Section: Pathology

Original Research Article

HISTOPATHOLOGICAL SPECTRUM OF GALLBLADDER LESIONS WITH P53 EXPRESSION AND HER2/NEU IMMUNOHISTOCHEMICAL CORRELATION IN GALLBLADDER ADENOCARCINOMA: A RETROSPECTIVE STUDY

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ABSTRACT

Background: Gallbladder lesions range from inflammatory and benign conditions to premalignant changes and invasive carcinoma. Tumor biomarkers such as p53 and HER2/neu may help characterize gallbladder adenocarcinoma and identify clinically relevant subgroups.

Aim: To evaluate the histopathological spectrum of gallbladder lesions in cholecystectomy specimens and to assess p53 expression and HER2/neu immunohistochemical status in gallbladder adenocarcinoma with clinicopathological correlation.

Materials and Methods: This hospital-based retrospective study (September 2019–June 2025) included 540 cholecystectomy specimens. Lesions were classified on hematoxylin and eosin sections. Gallbladder adenocarcinoma cases (n=9) were evaluated for age, gender, histological grade, pathological T stage, nodal status, TNM stage, lymphovascular invasion, and adjacent mucosal dysplasia. Immunohistochemistry for p53 and HER2/neu was performed in all adenocarcinoma cases. HER2 was scored as 0, 1+, 2+, or 3+ using standardized gastrointestinal HER2 interpretation criteria; scores of 2+ were considered equivocal. Results were expressed as frequencies and percentages.

Results: Non-neoplastic lesions predominated. Chronic calculous cholecystitis was the commonest lesion (463/540; 85.74%), followed by chronic cholecystitis (37/540; 6.85%) and acute cholecystitis (11/540; 2.04%). Gallbladder adenocarcinoma constituted 9/540 (1.67%). Among adenocarcinoma cases, 6/9 were <50 years and 7/9 were females. Most tumors were moderately differentiated (G2; 5/9). T stage distribution was T1 (3), T2 (4), T3 (1), and T4 (1). N0 status was observed in 8/9 and TNM stage I–II in 8/9 cases. Lymphovascular invasion was present in 6/9 and adjacent mucosal dysplasia in 5/9. Aberrant p53 immunohistochemical expression pattern was identified in 7/9 (77.8%) cases, predominantly nuclear overexpression (6/9; 66.7%). HER2 scores were 0:3, 1+:0, 2+:4, and 3+:2, with definite strong membranous overexpression (3+) in 2/9 (22.2%) cases. Deaths occurred in both HER2-expressing and non-expressing groups.

Conclusion: Cholecystectomy specimens predominantly showed non-neoplastic pathology, with chronic calculous cholecystitis as the commonest lesion. Gallbladder adenocarcinoma demonstrated frequent aberrant p53 expression and a subset showed HER2 overexpression, supporting molecular heterogeneity and the potential utility of biomarker evaluation. Equivocal HER2 (2+) cases warrant confirmatory testing for ERBB2 amplification.

Keywords: Gallbladder lesions; cholecystectomy; gallbladder adenocarcinoma; p53; HER2/neu; immunohistochemistry.

INTRODUCTION

Gallbladder pathology encompasses a wide range of lesions, from inflammatory and benign conditions to premalignant changes and invasive carcinoma. Cholecystectomy is among the most frequently performed abdominal surgeries, and routine histopathological evaluation often reveals a predominance of chronic inflammatory lesions, particularly those associated with cholelithiasis.[3-5] Several institutional series have emphasized the importance of systematic grossing and microscopic assessment because premalignant alterations and incidental carcinoma can be missed clinically and radiologically.[4-6]

Gallbladder carcinoma (GBC), although relatively uncommon globally, is the most common malignancy of the biliary tract, comprising approximately 80–95% of biliary tract cancers. It demonstrates marked geographic variation, with high-incidence regions including parts of South America and northern India, and is associated with poor outcomes due to late presentation and aggressive tumor biology. Chronic inflammation, gallstones, and mucosal injury are strongly implicated in gallbladder carcinogenesis, supporting a multistep pathway of epithelial transformation.

Histologically, the majority of gallbladder cancers are adenocarcinomas showing variable architectural patterns and degrees of differentiation.[8] The histopathological spectrum in cholecystectomy specimens has been widely documented, with chronic calculous cholecystitis consistently reported as the most common diagnosis, while adenomas, dysplasia, and carcinoma constitute a small but clinically significant fraction.[3-7] Such background data are essential because they provide context for interpreting the frequency of malignant lesions and for identifying precursor changes in routine practice.

At the molecular level, inactivation of tumor suppressor pathways is considered central to malignant progression. TP53 alteration is one of the most frequently implicated events in GBC, and immunohistochemical overexpression of p53 has been reported in a substantial proportion of gallbladder carcinomas, with associations noted with tumor aggressiveness and prognosis. Recent regional studies further support the role of p53 as a marker of malignant transformation, showing overexpression in carcinoma but not in benign inflammatory gallbladder lesions.

In addition to p53, HER2/neu (ERBB2) has emerged as a potentially actionable biomarker in biliary tract malignancies. HER2 overexpression/amplification has been documented in a subset of gallbladder cancers and is under active evaluation for targeted therapeutic strategies in biliary tract cancers. Reliable interpretation of HER2 immunohistochemistry requires standardized scoring to ensure reproducibility and clinical correlation. The immunohistochemical testing approach and scoring framework validated by Rüschoff et al. in gastric carcinoma has been widely adopted for gastrointestinal-type HER2 assessment and provides a structured basis for graded interpretation of HER2 staining intensity and distribution.

In this context, the present study was undertaken to document the histopathological spectrum of gallbladder lesions in cholecystectomy specimens and to evaluate p53 expression in gallbladder adenocarcinoma, while also assessing HER2/neu expression using standardized immunohistochemical grading and correlating HER2 status with clinicopathological parameters and outcomes.

Aim

To evaluate the histopathological spectrum of gallbladder lesions and to assess p53 protein expression and HER2/neu immunohistochemical status in gallbladder adenocarcinoma.

Objectives

1. To classify gallbladder lesions into non-malignant, benign, premalignant, and malignant categories based on histopathological examination
2. To assess p53 protein expression in histologically confirmed gallbladder adenocarcinoma
3. To evaluate HER2/neu expression in gallbladder adenocarcinoma and correlate it with clinicopathological parameters and patient outcomes.

MATERIALS AND METHODS

Study Design and Setting

This was a hospital-based retrospective study conducted in the Department of Pathology of a tertiary care teaching hospital.

Study Period

The study was carried out over a period of September 2019 to June 2025.

Study Material and Sample Size

A total of 540 cholecystectomy specimens received in the Department of Pathology during the study period were included. Among these, 9 cases were diagnosed as gallbladder adenocarcinoma and were subjected to detailed clinicopathological evaluation and immunohistochemical analysis.

Inclusion Criteria

- All cholecystectomy specimens received during the study period
- Specimens from patients of all age groups and both genders

Exclusion Criteria

- Patients who had received chemotherapy, radiotherapy, or targeted therapy prior to surgery
- Poorly fixed or autolysed specimens unsuitable for histopathological or immunohistochemical evaluation

Histopathological Examination

All specimens were fixed in 10% neutral buffered formalin, processed routinely, and embedded in

paraffin. Sections of 3–4 µm thickness were cut and stained with hematoxylin and eosin (H&E). Histopathological evaluation was performed to classify lesions into non-neoplastic, benign, premalignant, and malignant categories.

Gallbladder adenocarcinomas were graded according to the degree of differentiation (G1–G3). Tumor staging was performed based on pathological TNM staging, including depth of invasion (T stage), nodal status, and overall stage grouping. The presence of lymphovascular invasion and dysplasia in adjacent mucosa was specifically noted.

Immunohistochemistry

Immunohistochemical (IHC) staining was performed on formalin-fixed paraffin-embedded tissue sections from all gallbladder adenocarcinoma cases.

p53 Immunohistochemistry

p53 expression was assessed using a monoclonal anti-p53 antibody. Nuclear staining in tumor cells was evaluated, and cases showing definite nuclear positivity in tumor cells were considered p53 positive. The assessment was performed independently by pathologists, and discrepancies were resolved by consensus.

HER2/neu Immunohistochemistry

HER2/neu immunohistochemistry was performed using a standard protocol. Membranous staining of tumor cells was evaluated and scored as 0, 1+, 2+, or 3+ based on staining intensity and completeness. Scoring was performed according to the standardized immunohistochemical criteria proposed by Rüschoff et al.

For the purpose of analysis, HER2 scores of 2+ and 3+ were considered positive, while scores of 0 and 1+ were considered negative.

Clinicopathological Correlation

Clinicopathological parameters including age, gender, histological grade, depth of invasion, nodal status, TNM stage, lymphovascular invasion, and dysplasia in adjacent mucosa were correlated with **p53** and **HER2/neu** expression. Patient outcomes were categorized as alive or deceased based on available follow-up records.

Statistical Analysis

Data were entered into a spreadsheet and analyzed using descriptive statistics. Results were expressed as frequencies and percentages. Due to the limited number of carcinoma cases, inferential statistical testing was not emphasized, and correlations were interpreted descriptively.

RESULTS

A total of 540 cholecystectomy specimens received during the study period were evaluated to assess the histopathological spectrum of gallbladder lesions. Immunohistochemical analysis of p53 and HER2/neu was performed in cases diagnosed as gallbladder adenocarcinoma (n = 9), and the expression patterns were correlated descriptively with clinicopathological parameters and patient outcomes.

Histopathological Spectrum of Gallbladder Lesions

Table 1: Histopathological Spectrum of Gallbladder Lesions (n = 540)

Lesion Type	Number of Cases	Percentage (%)
Chronic calculous cholecystitis	463	85.74
Chronic cholecystitis	37	6.85
Acute cholecystitis	11	2.04
Acute on chronic cholecystitis	5	0.93
Xanthogranulomatous cholecystitis	3	0.56
Cholesterolosis	2	0.37
Empyema of gallbladder	2	0.37
Other rare lesions*	8	1.48
Adenocarcinoma	9	1.67
Total	**540**	**100**

*Includes follicular cholecystitis, gangrenous cholecystitis, adenoma, epithelial dysplasia, cholesterol polyp, and tuberculous cholecystitis.

The majority of gallbladder lesions were non-neoplastic, with chronic calculous cholecystitis constituting the predominant diagnosis. Malignant lesions were uncommon, with gallbladder adenocarcinoma accounting for 1.67% of cases.

Clinicopathological Characteristics of Gallbladder Adenocarcinoma

Table 2: Clinicopathological Profile of Gallbladder Adenocarcinoma (n = 9)

Parameter	Category	Number of Cases
Age	< 50 years	6
	> 50 years	3
Gender	Male	2
	Female	7
Histological grade	G1	1
	G2	5
	G3	3
Pathological T stage	T1	3
	T2	4

	T3	1
	T4	1
Nodal status	N0	8
	N1–N2	1
TNM stage	I–II	8
	III–IV	1
Lymphovascular invasion	Present	6
	Absent	3
Dysplasia in adjacent mucosa	Present	5
	Absent	4

Gallbladder adenocarcinoma predominantly affected females and patients below 50 years of age. Most tumors were moderately differentiated and diagnosed at early pathological stages (T1–T2; TNM stage I–II), although lymphovascular invasion was frequently observed.

p53 Immunohistochemical Expression Pattern in Gallbladder Adenocarcinoma

p53 immunohistochemistry was evaluated in all adenocarcinoma cases and interpreted using pattern-based reporting (wild-type vs aberrant patterns). The distribution of p53 expression patterns is shown in Table 3.

Table 3: p53 Immunohistochemical Expression Pattern in Gallbladder Adenocarcinoma (n = 9)

p53 IHC pattern	Number of Cases	Percentage (%)
Wild-type	2	22.2
Aberrant – nuclear overexpression	6	66.7
Aberrant – cytoplasmic (mutant pattern)	1	11.1
Aberrant – null pattern	0	0
Total	9	100

Overall, aberrant p53 staining patterns were identified in 7 of 9 cases (77.8%). Nuclear overexpression was the predominant abnormal pattern (66.7%), while cytoplasmic mutant-type staining was observed in 11.1%. No cases demonstrated a null expression pattern.

HER2/neu Immunohistochemical Expression

HER2/neu immunohistochemistry was performed in all gallbladder adenocarcinoma cases and graded as 0, 1+, 2+, or 3+ according to standardized gastrointestinal HER2 interpretation criteria described by **Rüschoff et al. (2010)**. The HER2 scoring distribution is shown in Table 4.

Table 4: HER2/neu IHC Scoring in Gallbladder Adenocarcinoma (n = 9)
(Scored as per Rüschoff et al., 2010)

HER2 IHC Score	Number of Cases	Percentage (%)
0	3	33.3
1+	0	0
2+	4	44.4
3+	2	22.2
Total	**9**	**100**

HER2 IHC score 2+ (equivocal) was observed in 4 cases (44.4%), while strong membranous overexpression (3+) was present in 2 cases (22.2%). No tumors demonstrated a 1+ score.

Patient Outcome in Relation to HER2/neu Expression

Patient outcomes according to HER2 IHC status are shown in Table 5.

Table 5: Outcome of Gallbladder Adenocarcinoma Patients in Relation to HER2 IHC Status

Outcome	HER2 IHC 2+/3+ (n = 6)	HER2 IHC 0/1+ (n = 3)
Death	3	1
Alive	3	2
Total	**6**	**3**

Mortality was observed in both HER2 IHC 2+/3+ and HER2 IHC 0/1+ groups. Due to the limited number of cases, statistical inference was not performed and findings were interpreted descriptively.

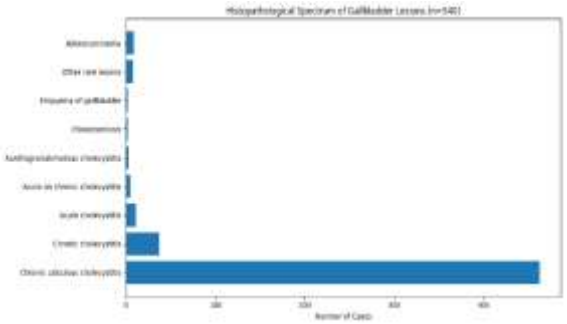


Figure 1: Histopathological spectrum (n=540) – horizontal bar graph

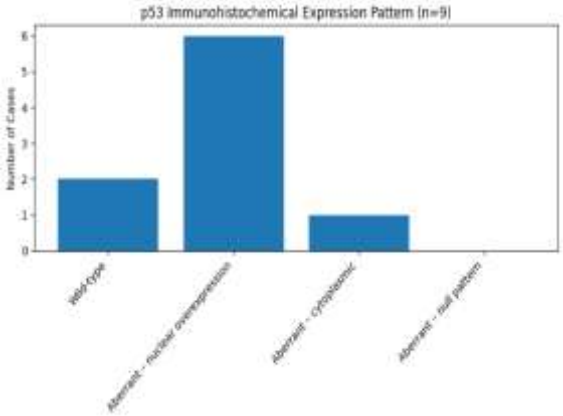


Figure 2: p53 IHC pattern distribution (n=9) – bar chart

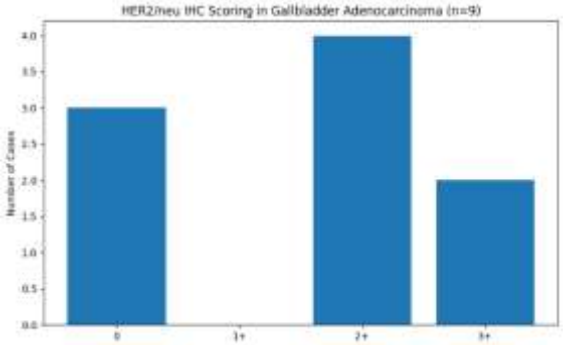


Figure 3: HER2 IHC score distribution (n=9) – bar chart

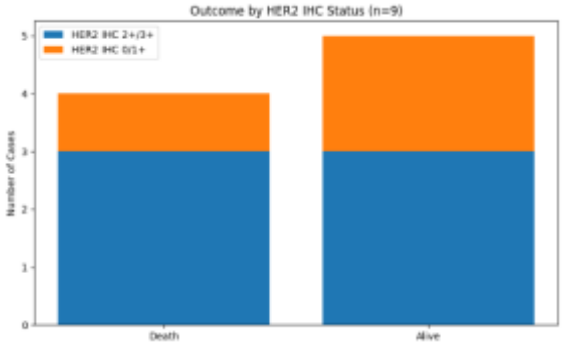


Figure 4: Outcome vs HER2 status (n=9) – stacked bar chart

Figure 5: Gross: cholecystectomy specimen

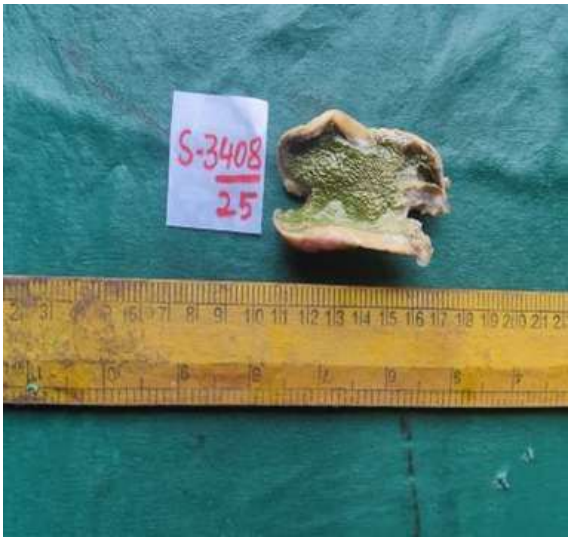


Figure 6: Mucosal surface shows numerous fine, yellow, granular spots imparting a characteristic "strawberry gallbladder" appearance

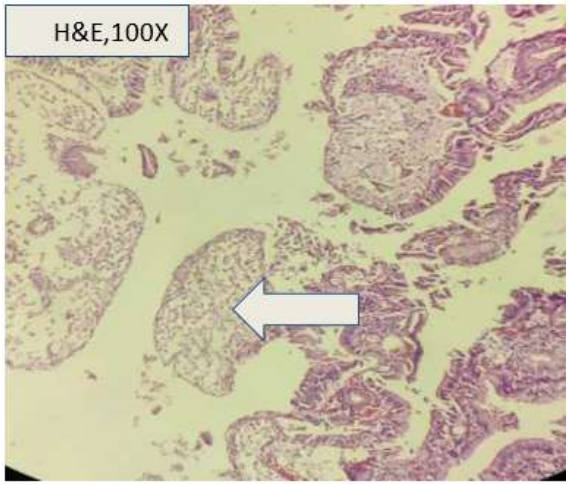


Figure 6A: Cholesterolosis Showing foamy macrophages

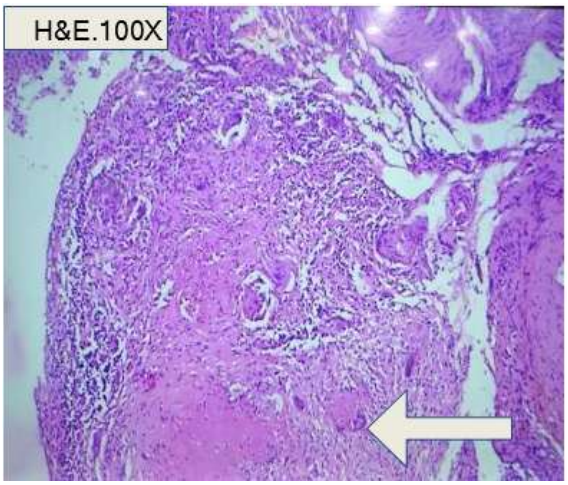


Figure 7: Tuberculous cholecystitis showing langhans giant cell

Figure 8: Radical cholecystectomy specimen - Gall bladder adenocarcinoma

Figure 8A: Gall bladder Adenocarcinoma with Cholelithiasis

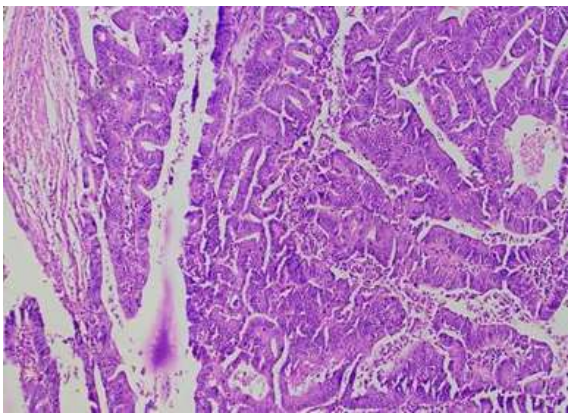


Figure 9: Gall Bladder Adenocarcinoma - Billiary Type

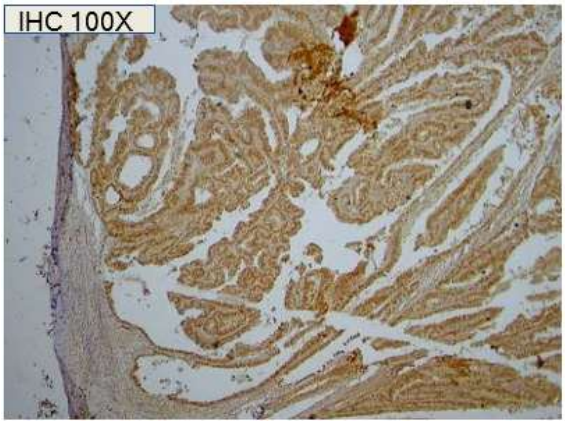
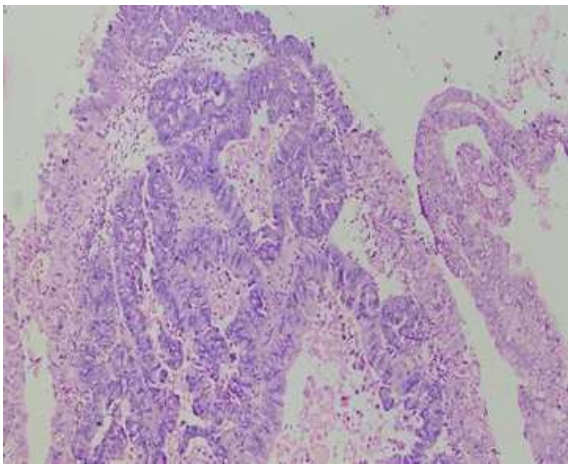
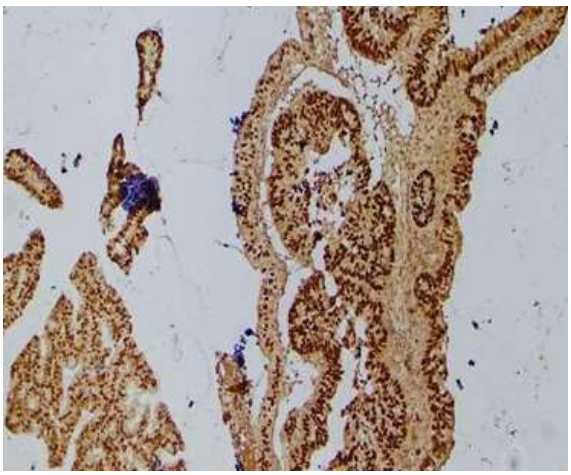


Figure 9A: P53 Overexpression in Gallbladder Adenocarcinoma





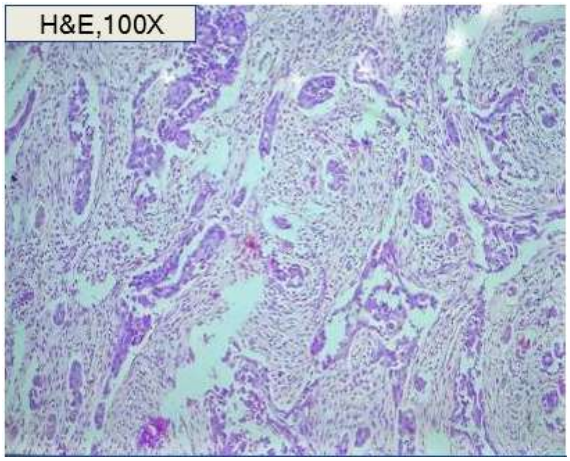


Figure 10: Gall bladder adenocarcinoma - moderately differentiated

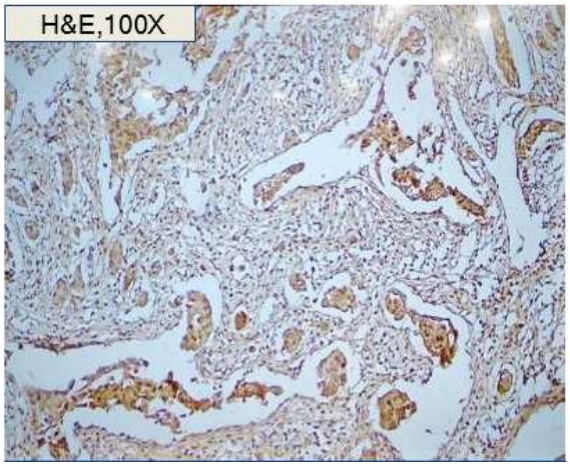


Figure 10A: P53 Wild Type in Gallbladder Adenocarcinoma

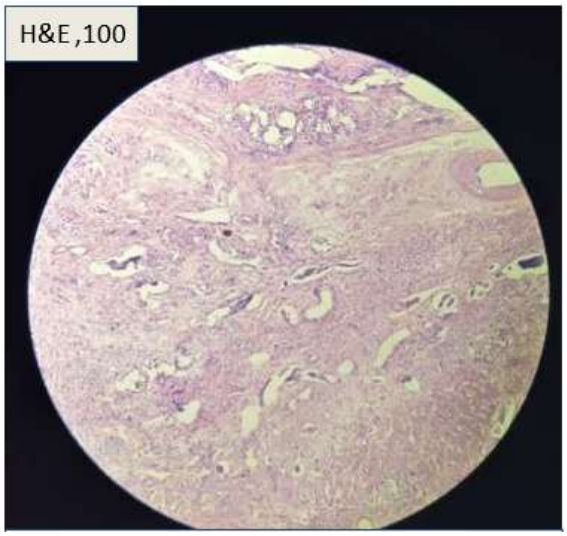


Figure 11: Gall bladder adenocarcinoma - moderately differentiated

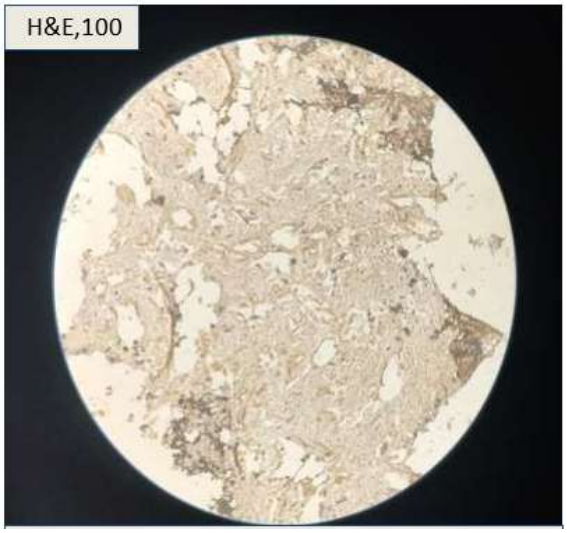


Figure 11A: P53 cytoplasmic (MUTANT TYPE) IN GALLBLADDER ADENOCARCINOMA

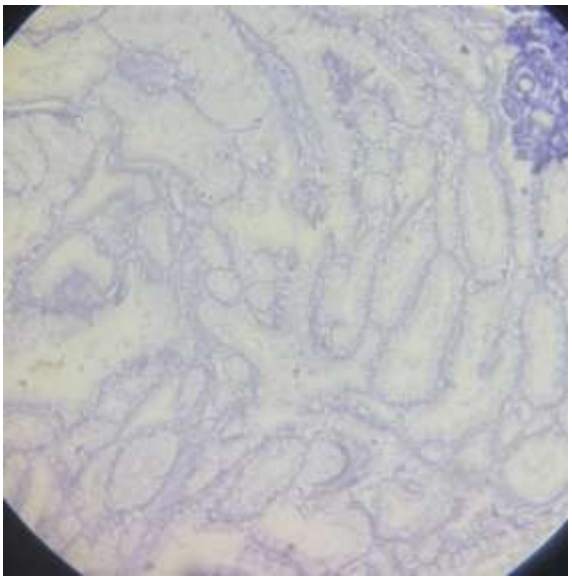


Figure 12 A: Her 2 – 0 200X (LM)

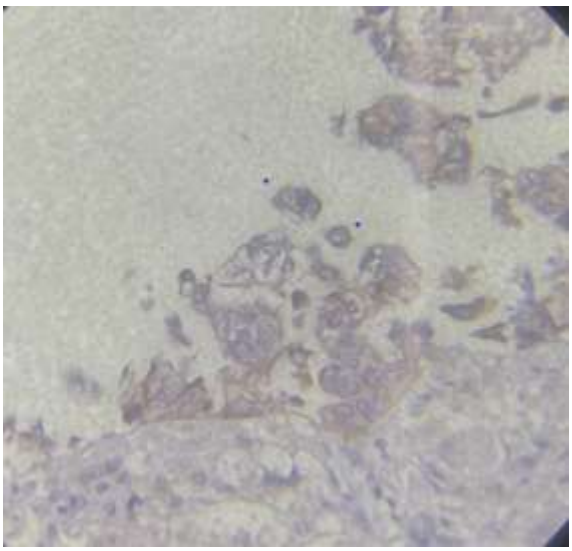


Figure 12B: Her 2- 1+ 400X(LM)

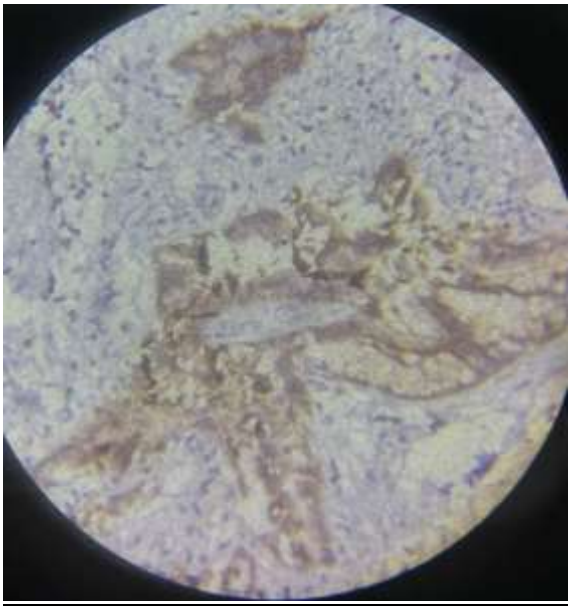


Figure 12C: Her 2-3+ 400X(LM)

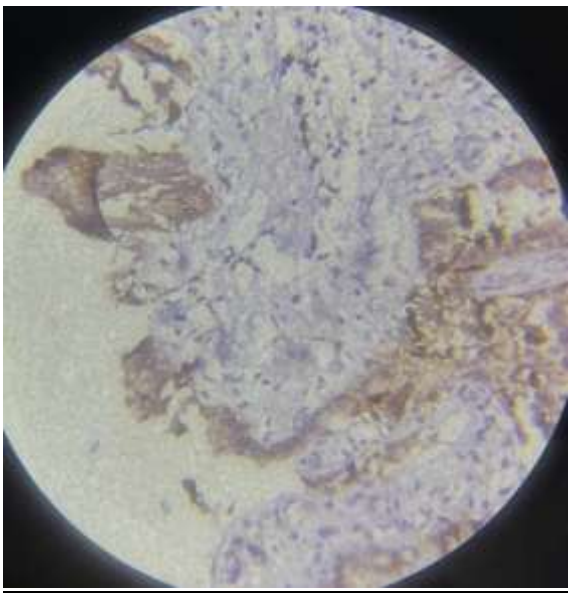


Figure 12D: Her 2 -3+ 400X(LM)

Figure 13: Gall bladder Adenocarcinoma.

DISCUSSION

This retrospective study evaluated the histopathological spectrum of gallbladder lesions in cholecystectomy specimens and further characterised gallbladder adenocarcinoma (n=9) using immunohistochemical biomarkers p53 and HER2/neu, correlating expression with clinicopathological variables and outcome. Although carcinoma constituted a small proportion of the overall cholecystectomy workload, the biomarker findings highlight molecular heterogeneity within gallbladder adenocarcinoma, particularly the high frequency of aberrant p53 expression patterns and the presence of HER2 expression in a subset of tumors. Given the modest number of carcinoma cases, comparison with larger cohorts is essential to interpret prevalence estimates and position these findings within the broader literature.

Histopathological spectrum and clinicopathological profile

The present series demonstrated a predominance of non-neoplastic gallbladder pathology, with chronic calculous cholecystitis constituting the most common diagnosis. Gallbladder adenocarcinoma accounted for 1.67% of specimens, supporting that carcinoma is uncommon in routine cholecystectomy material but remains clinically significant due to its aggressive behaviour and potential for incidental detection.

In the carcinoma subset, a female predominance was observed, and most tumors were diagnosed at early pathological stages (T1–T2; TNM stage I–II). Despite early-stage predominance, lymphovascular invasion was present in 66.7% of cases, suggesting that gallbladder adenocarcinoma may demonstrate adverse biological behaviour even when anatomically limited. Large cohort studies have similarly reported female predominance and substantial molecular heterogeneity in gallbladder carcinoma. Neyaz et al. (2018), in an Indian cohort of

268 gallbladder carcinomas, emphasized the need for marker-driven stratification in this disease.

p53 immunohistochemical expression and clinicopathological relevance

In the present study, p53 immunohistochemistry was interpreted using pattern-based reporting rather than simple positive/negative categorization. Aberrant p53 staining patterns were identified in 77.8% (7/9) of gallbladder adenocarcinomas. Diffuse strong nuclear overexpression was the predominant abnormal pattern (66.7%), while cytoplasmic staining consistent with mutant-type expression was observed in 11.1%. No cases demonstrated a null expression pattern, and 22.2% of tumors showed a wild-type staining pattern.

These findings reinforce the established role of TP53 pathway disruption as a frequent event in gallbladder carcinogenesis. Published studies have reported variable p53 positivity depending on cohort composition, antibody clone, and scoring thresholds. Neyaz et al. (2018) reported p53 positivity in approximately the mid-40% range. Ghosh et al. (2013) demonstrated p53 overexpression in 56.25% of gallbladder cancer cases and observed increasing positivity with higher grade, while benign inflammatory lesions lacked p53 overexpression. Legan et al. (2006) showed p53 accumulation in 48.1% of carcinomas and higher accumulation in poorly differentiated tumors, supporting the concept that p53 abnormalities increase along the dysplasia–carcinoma sequence. Genomic evidence further supports these observations, as de Bitter et al. (2022) reported TP53 mutations in 64% of gallbladder cancers and highlighted concordance between immunohistochemistry and genomic profiling. The present study's high frequency of aberrant p53 patterns is therefore concordant with the broader evidence supporting TP53 disruption as a major molecular event in gallbladder carcinoma.

HER2/neu expression, scoring considerations, and clinical implications

HER2/neu immunohistochemistry in this study demonstrated scores of 0 in 33.3%, 2+ in 44.4%, and 3+ in 22.2%, with no 1+ cases. HER2 scoring was performed using the standardized gastrointestinal HER2 interpretation criteria described by Rüschoff et al. (2010), which provide a reproducible framework for graded membranous staining interpretation.

A key interpretive issue is that HER2 IHC 2+ represents an equivocal category and requires confirmatory testing by in-situ hybridization (FISH/SISH/CISH) or molecular profiling for ERBB2 amplification to classify tumors as truly HER2-positive in most clinical frameworks. Therefore, while descriptive grouping of 2+ and 3+ cases yields an apparent HER2 IHC positivity of 66.7%, the most unambiguous "definite positive" subset in this cohort is the HER2 3+ group (22.2%). The high proportion of 2+ cases indicates a pool of tumors that may warrant confirmatory testing, particularly in settings where HER2-directed therapy is being considered.

Large published cohorts provide important context for HER2 prevalence in gallbladder carcinoma. Yoshida et al. (2016) evaluated HER2 in 211 resectable gallbladder cancers and reported IHC scores of 0 in 68.2%, 1+ in 13.3%, 2+ in 6.6%, and 3+ in 11.8%. They defined HER2 positivity as IHC 3+ or IHC 2+ with FISH positivity, yielding an overall HER2-positive rate of 16.6%. Importantly, heterogeneous HER2 staining was observed in approximately half of IHC 2+/3+ cases, highlighting intratumoral variability and sampling effects. Roa et al. (2014) reported HER2 overexpression in approximately 14% of advanced gallbladder cancers, supporting the presence of a clinically relevant HER2-driven subgroup. De Bitter et al. (2022) reported ERBB2 alterations in 9.3% of gallbladder cancers using genomic profiling approaches, reinforcing that HER2 activation represents a distinct actionable subset.

Thus, the present study supports the existence of HER2-expressing gallbladder adenocarcinoma, with strong overexpression (3+) in a minority and equivocal expression (2+) in a substantial proportion that would benefit from confirmatory testing.

Outcome in relation to HER2/neu expression

In this cohort, deaths occurred in both HER2 IHC 2+/3+ and HER2 IHC 0/1+ groups. Given the small number of patients and limited event counts, survival inference is not appropriate. In the literature, HER2 is generally regarded primarily as a predictive biomarker for therapeutic selection rather than a consistent standalone prognostic marker.

Integrative interpretation: p53 and HER2 together

The combined assessment of p53 and HER2/neu highlights two biologically relevant axes in gallbladder adenocarcinoma: frequent tumor suppressor pathway disruption (aberrant p53 in 77.8%) and a potential oncogenic signalling subset (HER2 3+ in 22.2%, with an additional 44.4% demonstrating equivocal 2+ staining). These findings support molecular heterogeneity in gallbladder adenocarcinoma and reinforce the potential value of integrating biomarker assessment into routine histopathological evaluation. p53 appears to function primarily as a marker of malignant transformation and aggressive tumor biology, whereas HER2 identifies a subset of tumors that may have therapeutic implications, contingent upon confirmatory testing in equivocal cases.

Strengths and Limitations

A key strength of the present study is the integration of routine histopathology, clinicopathological correlation, and biomarker assessment within a single institutional dataset. This combined approach enabled characterization of gallbladder adenocarcinoma beyond morphology alone and provided insight into molecular heterogeneity using p53 and HER2/neu immunohistochemistry.

The principal limitation is the small number of carcinoma cases (n=9), which restricts statistical power and precludes definitive conclusions regarding

prognostic associations. A second limitation is the lack of confirmatory HER2 testing by in-situ hybridization (ISH) or next-generation sequencing (NGS) for IHC 2+ cases. This is important because HER2 IHC 2+ represents an equivocal category and requires amplification confirmation for classification as truly HER2-positive in most clinical frameworks. Future studies should adopt a stepwise testing strategy (IHC screening followed by ISH/NGS for 2+ cases), include larger multicentre cohorts, and incorporate survival modelling to better define prognostic and predictive implications.

Summary of Comparative Position of the Present Results

In summary, the present study demonstrated a high frequency of aberrant p53 immunohistochemical patterns in gallbladder adenocarcinoma (77.8%), with nuclear overexpression being the predominant abnormal pattern (66.7%). This is concordant with published evidence indicating frequent TP53 pathway dysregulation in gallbladder carcinoma and supports the role of p53 as a marker of malignant transformation and aggressive tumour biology.

For HER2, the observed prevalence was strongly dependent on the positivity definition. Definite HER2 overexpression (3+) was observed in 22.2% of tumors, which lies closer to the range reported in larger series. In contrast, inclusion of HER2 IHC 2+ cases yields a higher apparent positivity (66.7%), but this likely includes tumors that would require ISH confirmation for ERBB2 amplification. These findings support the presence of a clinically relevant HER2-expressing subset in gallbladder adenocarcinoma and reinforce the need for confirmatory testing in equivocal IHC cases, particularly when therapeutic stratification is considered.

Table 7: HER2 positivity across published GBC cohorts

Study (Year)	Sample size (GBC n)	HER2 assessment	Definition of positivity	Key HER2 result
Present study (2019–2025)	9	IHC scored per Rüschoff et al.	3+ definite; 2+ equivocal	0: 33.3%, 2+: 44.4%, 3+: 22.2%
Yoshida et al. (2016)	211	IHC ± FISH	3+ or 2+ with FISH+	HER2 positive 16.6%
Roa et al. (2014)	191	IHC	Study-defined	~14% overexpression
Gupta et al. (2021)	50	IHC ± FISH	3+ positive; 2+ equivocal	HER2 positive 16% (all 3+)
Neyaz et al. (2018)	268	IHC	Study-defined	HER2 positive 21.6%
de Bitter et al. (2022)	642	Genomic profiling	ERBB2 alterations	ERBB2 alterations 9.3%

Table 8: p53 expression across published GBC cohorts

Study (Year)	Sample size (GBC n)	p53 method	Key p53 result
Present study (2019–2025)	9	IHC pattern-based reporting	Aberrant p53 = 77.8%; nuclear overexpression = 66.7%; cytoplasmic mutant = 11.1%; wild-type = 22.2%; null = 0%
Gupta et al. (2021)	50	IHC	p53 positive 74%
Ghosh et al. (2013)	80	IHC	p53 overexpression 56.25%
Neyaz et al. (2018)	268	IHC	p53 positive 44.8%
Legan et al. (2006)	27	IHC	p53 accumulation 48.1%
Chaube et al. (2006)	40	IHC	p53 positive 20%
de Bitter et al. (2022)	642	Genomic profiling	TP53 mutations 64%

Strengths and Limitations

A key strength of the present study is the integration of routine histopathology, clinicopathological correlation, and biomarker assessment within a single institutional dataset. This combined approach enabled characterization of gallbladder adenocarcinoma beyond morphology and provided insight into molecular heterogeneity using p53 and HER2/neu immunohistochemistry.

The principal limitation is the small number of carcinoma cases (n=9), which restricts statistical power and precludes definitive conclusions regarding prognostic associations. A second limitation is the lack of confirmatory HER2 testing by in-situ hybridization (ISH) or next-generation sequencing (NGS) for IHC 2+ cases. This is important because HER2 IHC 2+ represents an equivocal category and requires amplification confirmation for classification as truly HER2-positive in most clinical frameworks. Future studies should adopt a stepwise testing strategy (IHC screening followed by ISH/NGS for 2+ cases), include larger multicentre cohorts, and incorporate survival modelling to better define prognostic and predictive implications.

Summary of the Present Results

In summary, the present study demonstrated a high frequency of aberrant p53 immunohistochemical patterns in gallbladder adenocarcinoma (77.8%), with nuclear overexpression as the predominant abnormal pattern (66.7%). This finding is concordant with published evidence indicating frequent TP53 pathway dysregulation in gallbladder carcinoma and supports the role of p53 as a marker of malignant transformation and aggressive tumour biology (Ghosh et al., 2013; Legan et al., 2006; de Bitter et al., 2022).

For HER2, the observed prevalence was dependent on the definition of positivity. Definite HER2

overexpression (IHC 3+) was observed in 22.2% of tumors, which is closer to the range reported in larger cohorts. In contrast, inclusion of HER2 IHC 2+ cases yields a higher apparent positivity (66.7%); however, this likely includes tumors that would require ISH confirmation for ERBB2 amplification. These findings support the presence of a clinically relevant HER2-expressing subset in gallbladder adenocarcinoma and reinforce the need for confirmatory testing in equivocal IHC cases, particularly when therapeutic stratification is considered (Yoshida et al., 2016; Roa et al., 2014; Sung et al., 2021; de Bitter et al., 2022).

CONCLUSION

The present study demonstrates that the majority of gallbladder lesions encountered in routine cholecystectomy specimens are non-neoplastic, with chronic calculous cholecystitis constituting the predominant diagnosis. Gallbladder adenocarcinoma was an infrequent but clinically significant finding and showed distinct clinicopathological and biomarker characteristics.

Immunohistochemical evaluation revealed a high frequency of aberrant p53 expression patterns (77.8%) in gallbladder adenocarcinoma, with nuclear overexpression as the predominant abnormal pattern (66.7%). These findings support the role of TP53 pathway disruption as a common molecular event in gallbladder carcinogenesis and reinforce p53 as a practical marker of malignant transformation and aggressive tumour biology.

HER2/neu expression was observed in a subset of gallbladder adenocarcinomas, with definite strong membranous overexpression (IHC 3+) in 22.2% of cases and equivocal (IHC 2+) expression in 44.4%. This indicates that while unambiguous HER2 positivity is limited to a minority, a substantial proportion of tumors demonstrate intermediate expression that may warrant confirmatory testing for ERBB2 amplification. The variability in HER2 expression underscores the importance of standardized scoring criteria and highlights the potential clinical relevance of HER2 as a predictive biomarker in selected cases.

Overall, combined assessment of p53 and HER2/neu provides useful insight into the molecular heterogeneity of gallbladder adenocarcinoma. p53 appears to function primarily as a marker of malignant transformation, whereas HER2 identifies a biologically distinct subset of tumors with potential therapeutic implications. These findings support integration of biomarker evaluation with routine histopathological assessment and justify larger multicentre studies incorporating confirmatory HER2 testing and outcome analysis.

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