Only 55 out of 121 cases reported the site of the tumor. The sites reported included the fun-

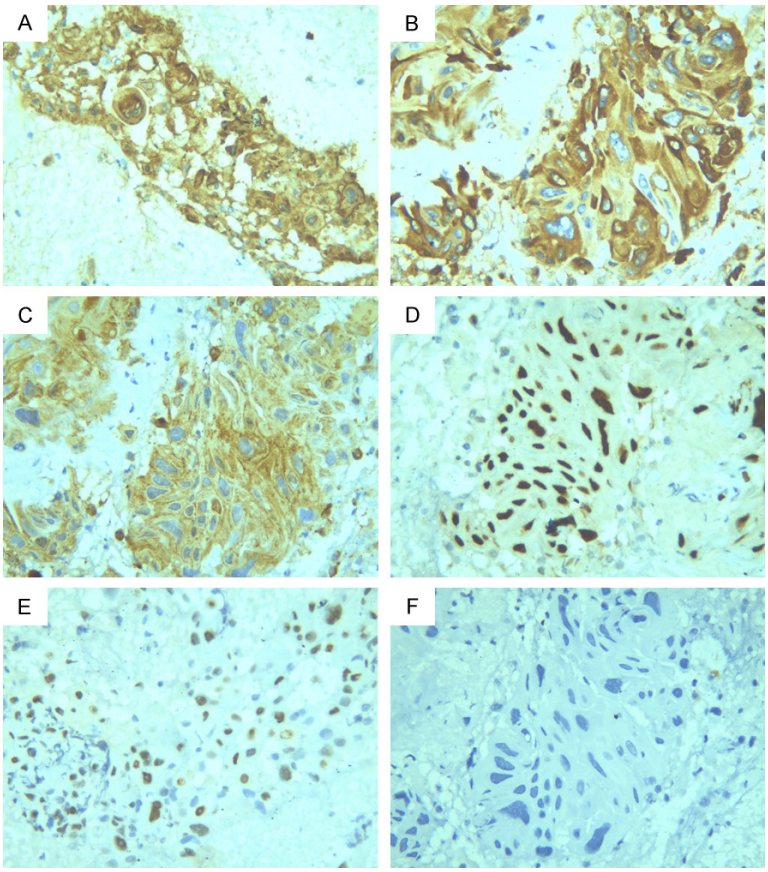

Figure 5. Immunohistochemistry figures of Case 2 from the First Affiliated Hospital of Guangxi Medical University. Image A shows AE1/AE3 (+), indicated by cytoplasm of cancer cells. Image B shows CK7 (+), indicated by cytoplasm of cancer cells. Image C shows CK19 (+), indicated by cytoplasm of cancer cells. Image D shows P40 (+), indicated by nucleus of cancer cells. Image E shows P63 (+), indicated by nucleus of cancer cells. Image F shows CK20 (-), indicated by cancer cells.

dus of the gallbladder (29.1%, 16/55 cases), the body (20%, 11/55), the neck (14.5%, 8/55), the body-fundus area (10.9%, 6/55), the neck-body area (9.1%, 5/55), the entire gallbladder (14.5%, 8/55) and the residual gallbladder (1.8%, 1/55). The largest diameter of the lesion ranged from 0.4 to 15 cm, averaging 5.25 cm. The gross appearance of the tumor was divided into 3 types. The first type, polypoid (52.7%, 29/55 cases), is described as being like a mushroom, nodule, or cauliflower in shape, manifesting as a locally space-occupying adenomatoid lesion on the gallbladder wall, jutting into the cavity and invading the gallbladder wall downward. The surrounding mucosa has ulcerated and necrosed. The section of the tumor is solid and brittle, sometimes accompanied by bleeding and necrosis. The second type is the infiltrating type (40%, 22/55), in which the gallbladder wall is thickened and hardened like leather locally or entirely. Finally, the ulcerative type (7.3%, 4/55) presents an irregularly thickened gallbladder wall, locally-formed ulcers, atrophic surrounding mucosa, and in some cases perforation. In Case 1 from in-house collection, the sample was a gallbladder in the cavity of which was discovered a large, grayish-white, cauliflower-like mass. The size of the tumor was 10 × 6 × 6 cm, infiltrating all layers of the gallbladder wall with a thickness of 0.5-0.8 cm. The fine-needle aspiration sample of Case 2 in-house was a pile of fragmentary, taupe tissue, measuring 1 × 1 × 0.4 cm.

Analysis of pathological features

Pathology confirmed that all 121 cases included were SCC. The cancer cells exhibited infiltrating patterns and formed cancer nests (**Figure 4A**), which showed squamous differentiation. No evidence of AC was noted. Among all 90 cases investigated for differentiation, 31 cases showed high differentiation (34.4%, 31/90 cases), 23 showed high-moderate differentiation (25.6%), 24 showed moderate differentiation (26.7%), 6 showed moderate-poor differentiation (6.7%), and 6 showed poor differentiation (6.7%). While 75 cases were described as keratinizing SCC, the other 46 cases did not mention whether keratinization appeared. Typical keratinocytes appeared in various sizes and were usually located in the center of the cancer nest in a polygonal shape. They were an abundant as well as highly eosinophilic cytoplasm (**Figure 4E**) with clear borders. Eosinophilic keratoses appeared in the center of some cancer nests, arranged in the stratified and concentric-circle form known as keratin pearls (34/75 cases, **Figure 4B**). There were apparent intercellular bridges between the squamous cells (39/75 cases, **Figure 4F**), with enlarged nuclei; thick-

Table 1. Kaplan-Meier univariate survival analysis

Parameters	Total (n)	Average survival time months (95% CI)	Median survival time months (95% CI)	X^2	*P*
Gender					
Male	20	55.7 (25.3-86.0)	15.0 (0.0-32.8)	1.100	0.294
Female	53	43.5 (27.2-59.8)	12.0 (8.9-15.1)		
Age					
≤ 65	30	57.3 (34.8-79.8)	17.0 (0.0-78.6)	2.809	0.094
> 65	43	36.9 (19.7-54.0)	11.9 (6.6-17.2)		
Race					
Chinese	35	32.3 (18.6-46.0)	11.9 (9.8-14.0)	0.515	0.473
Non-Chinese	38	57.4 (36.4-78.4)	17.0 (0.0-34.8)		
Gallstone					
Yes	37	48.7 (29.4-68.1)	12.0 (11.9-12.1)	0.621	0.431
No	12	15.7 (6.5-24.9)	16.5 (2.1-30.9)		
Growth pattern					
Polypoid	16	73.5 (42.9-104.1)	-	3.96	0.047
Infiltrating	10	10.0 (6.1-13.9)	10.0 (1.2-18.8)		
Tumor size					
< 5 cm	17	83.1 (55.3-111.0)	-	3.028	0.082
≥ 5 cm	15	17.7 (9.5-25.9)	12.0 (5.7-18.3)		
Pathological grade					
I	20	41.4 (16.2-66.6)	11.9 (2.5-21.3)	0.781	0.677
II	24	58.7 (30.9-86.4)	15.0 (0.0-55.5)		
III	11	49.6 (21.3-77.8)	12.0 (-)		
T stage					
T1-T2	11	84.9 (54.8-114.9)	-	3.055	0.080
T3-T4	50	46.6 (28.6-64.6)	15.0 (8.1-21.9)		
Lymph node					
Metastasis	15	33.8 (15.0-52.5)	16.5 (4.0-29.0)	2.339	0.126
N0 metastasis	25	82.8 (60.2-105.4)	120.0 (0-272.9)		
TNM stage					
I-II	11	84.9 (54.8-114.9)	-	3.055	0.080
III-IV	50	46.6 (28.6-64.6)	15.0 (8.1-21.9)		
Surgery					
R0	44	68.2 (49.1-87.4)	75.4 (7.0-143.8)	13.457	0.000
R1 or R2	9	9.3 (2.1-16.4)	4.9 (3.7-6.1)		
Adjuvant therapy					
Yes	17	35.2 (12.0-58.3)	15.0 (9.1-20.9)	1.078	0.299
No	13	40.3 (24.7-56.0)	-		

Note: TNM, tumor-node-metastasis.

ened, irregular nuclear membranes; and coarse pieces of deeply-stained chromatin, with a paving stone-like appearance. The mitosis of keratinocytes was rare in the studied cases. Keratinizing characteristics mainly appeared in highly- and moderately-differentiated SCC cases. Fibrous tissue proliferation and collagen deposition were frequently observed in the interstitial area around the cancer nests (**Figure 4A**), occasionally infiltrated by chronic inflammatory cells such as lymphocytes and plasma cells. In the 12 cases which showed the characteristics of poorly differentiated SCC, the keratinization of tumors was rather scarce. However,

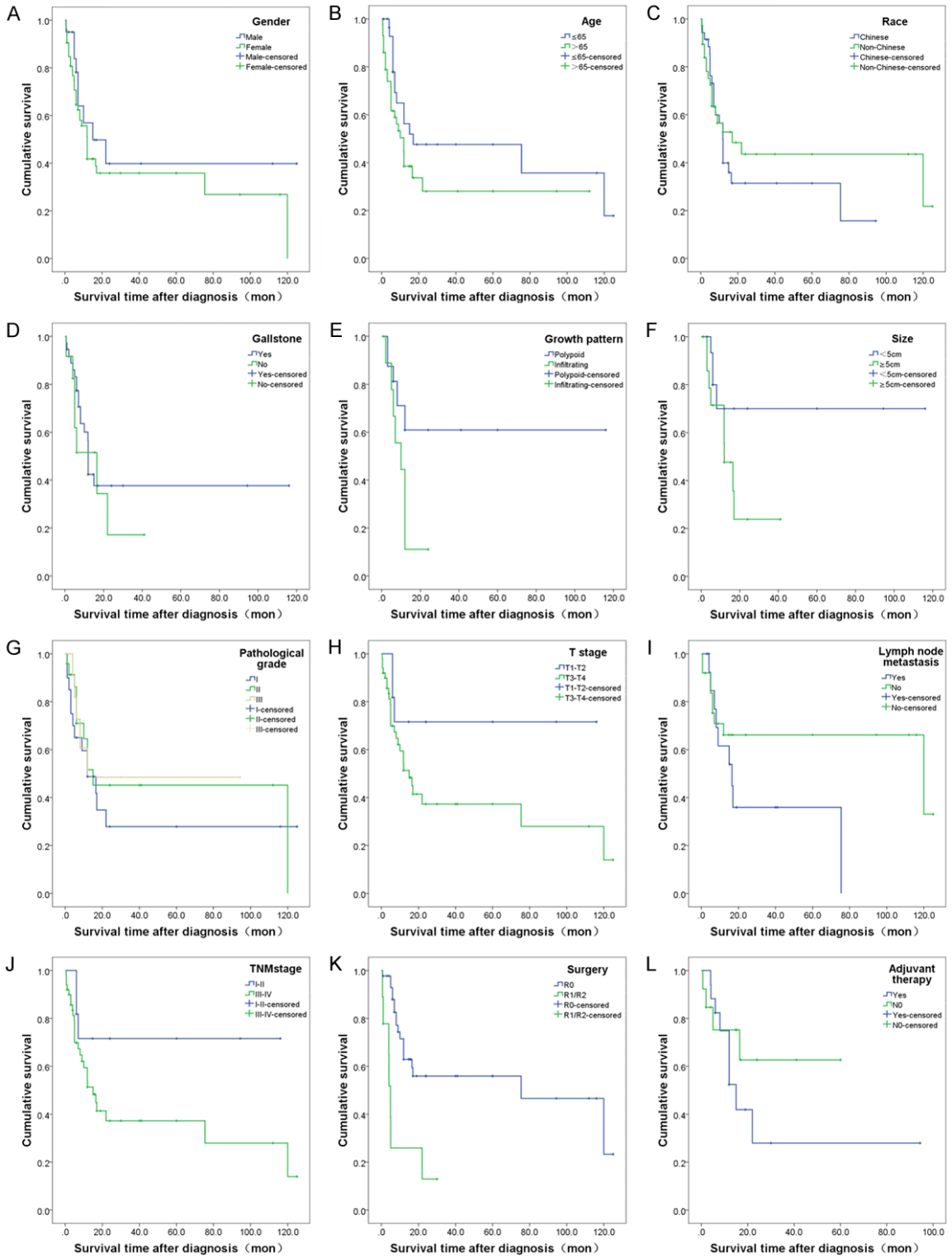

Figure 6. Survival curves according to gender (A), age (B), race (C), cholelithiasis (D), growth pattern (E), tumor size (F), pathological grade (G), T stage (H), lymph node metastasis (I), TNM stage (J), radical surgery (K), and adjuvant therapy (L).

the atypia of tumor and activity of mitosis increased, including pathological mitosis. In 1 case, special stains consisting of PAS and Alcian blue stain were applied, both with nega-

tive results, proving the sample included no infiltrating AC components. The results of immunohistochemical analyses were provided in 14 cases, with the following positive markers: AEI/AE3 (7/7 cases), p63 (5/6 cases), CK5/6 (5/5 cases), CK7 (3/5 cases), P40 (3/3 cases), CK10 (3/3 cases), CKI9 (2/2 cases), 34βE12 (2/2 cases), P53 (2/2 cases), CAI9-9 (1/1 case), EMA (1/1 case) and CKI4 (1/1 case). The negative markers were as follows: SMA (1 case), Hepat (1 case), CD68 (1 case), S-100 (4 cases), CgA (1 case), Syn (1 case), CK8/18 (1 case), CK8 (4 cases), CEA (3 cases), CK20 (2 cases), CAM5.2 (2 cases), B72.3 (2 cases), α-fetoprotein (2 cases), α-1-antitrypsin (2 cases), HMB 45 (2 cases) and vimentin (1 case).

With respect to the 2 cases from the local hospital, Case 1 was diagnosed as moderately-differentiated keratinizing GBSCC, which invaded all layers of the gallbladder wall. The surgical margin of the gallbladder neck was free of cancer cells. No tumor invasion was found in the liver tissue. The surrounding glandular epithelium showed moderate-severe dysplasia (**Figure 4C**). Metastatic SCC was discovered in 1 sentinel lymph node of the gallbladder (**Figure 4D**) but not seen in the 4 lymph nodes of the duodenum ligament. Case 2 was diagnosed as highly-differentiated keratinizing SCC. The immunohistochemistry results were: AE1/AE3 (+), CK19 (+), CK7 (+), P40 (+), P63 (+), CK20 (-) (**Figure 5**).

TNM stage of selected cases

The TNM staging of 96 cases were as follows: 5 at stage I (5.2%), 9 at stage II (9.4%), 34 at stage III (35.4%), and 48 at stage IV (50%). Moreover, there were 5 cases at T1 stage, 10 at T2 stage. 33 at T3 stage and 48 at T4 stage. No invasion of adjacent organs was detected in 21 cases; however, it was found in 75 cases, including the following organs: liver (84%, 63/75 cases), duodenum (33.3%, 25/75, including 1 case of duodenum ligament invasion), colon (28%, 21/75, including 13 cases of transverse colon invasion), stomach (12%, 9/75), omentum (10.7%, 8/75), abdominal wall (6.7%, 5/75), hepatic hilum (4%, 3/75), bile duct (4%, 3/75), pancreas (4%, 3/75), and peripheral ligaments (1.3%, 1/75). Regional lymph node metastases were observed in 21/54 cases (38.9%). No lymph node metastasis was discovered in the other cases. Only 4 out of 49 cases (8.2%) reported distant metastasis. The TNM stage of Case 1 in the local hospital was IIIb (T3N1M0) and that of Case 2 was IVb (T4NXM1).

Treatments received

In 86.0% of the cases (104/121 cases), patients received operations, and 55 of them were R0 radical operations. Adjuvant radiotherapy or chemotherapy was given in 10 cases. R1 surgical margin was reported in 6 cases. Palliative surgery was performed in 11 cases. No specific surgery type was described in 32 cases. In the cases that did not perform tumor resection, 2 cases found the tumor could not be resected by exploratory laparotomy and took only a part of the tumor for biopsy. In 15 cases, endoscopic, ultrasound-guided, fine-needle aspiration biopsy was performed.

Prognosis

Survival data was provided in 73/121 cases. Follow-up ranged from 0.5 to 125 months, with a mean survival time of 47.3 months and a median survival time of 12 months. Notably, the survival time was beyond 10 years in 2 cases [14, 15]. A Kaplan-Meier univariate survival analysis and a log-rank test (**Table 1**) showed the factors that influenced prognosis were tumor growth pattern ($P = 0.047$) and R0 radical surgery ($P < 0.001$) (**Figure 6**). Patients with tumors growing in a polypoid, or cauliflower-like, form had an average survival time of 73.5 months (95%, CI: 42.9-104.1). However, tumors growing in an infiltrating pattern indicated an average survival time of 10 months (95%, CI: 6.1-13.9), a significantly shorter time frame ($P = 0.047$). Patients with R0 surgical margins had significantly better prognoses, with a median survival time of 75.4 months (95%, CI: 7.0-143.8, $P < 0.001$). Nevertheless, the median survival time of patients who had R1 or R2 surgical margins was 4.9 months (95%, CI: 3.7-6.1). The data suggested no significant correlation between prognosis and clinicopathological features, including gender, age, race, cholelithiasis, tumor size, pathological grade, T stage, lymph node metastasis, TNM stage, and adjuvant therapy ($P > 0.05$). In in-house Case 1, the patient received R0 radical surgery but declined regular radiotherapy or

Table 2. 1985-2017 large-scale gallbladder cancer studies (n ≥ 100) in English reporting incidence of squamous cell carcinoma (SCC)

Author	Year	Region	Total patients	SCC patients	Percentage
Sons HU [4]	1985	Germany	287	-	3.7%
Henson DE [5]	1992	USA	2665	45	1.7%
Chan KM [6]	2007	Chinese Taiwan	157	2	1.3%
Kim WS [7]	2011	Korea	404	5	1.2%
Roa JC [2]	2011	Chile	606	8	1.3%
Gupta P [8]	2012	India	322	9	2.8%
Yadav R [9]	2013	India	437	5	1.1%
Mallick S [10]	2014	India	104	3	2.9%
Song HW [11]	2015	China	464	10	2.2%
Kumar R [12]	2017	India	645	7	1.1%
Wu ZC [13]	2017	China	1060	26	2.5%

chemotherapy. He was still disease-free at a 41-month follow-up. In Case 2, the patient showed no indication for surgery and refused the suggested radiotherapy and chemotherapy. He died 5 months after diagnosis.

Discussion

GBSCC is a rare kind of tumor. It accounts for 1.1-3.7% of all GBC in the English studies published between 1985 and 2017 (**Table 2**), each study including more than 100 cases of GBC. The First Affiliated Hospital diagnosed 93 cases of GBC between January 2008 and October 2017 using pathological approaches, finding only 2 cases of GBSCC. As a result, the GBSCC/GBC ratio in the hospital was 2.2%, consistent with the aforementioned statistics. However, some cases were misdiagnosed as GBSCC, including ASC containing a few AC components, beam-like poorly-differentiated AC, and undifferentiated carcinoma. These misdiagnoses produced an inaccurate GBSCC/GBC rate of up to 12.7% [16-18]. The pathogenesis of GBSCC is yet unclear. However, 2 probable hypotheses have been raised involving gallstones and parasite infections [19], the former being the most crucial risk factor for GBC. It has been reported that 95% of GBC cases are correlated with gallstones [20], and the present study discovered gallstones in 81% of the cases. The histological origin of GBSCC remains elusive, and there are multiple histogenesis hypotheses [18, 21]. Some researchers believe that GBSCC originates from the squamous metaplasia of pluripotent basal cells in gallbladder mucosa. In a case reported by Rekik et al., reciprocal transition occurs among the squamous epithelium, SCC in situ, and invasive carcinoma [22]. Other researchers tend to believe that AC occurs first, then gradually transforms into ASC by squamous metaplasia and, finally, SCC completely replaces AC [17, 23]. The co-existence of AC in situ and squamous metaplasia in an SCC case was observed by Hanada et al. [24]. In Case 1 from our institute, moderate-severe dysplasia of the columnar epithelium around invasive carcinoma was found, but no squamous metaplasia was discovered.

GBC is highly malignant and has a poor prognosis. It is frequently diagnosed in older women, and female patients are more than 3 times as common as male patients [20]. In the present study, the male-to-female ratio was 1:2.5. More than half of the patients in the study were aged 60-75. The average age was 62.9, and no difference existed between the average ages of male and female patients. No significant symptom can be observed at the early stages of GBSCC. The tumor was discovered accidentally in some patients after gallbladder resection indicated by gallstones [17] or acute cholecystitis [25]. The main clinical manifestations in the progression stage are upper-right abdominal pain (81.2%, mostly occasional or persistent, dull pain or colic), fever (25.7%), palpable mass in the upper right abdomen (swelling gallbladder, 22.8%), jaundice (17.8%), positive Murphy sign (17.8%), nausea (15.8%), vomiting (13.9%), weight loss (14.9%), and asthenia (7.9%). These manifestations are not typical or specific, and some of them are symptoms of acute cholecystitis [19, 23, 25], leading to delayed diagnosis as well as treatment. Consequently, clinicians should consider the possibility of GBSCC before diagnosing cholecystitis if older female patients complain of these symptoms.

Due to the lack of typical clinical manifestations, the clinical diagnosis of GBSCC mainly depends on imaging approaches. B-ultrasound, serving as a screening method, can discover

abnormalities such as a thickened gallbladder wall, space-occupying soft tissue lesion inside the gallbladder, and gallstones as soon as possible. The invasion of GBSCC into the liver and the swelling of adjacent lymph nodes can also assist in the diagnosis of GBC. However, some GBSCC cases are misdiagnosed as acute cholecystitis because ultrasound shows the thickened wall of the gallbladder [19, 23, 25]. CT scan and MRI are crucial for diagnosis, evaluating tumor progression and metastasis, and forming a surgical plan. As a valuable method for diagnosing, evaluating, and staging a tumor, PET-CT can provide images of functional metabolism and anatomy simultaneously to comprehensively evaluate the patient's condition. Utilizing PET-CT, Hu et al. diagnosed residual tumor at the incision after an operation and detected distant metastasis in 2 GBSCC cases [26]. In order to improve the prognosis and give proper treatment, it is essential to recognize the relapse of GBSCC accurately.

In laboratory tests reviewed in the present study, the serum alkaline phosphatase (ALP) was elevated in GBC (11/21 cases), which may have occurred due to the obstruction of the bile duct caused by a tumor. Reliable tumor markers are yet to be found, owing to low specificity [27]. Since serums CA19-9, CEA, CA125, and CA242 are elevated in GBC, the specificity for diagnosis may be increased by the combination of multiple biomarkers [28, 29].

Since no typical sign of GBSCC can be detected in an imaging examination, only the general condition can be observed. A confirmed diagnosis is dependent on the pathology. Concerning the gross appearance of GBSCC, the gallbladder is enlarged, and the tumor growth pattern is divided into 3 types (infiltrating, ulcerative, and polypoid). In the infiltrating type, the gallbladder wall is thickened and hardened like leather locally or entirely. Local ulceration is observed in some cases [30]. Sometimes it is difficult to distinguish the infiltrating type of GBSCC from chronic cholecystitis. However, the former usually manifests in an irregularly- and asymmetrically-thickened wall, with the structure of all layers damaged, vanishing, and replaced by grayish-white tumor tissues, which is different than chronic cholecystitis. Shaped like a mushroom, nodule, or cauliflower, polypoid type tumors manifest in a space-occupying lesion projecting into the cavity and invading gallbladder wall downward. The section is solid and brittle, sometimes combined with bleeding and necrosis. The lesion may be mistaken for gallbladder polyps or adenoma when it is rather small. Roppongi et al. discovered GBSCC of a tiny polypoid type (0.4 × 0.4 × 0.3 cm) by accident in a sample which was clinically diagnosed as a gallstone and cholecystitis [17]. In fact, the gross appearance of GBSCC at the early stages is not obvious. In the process of gross examination, pathologists are advised to cut through and fully expose all the gallbladder samples with cholelithiasis and completely inspect and palpate for abnormalities such as polyps and abnormal thickening of the wall.

Histopathology with immunohistochemistry is the most reliable method to confirm the diagnosis of GBSCC. As a malignant epithelial tumor, GBSCC is entirely composed of squamous cells, without any invasive glandular structure [2]. GBSCC can be divided into 2 types. The first, keratinizing carcinoma, possesses well-differentiated squamous cells which are arranged in a nest-like or beam-like structure. Only 1 keratin pearl found in the epithelium is sufficient to diagnose keratinizing carcinoma. Neoplastic squamous cells which do not form keratin pearls have abundant eosinophilic cytoplasm and apparent intercellular bridges. Almost every case included in the present study was of the keratinizing type. In the second type, unkeratinizing carcinoma, keratinization of single cell rather than keratin pearls is observed in the neoplastic squamous epithelium nest. The cell border is comparatively implicit. The nucleus is round or oval, with chromatin in coarse pieces. Mitosis is widely observed in the cells. In some SCC cases, cells are arranged in a solid lamellar form with a transparent cytoplasm. It is not difficult to diagnose keratinizing GBSCC. In immunohistochemistry, the positive expression of AE1/AE3, CK5/6, P63 and P40 helps the diagnosis of poorly-differentiated SCC. The negative expression of CK8/18, CK20 and CEA can exclude infiltrating AC components in the tumor.

The pathogenesis and progression of GBC comprise a continual and complex pathological process involving multiple factors and molecules. The process includes the deletion or inactivation of tumor suppressor genes, the amplific-

ation of oncogenes, abnormalities in DNA repair genes, the presence of microsatellite instability (MSI), epigenetic variation, and other molecular biological genetic processes [27]. However, the molecular mechanism of pathogenesis and progression of GBC remains unclear. Recent studies show that the genetic mutation of the ErbB signaling pathway (including EGFR and HER2) promotes the proliferation and invasion of GBC [27, 31-33]. In the high-throughput research of GBC, the mutation of TP53, PIK3CA, IK3CA and KRAS has also been detected [27]. In addition, Garcia et al. found an association between the prognosis of GBC and methylation of DLC1 and MGMT [34]. However, studies concerning the molecular mechanism of GBSCC are limited. Petracci et al. discovered 1 case of the C-Ki-ras mutation (33.3%) among 2 SCC cases and 1 ASC case [35]. Another recent study reported the mutation of ERBB2 and PTEN in GBSCC [36]. Dong et al. found CD109 is negatively expressed in normal gallbladder tissues and AC tissues but shows positive expression in 86.7% of SCC cases [37]. Consequently, they deduced that CD109 may promote the proliferation of GBSCC by suppressing the TGF-β signal component.

The 5-year survival rate of GBC, a lethal malignant tumor, is between 0 and 10% [29]. Previous research indicates that the prognosis of GBSCC is more pessimistic than the common type of GBC-AC-because the former is more invasive and that the average survival time after diagnosis is only 6 months [22]. In the present study, the median survival time of 73 GBC patients was 12 months. This improved prognosis was likely based upon the more frequently-performed radical surgery. Currently, surgery is the only effective curative treatment for GBC [38]. For patients at the Tis (carcinoma in situ) or T1a (invading mucosa) stage, simple cholecystectomy is appropriate. As for patients at the T1b (invading muscular layer) stage or higher, to attain a cancer-free surgical margin, radical R0 surgery should be performed, including resecting the gallbladder, the adjacent gallbladder bed, liver tissue (with the surgical margin of the liver being more than 2-3 cm from the gallbladder), and regional lymph nodes [38, 39]. Studies have reported the disease-free survival time of 2 GBSCC patients after receiving extended R0 radical cholecystectomy (both at IV stage) to be over 10 years [14, 15]. In Case 1 in-house, the intraoperative consultation led to diagnosis and confirmation that the surgical margin was cancer-free and that the operation had reached the standard of R0 radical surgery. The patient remained disease-free at the 41-month follow-up. It is also reported that radiotherapy may prolong survival time. Hou et al. reported that a GBSCC patient at an advanced stage and unable to receive surgery and only receiving radiotherapy had a disease-free survival time of 13 months but died 2 months later after a relapse [40]. With deeper exploration of oncogenes in GBC and signaling pathways, targeted therapy based on the oncogenes in GBC will play important roles in treatment [27, 41].

Conclusion

In the cases reviewed, GBSCC was frequently diagnosed in older women. The clinical manifestations were vague and lacked specificity, posing difficulties for early diagnosis. According to a Kaplan-Meier univariate survival analysis, patients with polypoid tumors who received R0 radical surgery had better prognoses. Clinicians are advised to be more aware of GBSCC and to diagnose it as early as possible using imaging and pathological approaches. The key to better prognosis is radical surgery with no tumor remaining.

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Disclosure of conflict of interest

None.

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