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SPECTRUM OF HISTOPATHOLOGICAL FINDINGS IN CHOLECYSTECTOMY SPECIMENS WITH SPECIAL REFERENCE TO CARCINOMA OF GALLBLADDER – A 5-YEAR RETROSPECTIVE STUDY

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Keywords:

Cholecystectomy, Chronic cholecystitis, Gallstones, Gallbladder carcinoma

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ABSTRACT: Background: Cholecystectomy is one of the most frequently performed abdominal surgeries worldwide for symptomatic gallstones and inflammatory gallbladder lesions. Routine histopathological examination remains the gold standard for detection of inflammatory, premalignant and malignant lesions, while immunohistochemistry is reserved for selected diagnostically challenging malignant cases. **Objective:** To analyze the spectrum of histopathological lesions in cholecystectomy specimens and to perform detailed histopathological and immunohistochemical analysis of gallbladder carcinoma cases. **Materials and Methods:** This retrospective cross-sectional record-based study included all cholecystectomy specimens received over a 5-year period from 1st June 2019 to 31st May 2024. Histopathology records, gross descriptions, and microscopic slides were reviewed. Cases with incomplete records or autolyzed specimens were excluded. Missing clinicodemographic or gross data fields were recorded as unavailable and excluded from variable-specific analysis without excluding the case from overall histopathological spectrum analysis. Statistical analysis included Chi-square test and comparative clinicopathological correlation. **Results:** Among 2063 specimens, chronic cholecystitis was the most common lesion followed by cholesterosis, acute on chronic cholecystitis and carcinoma gallbladder. Female predominance was observed in both overall lesions and carcinoma cases. Gallstones were significantly associated with carcinoma gallbladder. Premalignant lesions including dysplasia, intestinal metaplasia and ICPN (Intracholecystic papillary neoplasm) were also identified. A substantial proportion of carcinoma cases were incidental on microscopy without obvious gross growth. **Conclusion:** Routine gross and microscopic evaluation of all cholecystectomy specimens is essential for detecting incidental carcinoma and premalignant lesions. Immunohistochemistry serves as an adjunct in selected difficult malignant cases.

INTRODUCTION: Cholecystectomy is one of the most commonly performed abdominal procedures globally, most often indicated for symptomatic gallstones or cholecystitis.

The spectrum of pathological lesions involving the gallbladder, which may or may not be associated with gallstones, encompass entities like chronic and acute cholecystitis, empyema, mucocele and ominous ones like gallbladder carcinoma¹.

Different studies have highlighted the strong association of cholelithiasis with gallbladder carcinoma (GBC). Chronic trauma and inflammation are known to induce epithelial dysplasia and explain the subsequent progression to carcinoma *in-situ* and invasive carcinoma².

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Immunohistochemistry (IHC) is crucial for differentiating GBC from reactive or metastatic lesions^{3, 4}. Conducting a large-scale histopathological evaluation of cholecystectomy specimens allows for a comprehensive assessment of the prevalence and spectrum of gallbladder pathologies in a defined population. A large sample size improves the statistical reliability of observations and enables identification of relatively rare but clinically important lesions, including incidental carcinoma. The analysis of histopathological findings in relation to demographic parameters and gross features helps in understanding potential risk factors and disease patterns. The objective of the study is to analyze the spectrum of histopathological lesions in cholecystectomy specimens and to perform detailed histopathological and immunohistochemical analysis of gallbladder carcinoma cases in a tertiary care hospital.

MATERIALS AND METHODS:

Study Design and Period: This was a retrospective cross-sectional record-based study conducted in the Department of Pathology, Tezpur Medical College and Hospital, from 1st June 2019 to 31st May 2024. All consecutively received cholecystectomy specimens during the study period were retrieved from the histopathology register, laboratory information system, and archived slides/blocks.

Record Review Process: For each case, demographic details, gross findings, histopathological diagnosis, and carcinoma-related microscopic parameters were retrieved from pathology records and rechecked against archived reports. In carcinoma and premalignant lesions, original haematoxylin and eosin (H&E) stained slides were reviewed for confirmation.

Inclusion Criteria: All cholecystectomy specimens received for histopathological examination during the study period.

Exclusion Criteria: Specimens that were autolyzed and with incomplete clinical data were excluded from the study. For partially incomplete records, the case was retained for overall lesion spectrum analysis, while missing variables were excluded only from variable-specific statistical analysis.

Ethical Consideration: This study does not involve any *in-vivo* experiment on humans or animals. It is a retrospective record-based observational study for which ethical clearance was obtained from Institutional Human Ethical Committee, Tezpur Medical College and Hospital, Assam vide IEC Sl. No: 2024/083/TMC&H, dated 22/06/2024. As the study involved archival histopathology records and anonymized patient data, waiver of individual informed consent was granted by the ethics committee, and strict confidentiality was maintained.

Study Procedure: The specimens were processed routinely, fixed in 10% neutral buffered formalin followed by paraffin embedding, microtome sectioning at 4–5µm, and staining with haematoxylin and eosin (H&E) stain. Immunohistochemistry was performed as per standard protocol for cytokeratin 7 (CK7), cytokeratin 19 (CK19) and cytokeratin 20 (CK20) in cases diagnosed as carcinoma of gallbladder on routine H&E stained slides.

Study Variables: The study variables included age, gender, gross morphology features like presence or absence of gallstones, wall thickness, mucosal changes and visible growth.

The microscopic findings included histopathological diagnosis, tumor differentiation, lymphovascular and perineural invasion and positivity/negativity of immunohistochemical markers in IHC slides.

Data Analysis: Data was collected from histopathological records and analysed using SPSS (Statistical Package for the Social Sciences) version 23.0 (Released 2015, IBM Corp, Armonk NY) software (IBM Corp 2015).

Association between gallstones and gallbladder carcinoma were evaluated using Chi-square test (significance at $p < 0.05$). Visual representation included tables, charts, gross image and photomicrographs.

RESULTS: A total of 2063 cholecystectomy specimens were studied.

Gender-wise Case Distribution:

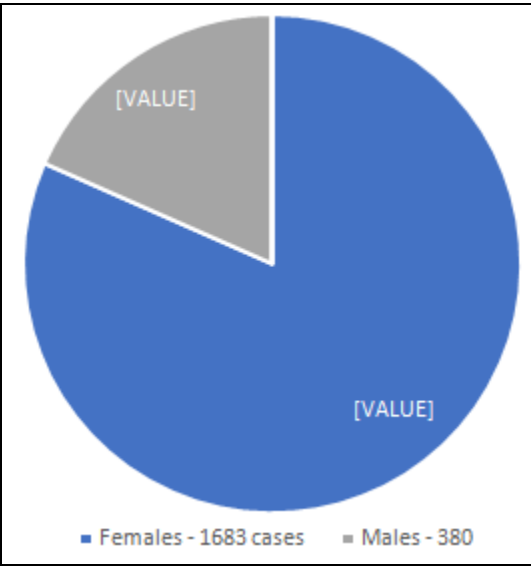


FIG. 1: PIE CHART DEPICTING GENDER-WISE DISTRIBUTION OF ALL CASES

Age-Specific Distribution of Gallbladder Lesions:

TABLE 1: TABLE DEPICTING AGE-SPECIFIC DISTRIBUTION OF GALLBLADDER LESIONS

Age group (years)	Total cases (n=2063)	Percentage (%)	Carcinoma cases (n=21)
≤20	35	1.7	0
21–30	285	13.8	1
31–40	620	30.1	4
41–50	710	34.4	7
51–60	295	14.3	5
>60	118	5.7	4

Gross Pathological Findings in Gallbladder Lesions:

TABLE 2: TABLE DEPICTING GROSS PATHOLOGICAL FINDINGS IN GALLBLADDER LESIONS

Gross parameter	Category	Cases (n=2063)	Percentage (%)
Gallstones	Present	1520	73.7
	Absent	543	26.3
Wall thickness	Normal	580	28.1
	Thickened	1483	71.9
Mucosal appearance	Normal	738	35.8
	Congested/ulcerated	1325	64.2

Histopathological Patterns:

TABLE 3: HISTOPATHOLOGICAL SPECTRUM OF GALLBLADDER LESIONS

Diagnosis	Cases (n=2063)	Percentage (%)
Chronic cholecystitis	1850	89.7
Cholesterolosis	97	4.7
Acute on chronic cholecystitis	39	1.9
Carcinoma gallbladder	21	1.0
Acute cholecystitis	12	0.6
Adenomyomatosis	10	0.5
Dysplasia	10	0.5
Mucocele	6	0.3
Inflammatory polyp	6	0.3
Adenomatous polyp	4	0.2
Intestinal metaplasia	4	0.2
Intracholecystic Papillary Neoplasm [ICPN]	4	0.2

Carcinoma Gallbladder Gender-Wise Distribution (n=21):

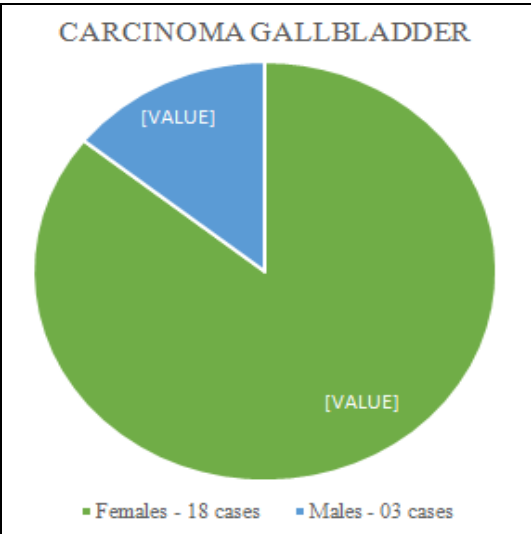


FIG. 2: PIE CHART DEPICTING CARCINOMA GALLBLADDER CASES WITH GENDER-WISE DISTRIBUTION

Gallstones in Carcinoma versus Non-Carcinoma Cases:

TABLE 4: TABLE DEPICTING GALLSTONES IN CARCINOMA VERSUS NON-CARCINOMA CASES

Gallstones	Carcinoma	Non-carcinoma	Total
Present	16	1504	1520
Absent	5	538	543
Total	21	2042	2063

Gross Findings in Carcinoma gallbladder:

TABLE 5: GROSS MORPHOLOGICAL PARAMETERS IN CARCINOMA GALLBLADDER CASES

Gross parameter	Category	Cases (n=21)	Percentage (%)
Wall thickness	<8.25mm	5	23.8
	>8.25mm	16	76.2
Visible growth	Present	8	38.1
	Absent	13	61.9
Gallstones	Present	16	76.2
	Absent	5	23.8

Chi-Square Test - Association between Gallstones and Gallbladder Carcinoma:

TABLE 6: CHI-SQUARE TEST

Parameter	Value
Chi-square (χ^2)	5.76
Degrees of freedom (df)	1
p-value	0.016

Histopathological Characteristics of Carcinoma Gallbladder:

TABLE 7: HISTOPATHOLOGICAL PARAMETERS OF CARCINOMA GALLBLADDER CASES

Histopathological Parameter	Cases (n=21)	Percentage (%)
Tumor differentiation		
Well differentiated adenocarcinoma	8	38.1
Moderately differentiated adenocarcinoma	6	28.6
Poorly differentiated adenocarcinoma	7	33.3
Lymphovascular invasion (LVI)		
Present	5	23.8
Absent	16	76.2
Perineural invasion (PNI)		
Present	2	9.5
Absent	19	90.5

Subanalysis of Parameters of Carcinoma Cases:

TABLE 8: TABLE DEPICTING ADDITIONAL PARAMETERS IN GBC CASES

Parameter associated with tumor	Cases (n=21)	Percentage (%)
Location		
Fundus	9	42.9
Body	7	33.3
Neck	5	23.8
Pathological staging		
pT1	4	19.0
pT2	10	47.6
pT3	7	33.4
Margin positive	3	14.3
Associated dysplasia/metaplasia	6	28.6
Lymph node positivity	2	9.5

Immunohistochemistry (IHC) Findings in Gallbladder Carcinoma Cases:

TABLE 9: IHC POSITIVITY RATES AND STAINING PATTERN

IHC marker	Positive Cases (n=21)	Positivity Percentage (%)	Staining pattern
CK7	20	95.2	Diffuse cytoplasmic
CK19	18	85.7	Diffuse cytoplasmic
CK20	4	19	Focal cytoplasmic

Gross Morphology Image:

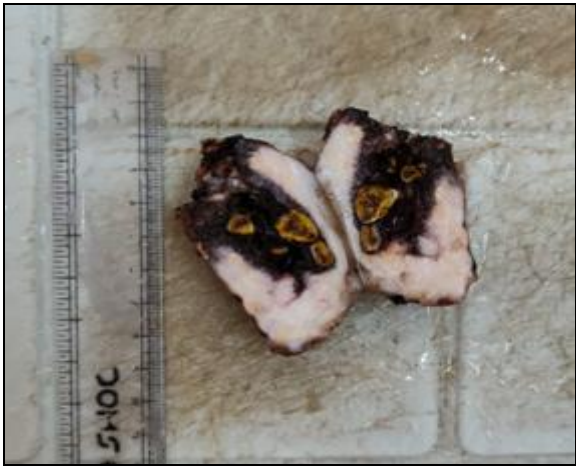


FIG. 3: GALLBLADDER SPECIMEN SHOWING GALLSTONES AND THICKENED WALL

Photomicrographs:

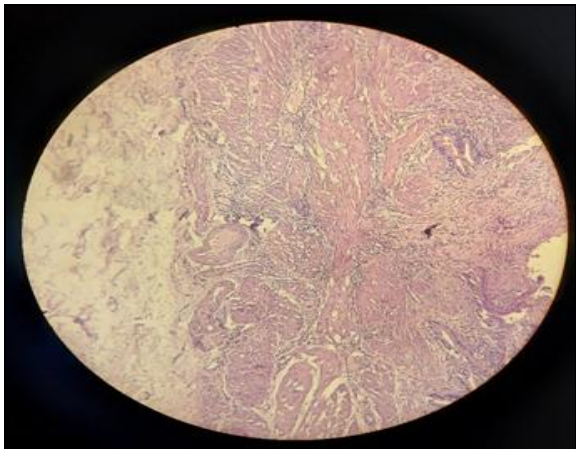


FIG. 4: CHRONIC CHOLECYSTITIS (H&E STAIN; MAGNIFICATION-10X; SCALE BAR: 1cm=200µm)

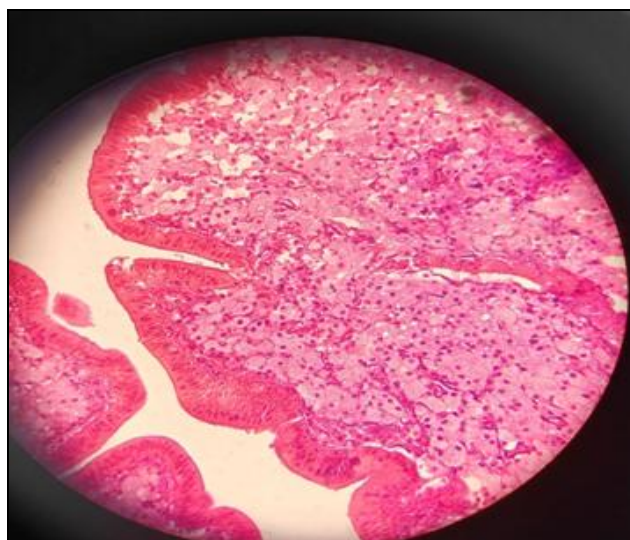


FIG. 5: CHOLESTEROSIS (H&E STAIN; MAGNIFICATION - 40X; SCALE BAR: 1cm=100µm)

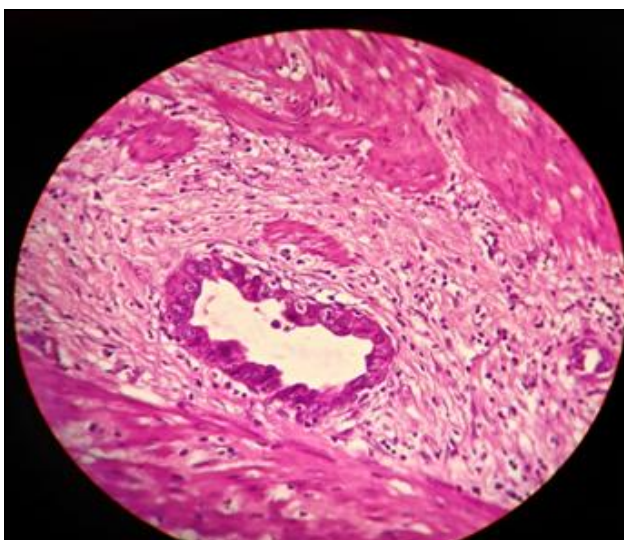


FIG. 6: GALLBLADDER CARCINOMA (H&E STAIN; MAGNIFICATION - 40X; SCALE BAR: 1cm=100µm)

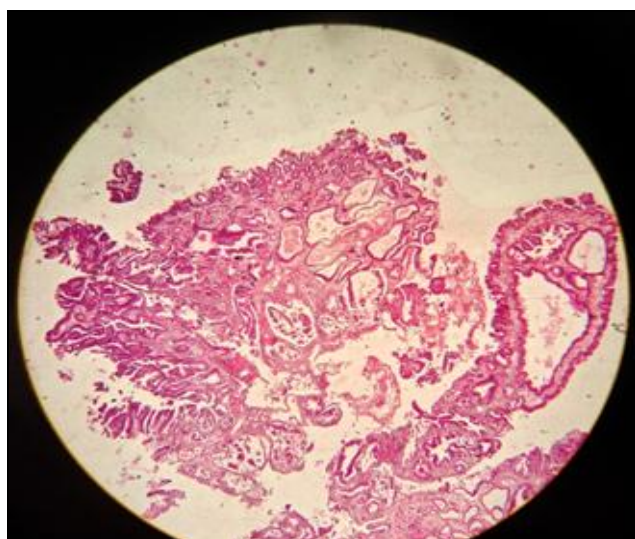


FIG. 7: ICPN (H&E STAIN; MAGNIFICATION-10X; SCALE BAR: 1cm=200µm)

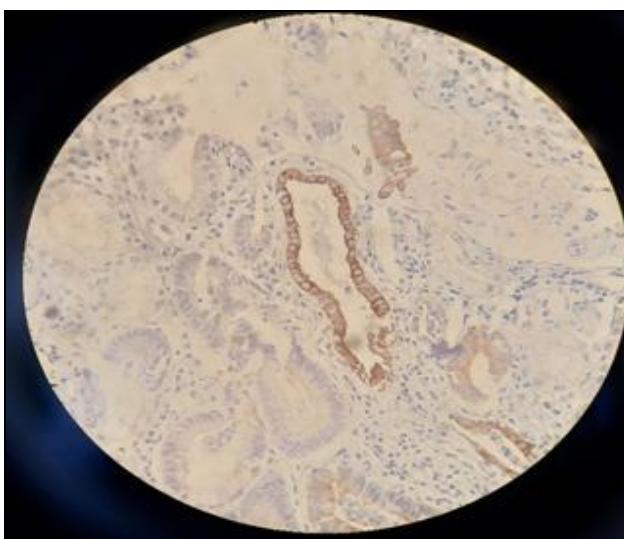


FIG. 8: IHC – CK7 POSITIVE (MAGNIFICATION-40X; SCALE BAR: 1cm=100µm)

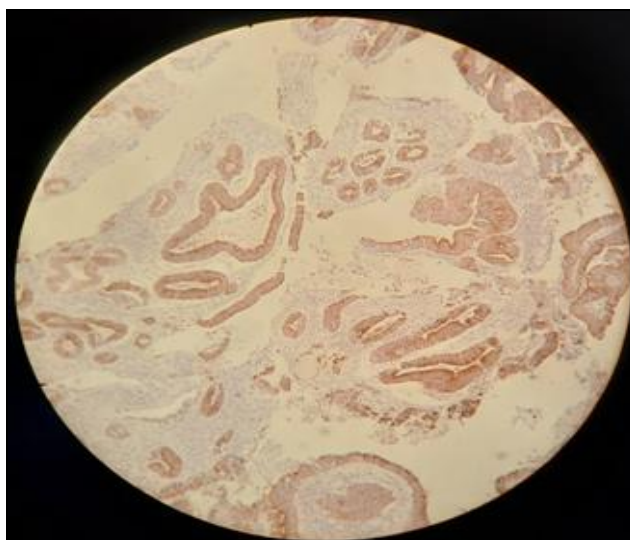


FIG. 9: IHC – CK19 POSITIVE (MAGNIFICATION-10X; SCALE BAR: 1cm=200µm)

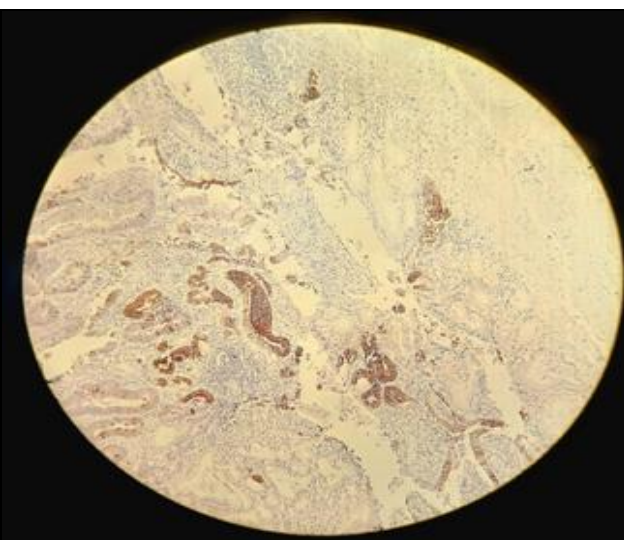


FIG. 10: IHC – CK20 POSITIVE (MAGNIFICATION-10X; SCALE BAR: 1cm=200µm)

DISCUSSION: A total of 2063 gallbladder specimens were evaluated histopathologically in the present study. Gallbladder diseases showed a marked female predominance, with females accounting for 81.6% and males 18.4%. Mondal et al. observed a higher prevalence of gallbladder disease among females undergoing cholecystectomy, with women constituting more than 80% of cases⁵. Degloorkar *et al.* also reported a significant female predominance in gallbladder pathology in their retrospective study of cholecystectomy specimens⁶. This female preponderance is widely attributed to hormonal influences, particularly estrogen, which increases cholesterol saturation in bile and predisposes to gallstone formation⁷.

The age of presentation between the 4th and 5th decades was seen in majority of the cases (34.4%) in the present study with GBC cases (33.3%) occurring predominantly in the same age interval. This is in concordance with various other studies conducted world over^{1, 3, 6, 7}.

In the present study, chronic cholecystitis was the most common histopathological diagnosis, accounting for nearly 89.7% of cases, followed by cholesterolosis, acute on chronic cholecystitis, and GBC. Mondal *et al.* documented chronic cholecystitis in approximately 79.8% of cases⁵. Gupta *et al.*¹⁰, Srinivasan and Sekar¹¹ and Kumar et.al¹² also reported chronic cholecystitis as the most frequent pathology in their histopathological study of gallbladder lesions. The predominance of chronic cholecystitis is largely attributed to the high prevalence of gallstones and chronic inflammation of the gallbladder mucosa. The incidence of gallbladder carcinoma in the present study was approximately 1%. This finding is comparable with previously published studies. Degloorkar *et al.*⁶ and Sangwan *et al.*⁸ reported gallbladder carcinoma in 0.46% and 1.9% respectively in their study of gallbladder specimens. Similarly, Mohan *et al.* observed carcinoma in a small percentage of cholecystectomy specimens, highlighting that incidental carcinoma remains an uncommon but clinically significant finding⁹. Among GBC cases in the present study, females constituted the majority (85.7%) which is consistent with the known gender predisposition of gallbladder

malignancy. Hundal and Shaffer reported that gallbladder carcinoma occurs two to six times more frequently in women compared to men¹⁴. Similarly, Misra et al. observed a strong female predominance in gallbladder carcinoma cases in their clinical study¹⁹. Gallstones were identified in 76% of carcinoma cases in the present study, suggesting a strong association between cholelithiasis and gallbladder carcinoma. Lazcano-Ponce *et al.* reported that gallstones are present in approximately 70–90% of gallbladder carcinoma cases¹³. Similarly, Dutta emphasized that chronic irritation and inflammation caused by gallstones play a major role in gallbladder carcinogenesis¹⁷.

Chi-square analysis demonstrated a statistically significant association between gallstones and carcinoma gallbladder ($\chi^2 = 5.76$, $df = 1$, $p = 0.016$). This statistical analysis concurs with similar observations made in other population based studies^{13, 17}. Additional exploratory analysis showed increasing age and gallbladder wall thickness were more frequently associated with GBC, though not subjected to multivariate testing.

In the present study, 76.2% of GBC cases demonstrated a wall thickness of >8.25 mm suggesting significant association between wall thickness and GBC. Shagor *et.al* reported in their study that a wall thickness cut-off value of ≥ 8.25 mm showed 81.8% sensitivity and 72.7% specificity for predicting gallbladder carcinoma, indicating its usefulness as a predictive marker²⁰.

Premalignant lesions such as intestinal metaplasia and dysplasia were also observed in a considerable number (28.6%) of GBC cases in the present study highlighting their importance as harbingers of malignancy. Wistuba and Gazdar¹⁸ and Randi *et al.*¹⁵ highlighted that chronic inflammation of the gallbladder mucosa can lead to metaplastic changes followed by dysplasia and eventual carcinoma. Another notable observation in the present study was that a considerable proportion of carcinoma cases (61.9%) did not show obvious gross tumour growth. This signifies the clinical importance of routine histopathological examination of all cholecystectomy specimens for detection of incidental GBC. Sangwan *et al.*¹⁸ and other studies¹¹ have reported that incidental gallbladder carcinoma may be detected only on microscopic

examination even when gross examination appears normal. Regarding tumor differentiation, the adenocarcinomas in the present study demonstrated variable degrees of differentiation, including well-differentiated, moderately differentiated, and poorly differentiated tumours. Previous studies have also reported that adenocarcinoma is the most common histological type of gallbladder cancer, with varying grades reflecting differences in tumor aggressiveness and prognosis¹⁶.

Subanalysis of additional parameters of GBC cases revealed fundal location (42.9%) of most tumours with majority of cases (47.6%) showing invasion of the subserosal connective tissue (pT2 staging). Several clinicopathological studies have similarly documented that the majority of gallbladder carcinomas arise in the fundus, with reported frequencies ranging widely but consistently demonstrating fundal predominance over other anatomical sites^{21, 22}. In addition, the distribution of tumor staging in the present study, with pT2 tumors forming the largest subgroup, is comparable to findings from surgical and histopathological series where T2 lesions constitute a major proportion of resectable gallbladder carcinomas^{14, 22, 23}.

Immunohistochemistry was performed only in histologically confirmed malignant cases where confirmation of primary biliary epithelial origin or exclusion of metastatic adenocarcinoma or reactive atypia was required. In the present study, out of a total of 21 cases, CK7 and CK19 showed diffuse cytoplasmic positivity in 95.2% and 85.7% cases respectively. Several studies have reported CK7 positivity in approximately 90–98% of gallbladder carcinomas, making it one of the most sensitive markers for tumours of pancreatobiliary origin²⁴⁻²⁶. CK19, another marker of biliary epithelial differentiation, has also been shown to be positive in 85–98% of cases, supporting its diagnostic utility in biliary tract malignancies^{24, 26}.

In contrast, CK20 showed focal positivity in only 19% of cases in the present study. CK20 positivity has been reported in approximately 15–30% of cases, and when present, the staining is usually focal and less intense^{24, 25}. This immunohistochemical pattern is particularly useful in differentiating primary gallbladder carcinoma

from metastatic colorectal carcinoma, which usually demonstrates a CK7-negative and CK20-positive staining pattern^{25, 27, 28}.

CONCLUSION: The present study demonstrates the importance of routine study of gross morphology and histopathological examination for the detection of premalignant lesions and incidental GBC. Immunohistochemistry plays a crucial role in differentiating between primary GBC and metastatic lesions and/or reactive atypia.

Collectively, these findings underscore the indispensable role of comprehensive pathological assessment in guiding appropriate clinical management and highlight the necessity of systematic evaluation of all cholecystectomy specimens, irrespective of the preoperative clinical impression. The study is limited by its retrospective design, single centre nature and lack of imaging correlation. However, the large sample size and methodical histopathological and immuno-histochemical evaluation reinforce the significance of the study findings.

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Authors' Contribution: All the authors have significantly contributed in concept, study designing, data acquisition, data analysis, and writing of the manuscript.

Data Availability: Data are available with the authors and can be retrieved as and when required.

CONFLICT OF INTEREST: There are no conflicts of interest for this research work

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