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Surgical Significance of Superficial Cancer Spread in Early Gallbladder Cancer

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Background: A considerable percentage of gallbladder cancers are accompanied by superficial cancer spread adjacent to the main tumor, therefore cholecystectomy for early gallbladder cancer must be performed carefully to avoid leaving cancer cells at the surgical margins.
Methods: Thirty-two patients with gallbladder cancer invading no more than perimuscular connective tissue underwent surgical resection at our medical center. After the operation, resected specimens were investigated macroscopically and microscopically to clarify the clinicopathological features and the risk factors of superficial cancer spread.
Results: Sixty-six percent of all cases (21 cases) had superficial cancer spread. Comparison between the cases having superficial cancer spread and the cases without it revealed that the macroscopic morphology of the primary tumor and the depth of cancer invasion in the gallbladder wall were significantly different between the two groups. Furthermore, multivariate analysis indicated that 'superficial raised type' in macroscopic morphology was an independent predictive factor for having superficial cancer spread. Superficial cancer spread from the main tumor located in the neck of the gallbladder grows predominantly in the direction of the fundus. More advanced gallbladder cases were accompanied by more extensive superficial spread.
Conclusions: Superficial cancer spread is frequently observed adjacent to the gallbladder cancer, especially in the superficial raised type. A negative margin should be confirmed by intraoperative frozen section histology while performing cholecystectomy.

Key words: gallbladder cancer – superficial cancer spread – surgical margin – superficial raised type

INTRODUCTION

Gallbladder cancer (GBC) is the most common malignant tumor of the biliary tract (1). Although surgical resection is the only curative modality for this cancer, curative resection rates are as low as 10–30% (2). Likewise, the overall 5-year survival rates are reported to be <5% in advanced cases (3,4). However, in the case of early GBC in which the depth of cancer invasion is limited to the lamina propria (pT1a) or the muscle layer (pT1b) of the gallbladder wall without nodal involvement, hematogenous metastasis or peritoneal implantation, a 5-year survival rate of >80% can be expected even when a simple cholecystectomy is performed (5,6). When performing a laparoscopic cholecystectomy for polyps or stones, incidental early GBC is not rare. Wakai et al. (6) indicated that neither additional partial hepatectomy (resection of gallbladder bed) nor lymphadenectomy was necessary for pT1-GBC, but that simple cholecystectomy alone was sufficient to accomplish curative surgery. However, in such cases, paying careful attention to the surgical margins of the cystic duct is essential, because the status of the tumor at the surgical margin is the most important prognostic factor in such early cancers (5,7). Albores-Saavedra et al. (1) and Tsuchiya (8) reported that gallbladder epithelium (mucosal layer) around the main tumor was frequently replaced by carcinoma *in situ*, just like the spread of superficial cancer. A similar type of cancer extension has been well documented in cancers of the stomach, esophagus and pancreas. Usually it is difficult to identify the range of superficial cancer spread by intraoperative macroscopic inspection. Fahim et al. (9) reported some cases of GBC in which postoperative histology revealed that superficial cancer spread continuously extended into the extrahepatic bile duct via the cystic duct. In order to prevent positive surgical margins in performing cholecystectomy, precise investigation of superficial cancer spread is indispensable. In the present

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article, superficial cancer spread was studied histopathologically on surgically resected specimens to clarify the clinicopathological features and the type of GBC that is more likely to show superficial cancer spread. These data will give us important information for the selection of the operative procedure, especially for less advanced GBC cases.

PATIENTS AND METHODS

Thirty-two patients with GBC underwent surgical resection at the Osaka Medical Center for Cancer and Cardiovascular Diseases from November 1982 to January 2004. In the present study, GBCs invading the lamina propria (TNM stage T1a, $n=6$), muscle layer (T1b, $n=6$) or perimuscular connective tissue (T2, $n=20$) were studied. The patients included 20 women and 12 men with a mean age of 66.7 years (range 43–82). Prior to surgery, GBC had been suspected or diagnosed by computed tomography (CT) or ultrasonography (US). Five patients with pT1a and two with pT1b underwent simple cholecystectomy, and one with pT1a and four with pT1b underwent cholecystectomy with wedge resection of the gallbladder bed. Of the pT2 cases, patients underwent either simple cholecystectomy ($n=7$), cholecystectomy with wedge resection of the gallbladder bed ($n=7$), cholecystectomy with bile duct resection ($n=5$) or pancreatoduodenectomy ($n=1$). In all cases, the cystic duct was resected together with the gallbladder. The main location of the cancer was defined as follows: Gn, neck cancer located in the proximal third of the cystic duct; Gb, body cancer located in the middle third; and Gf, fundus cancer located in the fundic third. The cancers were classified macroscopically as pedunculated type, sessile type, superficial raised type or flat type, according to the classification of Tsuchiya (8). Briefly, the pedunculated type was defined as a polypoid tumor with a stalk, and the sessile type as a papillary and protruding lesion without an obvious stalk but with a broad base. The superficial raised type was defined as a smoothly elevated lesion rising 1.5–3 mm from the surrounding mucosa, and the flat type as a lesion showing the same height as the adjacent non-tumorous epithelium.

For histopathological analysis, 5 mm stepwise tissue sections were obtained (mean ± SD, 11.1 ± 3.3 sections/case) from the entire resected gallbladder. They were embedded in paraffin, cut into 4 μm slices and stained with hematoxylin and eosin. All 32 patients showed adenocarcinoma on histology, and the deepest layer of cancer invasion in the gallbladder wall was determined by examining multiple sections from each patient. Superficial cancer spread was investigated by microscopic examination of all specimens and was recorded as positive if cancerous epithelia were observed in the surrounding mucosa of the macroscopic lesion (Fig. 1A). With regard to the distinction between superficial carcinoma and neighboring non-malignant epithelial cells, abrupt transformation (front formation) was employed in comparing (i) nuclear chromatin; (ii) N/C ratio; (iii) length of nuclei; (iv) mitosis; and (v) polarity, according to criteria proposed by Albores-Saavedra et al. (1) and Bivins et al. (10). The maximum distance of the superficial cancer spread measured from the edge of the macroscopic lesion was defined as the spreading distance (B in Fig. 1B) and recorded in the positive cases. The distance from the macroscopic or microscopic edge of the tumor to the cystic duct was also investigated (C or D in Fig. 1B).

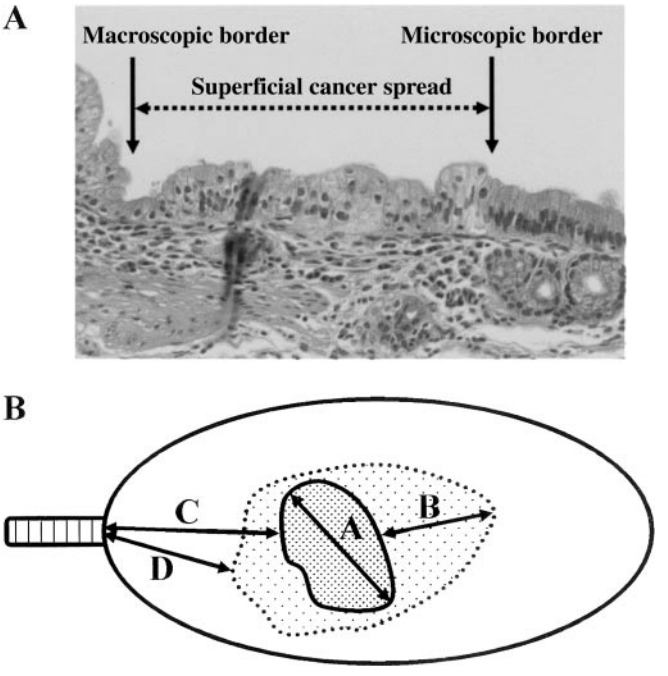

Figure 1. (A) Superficial spread of gallbladder cancer (GBC). Carcinoma *in situ* continuously spreading in the mucosal layer from the macroscopic lesion. The microscopic border was investigated at a magnification of 200× (HE-stained section). (B) Schema of superficial GBC spread. A, maximum diameter of the macroscopic lesion; B, maximum microscopic cancer spread from the border of the macroscopic lesion; C, distance from the cystic duct to the macroscopic border of the lesion; D, distance from the cystic duct to the microscopic border of the cancerous epithelium.

The study protocol was approved by the Human Ethics Review Committee of Osaka Medical Center for Cancer and Cardiovascular Diseases, and a signed consent form was obtained from each subject.

Medical records and survival data were obtained for all 32 patients. The follow-up period ranged from 1 to 148 months (mean ± SD, 45.7 ± 28.7). Statistical evaluation was performed by one-way factorial ANOVA (analysis of variance) test or unpaired *t*-test using the StatView software (SAS Institute Inc., Cary, NC). Multivariate analysis using logistic regression modeling was also performed to determine the independent predictive factor(s) for superficial spread. All data are expressed as mean ± SD. A P-value of <0.05 was defined as statistically significant.

RESULTS

Among 32 patients, 21 (66%) exhibited superficial cancer spread around the macroscopic tumor. The macroscopic tumors occupied an area of 3.2 ± 1.6 cm (range 0.8–7.0) in diameter at the macroscopic level (A in Fig. 1B), whereas the microscopic spread from the border of the macroscopic lesion

(B) was 2.3 ± 2.2 cm (range 0–7.5). The average distances from the cystic duct to the macroscopic border of the lesion (C) or to the microscopic border of the cancerous epithelium (D) were 4.6 ± 2.5 cm (range 0.5–8.5) and 3.4 ± 2.3 cm (range 0.5–8.0), respectively.

Thirty-two cases were classified into two groups according to the presence or absence of superficial cancer spread (Table 1). There were no significant differences between the two groups with regard to age, sex, maximum diameter of the main tumor, location, histological differentiation and nodal involvement. However, the macroscopic morphology of the primary tumor was significantly different between the two groups ($P < 0.05$): the group with superficial cancer spread comprised one (5%) pedunculated type and 15 (71%) superficial raised type, whereas the group without superficial cancer spread consisted of five (45%) pedunculated type and only one (9%) superficial raised type. According to the depth of cancer invasion in the gallbladder wall (pT category), the former group consisted of one (5%) pT1a, three (14%) pT1b and 17 (81%) pT2 cancers, whereas the latter group consisted of five (45%) pT1a, three (27%) pT1b and three (27%) pT2 cancers. This difference was also statistically significant ($P < 0.05$).

In order to identify the independent predictive factor(s) for superficial spread, multivariate analysis was employed using the factors of macroscopic type and the depth of cancer invasion (Table 2). Superficial raised type was selected as a significant predictive factor for superficial cancer spread with a relative risk of 35.4 ($P = 0.03$), while pT classification was not significant.

In the present study, investigation of the resected specimens revealed that no cases had cancer cells at the surgical margins macroscopically or microscopically, and no recurrences from the surgical margins were observed in any cases throughout the follow-up period. The cancer spread even from the GBCs located at the neck of the gallbladder (Gn) does not invade the cystic duct, suggesting that the mode of spread may be different according to the location of the main tumor. We therefore investigated the spreading distances and the predominant directions of the spread in Gn, Gb and Gf GBCs. The average distances of the spread were similar among the three groups. While 50% (six cases) of Gf GBCs and 33% (two cases) of Gb GBCs predominantly spread in the direction of the cystic duct, no Gn GBCs predominantly spread in that direction (Table 3).

We next investigated the correlation between superficial spread and cancer progression. Table 4 shows the relationships between the depth of cancer invasion and superficial spreading distance in superficial raised type GBC. Although the number of cases was small in each group, the distance of cancer spread in pT2 cases (3.2 ± 1.6 cm) was greater than in pT1a or pT1b cases.

Table 1. Univariate analyses of backgrounds between two groups classified according to the presence or absence of superficial cancer spread

	Superficial cancer spread		*P*-value
	Yes	No	
No. of cases	21	11	
Age	65.6 ± 10.3	68.7 ± 7.3	0.38
Gender (M:F)	6:15	6:5	0.25
Maximum diameter of the main tumor (cm)	3.4 ± 1.6	2.9 ± 1.6	0.35
Location			0.45
Gn	3 (14%)	2 (18%)	
Gb	6 (29%)	1 (9%)	
Gf	12 (57%)	8 (73%)	
Histology			0.68
Well	17 (81%)	10 (91%)	
Moderately	3 (14%)	1 (9%)	
Poorly	1 (5%)	0 (0%)	
Nodal involvement			0.07
Positive	6 (29%)	0 (0%)	
Negative	15 (71%)	11 (100%)	
Macroscopic type			0.002*
Pedunculated type	1 (5%)	5 (45%)	
Sessile type	5 (24%)	5 (45%)	
Superficial raised type	15 (71%)	1 (9%)	
pT			0.006*
1a	1 (5%)	5 (45%)	
1b	3 (14%)	3 (27%)	
2	17 (81%)	3 (27%)	

*$P < 0.05$.

DISCUSSION

Although advanced GBC is associated with a high mortality rate, a simple cholecystectomy is an adequate treatment for patients with early GBC, provided that the resection margin is

Table 2. Multivariate analysis of predictive factors for superficial cancer spread

Factor	Relative risk	95% confidence limits	*P*-value
Macroscopic type			
Pedunculated type	Reference		
Sessile type	2.48	0.152–40.568	0.52
Superficial raised type	35.40	1.41–886.76	0.03*
pT			
1a	Reference		
1b	5.22	0.19–145.79	0.33
2	16.14	0.78–336.44	0.07

*$P < 0.05$.

Table 3. The spreading distances and the predominant directions of the spread according to the location of the main tumor

Location (cases)	Superficial cancer spread			Spreading distance (cm)
	Yes		No	
	Neck	Fundus		
Gn (5)	0 (0%)	3 (100%)	2	2.6 ± 2.8
Gb (7)	2 (33%)	4 (67%)	1	2.0 ± 1.0
Gf (20)	6 (50%)	6 (50%)	8	2.4 ± 2.4

Table 4. Relationships of the depth of cancer invasion and the distance of superficial cancer spread in superficial raised type

pT	Cases	Spreading distance (cm)
pT1a	1	1.5
pT1b	2	1.8 ± 2.5
pT2	13	3.2 ± 1.6

not involved (5). Because GBCs are reported to spread superficially adjacent to macroscopic lesions, precise investigation of the spread is important to prevent positive surgical margins. Our results showed that 66% of pT1–2 GBC cases were associated with superficial cancer spread adjacent to the macroscopic lesions and that the superficial raised type of tumor was an independent predictive factor for the presence of superficial spread.

A smaller percentage of pedunculated type cancers were associated with superficial spread than sessile type or superficial raised type cancers. Our results suggest that in the case of the superficial raised type, careful attention is needed not to leave superficial cancer at the surgical margin in performing cholecystectomy because superficial spread adjacent to the macroscopic tumor is likely in such cases. However, 17% of the pedunculated type and 50% of the sessile type also had superficial spread, indicating that even in the case of pedunculated type or sessile type GBCs, negative surgical margins should be confirmed by intraoperative frozen section histology.

Although several cases in our study were associated with superficial spread, spread into the cystic duct was not observed in any cases. This may be explained by the fact that the spread from Gn GBCs grows predominantly in the direction of the fundus (Table 3). However, this observation may not be true in the case of advanced GBCs, as Fahim et al. (9) reported that six cases (4%) out of 151 surgically resected GBCs had intraductal spread into the extrahepatic bile ducts. However, many of the cases in that series were of far advanced GBCs. It may therefore be possible to say that at least in the cases with pT1–2 GBC, invasion into the cystic duct from the macroscopic lesion is very rare, regardless of the macroscopic classification. This observation is of importance for early or incidental GBC cases because simple cholecystectomy is the first line treatment for such cases. The present study also revealed that the border of the superficial spread is close to the cystic duct in some cases; however, the whole cystic duct and the tip of the gallbladder wall sometimes remain unresected, especially in laparoscopic colecystectomy. Surgeons should therefore take care to remove the tip of the gallbladder wall completely in laparoscopic colecystectomy, otherwise negative surgical margins should be confirmed by intraoperative frozen section histology.

In this study, we enrolled relatively early cases of GBC, which had invasion no deeper than perimuscular connective tissue. Advanced cases often show other types of cancer extension, including lymph node metastasis and direct invasion into the hepatic bed, and also may have intraductal spread by vascular invasion or lymphatic invasion in the submucosal layer. Toyonaga et al. (11) reported that the postoperative survival time of pT2 GBC patients who underwent a radical second operation after simple cholecystectomy did not differ from that of patients without an additional operation, suggesting that simple cholecystectomy may be a feasible procedure for T1–2 GBC patients. We therefore enrolled only T1–2 GBCs, in which the prognosis after simple cholecystectomy depends mainly on the status of the surgical margin in the cystic duct, and excluded advanced GBC cases, in which the prognosis depends rather on lymph node metastasis or direct invasion into the hepatic bed. Investigating superficial cancer spread using T1–2 GBC cases is therefore important; however, our results also revealed that lymph node metastases were present in 30% (six cases) of pT2 GBCs, suggesting that simple cholecystectomy was insufficient for such cases.

It remains unknown whether superficial spread is the lateral growth or the precursor of the macroscopic lesion. Previously, some authors have reported that carcinoma *in situ* adjacent to the main lesion may be a precursor and can develop into invasive GBC (1,12). In the present study, although the numbers of cases investigated was too small to show the statistical significance, more advanced (pT2) GBC cases were accompanied by more widely extended superficial spread, suggesting that superficial spread is not a precursor of the GBC but rather the lateral growth from the macroscopic lesion. Further study of this question is required using a larger number of cases.

In summary, superficial raised type GBCs are frequently accompanied by superficial cancer spread, to which we need to pay attention in order to avoid positive surgical margins at cholecystectomy. Although evaluation of macroscopic morphology is useful to some extent to predict the existence of superficial cancer spread, negative surgical margins should be confirmed by intraoperative frozen section histology.

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