Squamous Cell and Adenosquamous Carcinoma of Gall Bladder: a Clinicopathological Study of 8 Cases Isolated in 94 Cancers

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Abstract Adenosquamous/squamous cell carcinoma (AS/SCC) of the gall bladder is a rare histopathologic subtype that accounts for 1 to 12% of all the gall bladder carcinomas (GBC). Literature on these malignancies is limited. A retrospective analysis of 8 cases (study group) of pathologically proven AS/SCC of the gallbladder among 94 patients who underwent surgery for GBC from January 2001 to December 2014 was done. Twenty-four conventional gall bladder adenocarcinoma cases were selected as control group after matching for age and gender. Mean patient age was 57.8 years and six were females. Clinical presentation did not seem to be too different among the study and control group. Average tumor thickness was significantly higher in AS/SCC cases as compared to the control group (mean 3.1 versus 1.44 cm; $p = 0.001$). Immunohistochemistry for Ki-67 in adenosquamous carcinoma of the gall bladder showed much higher proliferative index in squamoid area than the adenocarcinoma areas (mean 31.3 versus 16.8%; $p = 0.0004$). Study group showed 87.5% patients were in higher stage whereas only 37.5% of the patients in control group presented at a higher stage. AS/SCC of the gall bladder presented at an advanced stage. Higher proliferation index in squamous component of adenosquamous carcinomas may account for higher T stage in these malignancies.

Keywords Squamous · Adenosquamous · Gall bladder · Carcinoma

Introduction

Gall bladder cancer (GBC) is a rare but aggressive malignancy with marked ethnic and geographic variations. The highest rates of gall bladder cancer are observed in Northern India and Pakistan, East Asia, Eastern Europe, and South America including Columbia and Chile. Incidence of Gall bladder cancer in India is highly variable and it varies among women from 9 per 100,000 per year in northern India in comparison to as low as 1 per 100,000 per year in Southern India. [1] About 9 of the 10 gall bladder cancers are adenocarcinomas (AC) which are usually well to moderately differentiated. Other malignant epithelial tumors of the gall bladder include papillary adenocarcinoma, intestinal type adenocarcinoma, mucinous adenocarcinoma, clear cell adenocarcinoma, signet ring cell carcinoma, small cell carcinoma, and undifferentiated carcinoma [2].

Adenosquamous/squamous cell carcinoma (AS/SCC) of the gall bladder are a rare histopathologic subtype and account for nearly 1 to 12% of all cases [3–6]. The literature available is limited and suggests that these rare tumors are more aggressive in comparison to adenocarcinoma and often infiltrate the adjacent viscera [7]. Adenosquamous cell carcinoma comprises of a mixture of glandular and squamous elements and is described as a tumor wherein the squamous component is between 25 and 99%. If the squamous component is less than 25%, then, the tumors are described as adenocarcinoma with focal squamous change. Pure squamous cell carcinomas are even rarer and are described in literature mainly in the form of case reports [8]. These tumors exhibit exclusively squamous differentiation without any recognizable invasive glandular component [9]. Since adenosquamous/squamous cell

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carcinoma of the gall bladder are rare and its clinicopathologic characteristics and behavior need to be further explored, the present study was carried out.

Material and Methods

Pathology material (reports and slides) from 94 consecutive invasive carcinomas of gall bladder identified in Bhopal Memorial hospital and Research Centre between January 2001 to December 2014 were retrieved and evaluated. Squamous differentiation was observed in 10 of the 94 cases. Of these, six cases were of adenosquamous type (25–99% of the tumor was squamous) and two cases were pure squamous cell carcinoma. The remaining two cases were excluded from the study as they exhibited focal squamous change (less than 25%). Two groups were constituted in the study. The study group comprised of 8 cases (AS/SCC) and the control group comprised of 24 cases of adenocarcinoma. Age and sex matched control cases were randomly selected from the excluded cases without any squamous component. Incidental and early carcinomas (pT1) were excluded from the control group to eliminate the bias for comparison with advanced stages of AS/SCC's. Both the study and control groups were analyzed for the clinical and pathological parameters. The study was performed in accordance with the institutional ethical committee requirements.

Immunostain was performed on paraffin embedded sections using peroxidase-based Ultravision Quanto Detection System (Thermo Scientific, UK). Monoclonal rabbit anti-Ki-67 antibody (Clone SP6) from Spring Bioscience, CA, USA was used. The percentage of positively stained tumor cell nuclei out of the total tumor cells counted ($n = 1000$) from 10 randomly selected fields; $>20\%$ stained cells was considered as high Ki-67 index group.

Statistical analysis was performed using statistical program GraphPad Prism 7. Mean values were compared using paired and unpaired t test. Proportions were compared using chi-square test without Yates correction. A p value of <0.05 was considered statistically significant.

Results

Incidence and Clinical Features Squamous differentiation was identified in 8 of 94 cases of invasive gall bladder carcinoma (8.5%). Of these cases, 2% were pure squamous cell carcinomas and 6% were adenosquamous cell carcinomas. Among these patients, two were males and six were females with a M:F ratio of 1:3. The ratio of male and female in entire group ($n = 86$) of GBC was 1:2.18. Mean age of the patients in study group was 57.8 years as compared with 56.65 years in the gall bladder adenocarcinoma cases ($n = 86$). Age range of

50–71 years was observed in study group. Six (75%) of these patients presented with abdominal pain and four (50%) had nausea and vomiting. Four of these patients also had fever with raised total leucocyte count (TLC). Preoperative clinical suspicion of malignancy was present in 88% of cases, only in one case preoperative diagnosis of empyema gall bladder/acute calculous cholecystitis was made. Gallstones were observed in four (50%) cases [Table 1].

Gross Examination The tumor was located only in fundus in three of the cases. In one other case, the tumor involved fundus and body of gall bladder and in four of the cases, the entire length of the gall bladder was involved from fundus to neck. Radical cholecystectomy with liver wedge resection was done in all eight cases. Mucosal ulceration with infiltrative growth was observed in six cases, whereas proliferative/polypoidal growth was noted in two cases. The tumor was adhered and directly infiltrating the liver in six cases. Average tumor size and tumor thickness in study group was 5.5 and 3.1 cm, respectively, in comparison to size of 3.8 and 1.44 cm in control group of adenocarcinomas (p value 0.087 and 0.001).

Histopathology Examination Both the pure squamous cell carcinomas exhibited abundant keratinization with pearl formation, prominent intercellular bridges, and presence of keratohyaline granules with numerous dyskeratotic cells (Fig. 1). Among the cases of adenosquamous carcinoma, significant amount of keratin was observed in 1 case (16%), two

Table 1 Comparison of clinical, gross, and microscopic features of squamous cell/adenosquamous carcinoma (study group A) and ordinary adenocarcinoma of the gall bladder (control group B)

	Group A ($N = 8$)	Group B ($N = 24$)	p value
A. Clinical features			
Abdominal pain	6	23	0.080
Nausea and vomiting	4	7	0.282
Fever	2	5	0.805
B. Tumor site			
Fundus	3	6	0.495
Fundus and Body	1	3	1.000
Body and Neck	0	4	0.217
Fundus, body, neck	4	10	0.680
Fundus and Neck	–	1 (multifocal)	0.557
C. Mean tumor size (cm)	5.5 ± 0.96	4.0 ± 0.39	0.087
D. Mean tumor thickness (cm)	3.1 ± 0.7	1.44 ± 0.13	0.001
E. Microscopic features			
Peritumoural eosinophils	3 (37.5%)	1 (4.16%)	0.013
Vascular invasion	4 (50%)	14 (58.3%)	0.680
Perineural invasion	3 (37.5%)	13 (54.16%)	0.414

Fig. 1 Pure squamous cell carcinoma of gall bladder infiltrating its wall

of the cases were poorly differentiated and three were moderately differentiated. Clear cell change was observed in 3 cases (37.5%) of adenosquamous carcinoma that was more prominent in squamous areas (Fig. 2a). Sebaceous differentiation was observed in 12.5% cases. In one of the cases, prominent goblet cell change was noted along with marked intestinal metaplasia in adjacent gall bladder mucosa. One of the cases exhibited large pools of extra cellular mucin with several cells showing deposits of intra cellular mucin. Focal areas of sarcomatoid change were observed in only one of the cases. Two of the cases showed large geographic areas of necrosis occupying >50% of tumor. One of these cases was pure squamous cell carcinoma and the other case was of adenosquamous carcinoma. Most of the cases (87.5%) revealed comedo-like central necrosis in the squamous nests. Prominent peritumoral eosinophils were observed in both the pure squamous cell carcinoma and 33% cases of adenosquamous cell carcinoma (Fig. 2b). Only one of the

cases of gall bladder adenocarcinoma of control group showed significant eosinophils that were possibly due to associated parasitemia and presence of hydatid cyst in liver. Prominent lymphocytic infiltration was observed in 75% of cases of study group and 41% of cases of gall bladder adenocarcinoma. Xanthogranulomatous response was noted in only one (12.5%) of the AS/SCC cases whereas in three cases of control group xanthogranulomatous reaction was noted and it accounted for a similar percentage as observed in study group.

In all the six cases of adenosquamous carcinoma, immunohistochemical stain for Ki-67 was performed to compare the percentage of positive nuclei in both adenocarcinoma and squamous areas (Fig. 3a, b, c). Only one of the cases showed high Ki-67 index in adenocarcinoma group whereas all the six cases showed high Ki-67 index in squamous component. The average index obtained in squamoid areas was 31.3% and was significantly higher than adenocarcinoma areas that demonstrated an average index of 16.8% (p value = 0.0004). The invading front of malignant cells invading the liver in adenosquamous cell carcinoma showed squamoid differentiation in five of the six cases.

Adjacent gall bladder mucosa demonstrated squamous metaplasia in both the pure squamous cell carcinoma [Fig. 4] and in 33% cases of adenosquamous cell carcinoma. Lymphovascular and perineural invasion was noted in 3 cases (37.5%) each in the study group (Fig. 5), as compared to 11 cases (46%) showing lymphovascular invasion and 13 cases (54%) showing perineural invasion in gall bladder adenocarcinoma control group (p value 0.680 and 0.414, respectively). All the cases in the study group presented in advanced stage. None of the cases was in T1 stage, 2 cases (25%) were in T2, 5 cases (62.5%) were in T3, and one (12.5%) of the cases was in T4 stage; whereas, in the gall bladder adenocarcinoma control group, 20 cases (83.3%) were in T2, 3 cases (12.5%) were in T3, and one (0.04%) of the cases was in T4. Subset of early

Fig. 2 **a** Photomicrograph showing clear cell change in squamous nest in a case of pure squamous cell carcinoma. **b** Prominent peritumoral eosinophilic infiltrate around the malignant squamous epithelial cells

Fig. 3 **a** Photomicrograph from a case of adenosquamous cell carcinoma showing gradual transition from adenocarcinoma to squamous cell carcinoma. **b** Immunostain for Ki-67 in adenocarcinoma areas showing lower proliferative index. **c** Higher proliferative Ki-67 index in the squamous component of adenosquamous carcinoma

Fig. 4 Arrows showing transition from columnar to metaplastic squamous epithelium showing atypia with an infiltrating squamous cell carcinoma (*inset* shows prominent keratohyaline granules with wet keratin)

Fig. 5 Photomicrograph of a large nerve bundle showing infiltration by neoplastic glands in a garlanding pattern

gall bladder carcinoma (pT1) was not included in the study among the control group to remove the potential bias while comparing the cases of squamous/adenosquamous cell carcinoma (Table 2). All the cases with liver metastasis showed areas of squamous differentiation in liver tissue.

Lymph nodes were submitted for histopathological examination in 7 cases and of these 4 cases showed nodal metastasis. Squamous differentiation in metastatic nodal deposits was

observed in only one of the cases of adenosquamous cell carcinoma. No lymph nodes metastases were seen in pure squamous cell carcinoma cases. Postoperative period and recovery was uneventful in all the above cases. Since radiotherapy facilities are not available in our institute; the patients were referred to an outside hospital and follow-up of the patients was not available. Thus, the survival analysis of the patients under study could not be performed.

Discussion

Pure squamous cell carcinomas of gall bladder are extremely rare and adenosquamous carcinomas also constitute an uncommon subset of gall bladder carcinomas [10]. According to Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute, out of 3038 patients with gall bladder cancer, only 45 (1.7%) were squamous cell carcinoma [6, 11].

Limited data is available in medical literature regarding the clinicopathologic features and behavior of these tumors. In the present study, the incidence of adenosquamous carcinoma (defined as 25–99% of the tumor being squamous) was 6%, and that of pure squamous cell carcinoma (without any documented invasive glandular component) was 2%. Worldwide substantial variation has been noted in the incidence of these tumors; ranging from 1.4 to 10.6% of all cases of gall bladder carcinoma and this could be attributed partly also to definitional differences [9, 12].

As per observations made in the present study, clinical presentation of adenosquamous carcinoma/squamous cell carcinoma group in study group did not seem to be too different than ordinary gall bladder adenocarcinoma, and these findings are in accordance with the previous studies [13, 14]. Mean age of presentation in the study group of AS/SCC was 57.8 years whereas it was 56.65 years in gall bladder adenocarcinoma cases. Average age of patients with adenosquamous/squamous cell carcinoma in other studies was about 60 years. [10, 15] Most common site of these tumors was fundus (37.5%) and the entire length of gall bladder was involved in 50% of the cases with involvement of the cystic duct. Association with gall stones was identified in 50% of cases and is in concordance with literature [15].

The average tumor size in study group was larger at the time of presentation as compared to the conventional gall bladder adenocarcinoma (mean, 5.5 versus 4.0 cm; $p = 0.087$). Tumor thickness was also significantly higher in AS/SCC cases as compared to the control group (mean, 3.1 versus 1.44 cm; $p = 0.001$). Similar findings were observed by R. Kalayarsan et al. who noted median tumor size of 7.9 cm in AS/SCC group versus 4.8 cm in AC group [12].

The histologic differentiation of both elements in adenosquamous carcinomas tends to be of moderate degree,

Table 2 Comparison of pathologic TNM staging between study (squamous cell/adenosquamous carcinoma) and control (ordinary adenocarcinoma) group

Stage	Group A (n = 8)	Group B (n = 24)	p value
T stage (%)			
T1	—	—	—
T2	2	20	0.002
T3	5	3	0.004
T4	1	1	0.399
N stage (%)			
Nx	—	2	—
N0	5	15	1.000
N1	3	7	0.659
N2	0	0	—
Stage (%)			
I	—	—	—
II	1	15	0.014
IIIa	3	1	0.013
IIIb	3	7	0.659
IVa	1	1	0.399
IVb	—	—	—

rather than anaplastic or poorly differentiated. Keratin pearls are often present in the squamous component and mucin is usually demonstrable in the neoplastic glands [2]. Adenosquamous carcinoma with human chorionic gonadotropin production, foci of spindle cells, neuroendocrine and gastric foveolar type of differentiation have been described [16–18]. In our study, out of the six cases of adenosquamous carcinoma, four were moderate or well differentiated. One of the cases showed large pools of mucin in adenocarcinoma areas. Clear cell change and sebaceous differentiation were observed in squamous areas.

Histogenesis of these tumors remains uncertain and usually is thought to develop in response to irritation resulting in squamous metaplasia or originate from preexisting adenocarcinoma [19]. Pure squamous cell carcinoma often arises in association with squamous metaplasia [16]. Similar findings were noted in the present study; both the pure squamous cell carcinomas had areas of squamous metaplasia whereas adenosquamous carcinomas showed abrupt transformation from adenocarcinoma to squamous cell carcinoma. In a study by Nishihara et al., squamous cell component of adenosquamous carcinoma of the gall bladder arose by a stepwise molecular progression of the pre-existing adenocarcinoma [3]. Intraepithelial neoplasia can be found in the metaplastic squamous mucosa [20].

Eosinophil prominence has been noted in squamous cell carcinoma from sites such as esophagus, head and neck region and has been implicated due to chemotactic features [21]. Similarly, peritumoral eosinophilic infiltration was observed in a study by Roa et al. [9]. The present study also showed significant increase in peritumoral eosinophils in AS/SCC in comparison to the study group (p value = 0.013).

Patients with adenosquamous /squamous cell carcinoma of the GB are frequently diagnosed at an advanced stage with a bulky tumor and adjacent organ involvement. This has been attributed to a high proliferative index of the squamous component in these tumors [7]. In a Japanese study by Nishihara K et al., of 20 cases of adenosquamous carcinoma of gall bladder, the positive rate of immunostaining for proliferating cell nuclear antigen in the squamous areas (mean 20.55%) was higher than that of adenocarcinoma areas (mean 11.40%; $p = 0.0029$) indicating that the squamoid areas had a greater proliferative capacity than that of adenocarcinoma [3]. In the present study, the Ki-67 proliferation index was much higher in squamoid areas than the adenocarcinoma areas (mean 31.3 versus 16.8% $p = 0.0004$). The invading front of the gall bladder carcinoma showed squamoid differentiation in 66% of cases. Both the above evidences suggest that squamoid part of adenosquamous cell carcinoma is more aggressive than the adenocarcinoma component. In a study by Hidalgo et al., Ki-67 protein expression in gall bladder carcinoma was not related to histological differentiation, level of GB cancer invasion or patient survival [22].

Mechanism of spread of these tumors is not certain and controversial. Although the patients with adenosquamous/

squamous cell carcinoma present at an advanced stage, the nodal metastases were not common and squamoid areas were observed in only one case with nodal metastases [23]. Nodal metastases were observed in 37.5% of cases in study group and 32% cases in control group. On the contrary, some of the authors have described an aggressive biology with a higher incidence of nodal metastases. Fewer studies have reported node positive disease in 85.7% cases of adenosquamous cell/squamous cell carcinoma [24]. Whereas, other studies have reported an incidence of nodal metastases as 57.1% [7].

These tumors are usually silent in initial stages and are detected in advanced stages. In a study of 29 patients by Oohashi Y et al., 23 patients were categorized with T3 or T4 tumors that invaded adjacent organs [25]. In the current study, six of the 8 patients (75%) were in higher stage (T3 or T4) in the study group whereas in the control group 16.6% patients (4/24) were in the higher stage. This supports the fact that AS/SCC usually present at an advanced T stage.

Kim et al. have suggested that although AS/SCC of the gall bladder present at advanced stage, curative surgical resection of AS/SCC of the gallbladder might result in disease-free survival rates that are comparable with those of conventional GBC [10]. On the contrary, Oohashi et al. have reported a 5-year survival rate of 49% with radical resection compared to a 3-year survival of 8% with primary tumor resection alone. They suggested that the overall prognosis of adenosquamous carcinoma/squamous cell carcinoma appears to be even worse than that of ordinary adenocarcinomas [25].

To summarize, adenosquamous carcinoma/squamous cell carcinoma of the gall bladder are uncommon, together constituting ~8% of the gall bladder malignancies. They often present at an advanced (pT2, pT3, and pT4) stage at the time of diagnosis, and it may be partly attributed to the higher proliferative rate of squamous component in adenosquamous carcinoma of gall bladder. More studies are needed to determine the etiopathogenetic and molecular differences, if any, of this histologic type compared with conventional adenocarcinomas.

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Compliance with Ethical Standards

Conflict of Interest All the authors declare that they have no conflict of interest.

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