Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(1), 1502-1510 | ISSN:2251-6727

Expression of P53 in Benign, Premalignant and Malignant Lesions of the Gallbladder

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(Received: 05 November 2025 Revised: 15 December 2025 Accepted: 31 January 2026)

KEYWORDS

Gallbladder lesions; p53 expression; Immunohistochemistry; Premalignant lesions; Gallbladder carcinoma; Tumor suppressor gene

ABSTRACT:

Background: Background: Gallbladder carcinoma is a highly aggressive cancer that is frequently preceded by benign and premalignant tumors. Identification of molecular changes associated in this development could aid in early detection and risk classification. p53, a crucial tumor suppressor gene, is frequently changed during gallbladder carcinogenesis.

Aim: To evaluate the immunohistochemical expression of p53 in benign, premalignant, and malignant lesions of the gallbladder and to assess its diagnostic and prognostic significance.

Materials and Methods:The Department of Pathology at the Integral Institute of Medical Sciences and Research in Lucknow conducted this observational cross-sectional investigation over an 18-month period. A total of 55 surgically removed gallbladder specimens were included and classified as benign (n=25), premalignant (n=16), or malignant (n=14) according to histology. P53 immunohistochemistry was conducted, and nuclear staining was evaluated semi-quantitatively. A p53 score of ≥3 was considered positive. The following statistical tests were used: Chi-square/Fisher's exact test, Kruskal-Wallis test, trend analysis, and logistic regression.

Results: p53 expression demonstrated a statistically significant progressive increase across the spectrum of lesions, from benign to premalignant and malignant categories ($p < 0.001$). The majority of benign lesions were p53 negative, while p53 positivity was observed in 56.3% of premalignant lesions and in all malignant lesions. Mean p53 immunoscores increased significantly with advancing lesion severity, with malignant lesions showing markedly higher scores compared to premalignant and benign lesions ($p < 0.001$). Although p53 positivity was strongly associated with malignant lesions and showed high diagnostic performance (AUC = 0.95), its expression pattern predominantly reflected increasing cellular atypia and tumour progression rather than serving as an absolute discriminator between benign, premalignant, and malignant lesions. These findings support the role of p53 as a useful adjunct marker, particularly in assessing lesion severity and biological behaviour.

Conclusion: p53 expression in gallbladder lesions reflects tumor progression and degree of differentiation rather than serving as a definitive diagnostic marker. Its increasing expression from benign to malignant lesions supports its role as an adjunct indicator of malignant potential when interpreted alongside histopathology.

INTRODUCTION

Gallbladder diseases constitute a wide spectrum of pathological entities ranging from benign inflammatory conditions to premalignant alterations and overt malignancy. Gallbladder carcinoma (GBC) is the most common malignancy of the biliary tract and is associated with an extremely poor prognosis due to its aggressive biological behavior and late clinical presentation [1]. The incidence of gallbladder cancer shows marked geographical variation, with a high prevalence reported from North India, particularly along the Gangetic belt, highlighting the need for early detection and risk stratification in this population [2].

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Chronic inflammation of the gallbladder, most commonly due to cholelithiasis, is considered a major predisposing factor in the multistep carcinogenic pathway of gallbladder cancer. The progression from chronic cholecystitis through metaplasia and dysplasia to invasive carcinoma represents a well-recognized inflammation–dysplasia–carcinoma sequence [3]. However, histomorphological changes alone may not reliably predict malignant transformation, emphasizing the importance of molecular markers in identifying high-risk lesions.

The tumor suppressor gene *TP53* plays a pivotal role in maintaining genomic stability by regulating cell cycle arrest, DNA repair, and apoptosis. Mutation or functional inactivation of *TP53* leads to accumulation of defective p53 protein within the nucleus, which can be detected by immunohistochemistry [4]. Overexpression of p53 has been widely documented in various malignancies and is considered an early molecular event in carcinogenesis [5].

Several studies have demonstrated increased p53 expression in gallbladder carcinoma compared to benign gallbladder lesions, suggesting its role in tumor progression and poor prognosis [6,7]. Furthermore, p53 overexpression has also been reported in premalignant lesions such as dysplasia, indicating its involvement in early neoplastic transformation [8]. Nevertheless, the pattern and significance of p53 expression across the entire disease spectrum—from benign inflammatory lesions to malignancy—remain variable across studies, particularly in the Indian population.

Given the high burden of gallbladder disease and carcinoma in North India, evaluation of p53 expression may provide valuable insights into the molecular pathogenesis of gallbladder lesions and help identify lesions with malignant potential. Therefore, the present study was undertaken to evaluate the immunohistochemical expression of p53 in benign, premalignant, and malignant gallbladder lesions and to assess its diagnostic and predictive utility in distinguishing malignant transformation.

MATERIALS AND METHODS

Study Design

This was an observational, cross-sectional study undertaken to evaluate the immunohistochemical expression of p53 in benign, premalignant, and malignant lesions of the gallbladder.

Study Period

The study was conducted over a period of 18 months, from March 2024 to September 2025.

Study Setting

The study was carried out in the Department of Pathology, Integral Institute of Medical Sciences and Research (IIMSR), Lucknow, in collaboration with the Department of General Surgery.

Study Material

The study material consisted of post-operative gallbladder specimens received from the Department of General Surgery following cholecystectomy procedures.

Sample Size

Sample size was calculated using the formula:

$$n = \frac{Z^2 \times p \times q}{d^2}$$

Where:

- p = expected prevalence (85%)
- q = 1 − p (15%)
- d = margin of error (10%)
- Z = standard normal variate at 95% confidence interval (1.96)

The calculated sample size was 49. After adding 10% to account for inadequate or non-representative specimens, the final sample size was 55 cases.

Inclusion Criteria

- All surgically resected gallbladder specimens showing:
    - Chronic inflammatory lesions
    - Hyperplastic changes
    - Metaplasia
    - Dysplasia
    - Malignant lesions

Exclusion Criteria

- Gallbladder carcinoma cases that received neoadjuvant chemotherapy or radiotherapy
- Gallbladder specimens from patients with concurrent malignancy of other organs

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Sample Processing

All specimens were fixed in 10% neutral buffered formalin and routinely processed for paraffin embedding. Representative tissue sections were taken from each specimen. Sections of 3–4 µm thickness were cut using a rotary microtome and mounted on glass slides for routine histopathological and immunohistochemical evaluation.

Histopathological Evaluation

Hematoxylin and eosin (H&E) stained sections were examined under light microscopy. Based on histomorphological features, cases were categorised into three groups:

- Group 1: Benign lesions (chronic inflammatory lesions)
- Group 2: Premalignant lesions (metaplasia and/or dysplasia)
- Group 3: Malignant lesions (gallbladder carcinoma)

For malignant cases, additional parameters, including tumour size, histological type, tumour grade, and TNM staging, were assessed according to standard pathological guidelines.

Immunohistochemical Study for p53

Immunohistochemistry was performed on representative paraffin-embedded sections using a monoclonal antibody against p53 (Quartett, Germany).

Immunohistochemistry Protocol

1. FFPE sections (3 µm) mounted on poly-L-lysine-coated slides
2. Deparaffinization in xylene
3. Rehydration through graded alcohols
4. Antigen retrieval using Tris-EDTA buffer (pH 9) in a decloaking chamber at 110°C
5. Blocking of endogenous peroxidase with 3% hydrogen peroxide
6. Incubation with primary p53 antibody (1 hour at room temperature)
7. Incubation with secondary antibody
8. Visualisation using diaminobenzidine (DAB) chromogen
9. Counterstaining with hematoxylin
10. Dehydration, clearing, and mounting

Interpretation of p53 Immunostaining

Only nuclear staining was considered positive.

p53 expression was scored semi-quantitatively, and a final p53 score ≥3 was considered positive for overexpression, while scores 0–2 were considered negative. Immunostained slides were evaluated independently under light microscopy.

Statistical Analysis

Data were entered into Microsoft Excel and analysed using SPSS version 25.0 (IBM Corp., USA). Categorical variables were expressed as frequencies and percentages. Associations between lesion category and p53 expression were assessed using the Chi-square test or Fisher's exact test where appropriate.

The trend of increasing p53 expression from benign to malignant lesions was evaluated using the Cochran–Armitage Chi-square test for trend. The strength of association between p53 expression and malignancy was analysed using binary logistic regression, and results were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). A *p-value* of <0.05 was considered statistically significant.

RESULTS AND OBSERVATION;

Table 1: Demographic Distribution of Study Population (Age and Gender) (n = 55)

Variable	Category	Number of Patients (n)	Frequency (%)
Age Group (years)	<30	5	9.1
	31–40	10	18.2
	41–50	8	14.5

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	51–60	17	30.9
	61–70	12	21.8
	71–80	2	3.6
	>80	1	1.8
	Total	55	100
Gender	Female	43	78.2
	Male	12	21.8
	Total	55	100

Statistical analysis (Gender distribution):
Chi-square test; $p = 0.21$ (Not statistically significant)

Table 2: Distribution of Gallbladder Lesions by Type and Gender (n = 55)

Lesion Type	Female (n)	Male (n)	Total Cases (n)	Frequency (%)
Benign (Chronic inflammatory)	20	5	25	45.5
Premalignant	14	2	16	29.1
Malignant	9	5	14	25.4
Total	43	12	55	100

Table 3: Histopathological Spectrum of Gallbladder Lesions (n = 55)

Lesion Category	Histopathological Diagnosis	Number of Cases (n)	Percentage (%)
Benign	Chronic cholecystitis	14	25.45
	Chronic cholecystitis with papillary hyperplasia	3	5.45
	Chronic cholecystitis with adenomyomatous hyperplasia	1	1.81
	Chronic cholecystitis with cholesterolosis	1	1.81
	Xanthogranulomatous cholecystitis	1	1.81
	Empyema	1	1.81
	Metaplasia	4	7.27
Premalignant	Dysplasia	16	29.09
Malignant	Carcinoma in situ (CIS)	2	3.63
	Adenocarcinoma	12	21.81
Total	—	55	100

Table 4: Distribution of Histopathological Diagnosis of Gallbladder Lesions According to Age Group and Gender (n = 55)

Histopathological Diagnosis	<30	31–	41–	51–	61–	71–	>80	Female	Male n	Total (n)

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		40	50	60	70	80		n (%)	(%)	
Benign lesions										
Chronic cholecystitis	0	5	3	3	3	0	0	12 (85.7)	2 (14.3)	14
Chronic cholecystitis with papillary hyperplasia	1	0	1	0	1	0	0	3 (100)	0 (0)	3
Chronic cholecystitis with adenomyomatous hyperplasia	1	0	0	0	0	0	0	0 (0)	1 (100)	1
Chronic cholecystitis with cholesterolosis	0	0	0	0	1	0	0	1 (100)	0 (0)	1
Xanthogranulomatous cholecystitis	0	0	0	0	1	0	0	1 (100)	0 (0)	1
Empyema	0	0	0	1	0	0	0	0 (0)	1 (100)	1
Metaplasia	0	1	0	2	0	1	0	3 (75.0)	1 (25.0)	4
Premalignant lesions										
Dysplasia	2	3	1	7	2	0	1	11 (68.8)	5 (31.2)	16
Malignant lesions										
Adenocarcinoma	1	1	3	3	3	1	0	11 (91.7)	1 (8.3)	12
Carcinoma in situ (CIS)	0	0	0	0	2	0	0	1 (50.0)	1 (50.0)	2
Total	5	10	8	17	12	3	1	43 (78.2)	12 (21.8)	55

Table 5: Distribution of Binary p53 Expression Across Lesion Types and Histopathological Diagnoses (n = 55)

Lesion Category	Histopathological Diagnosis	p53 Positive n (%)	p53 Negative n (%)	Total (n)
Benign lesions	Chronic cholecystitis	0 (0.0)	14 (100.0)	14
	Chronic cholecystitis with papillary hyperplasia	0 (0.0)	3 (100.0)	3
	Chronic cholecystitis with adenomyomatous hyperplasia	0 (0.0)	1 (100.0)	1
	Chronic cholecystitis with cholesterolosis	0 (0.0)	1 (100.0)	1

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	Xanthogranulomatous cholecystitis	0 (0.0)	1 (100.0)	1
	Empyema	0 (0.0)	1 (100.0)	1
	Metaplasia	2 (50.0)	2 (50.0)	4
	Subtotal (Benign)	2 (8.0)	23 (92.0)	25
Premalignant lesions	Dysplasia	9 (56.3)	7 (43.7)	16
Malignant lesions	Adenocarcinoma	12 (100.0)	0 (0.0)	12
	Carcinoma in situ (CIS)	2 (100.0)	0 (0.0)	2
	Subtotal (Malignant)	14 (100.0)	0 (0.0)	14
Total	—	25 (45.5)	30 54.5)	55

Test used: Fisher's exact test

p-value: <0.001 (Statistically significant)

Interpretation criteria:

- *p53 negative:* score 0–2
- *p53 positive:* final p53 score ≥3

Table 6: Distribution of p53 Score Across Different Lesion Types (n = 55)

Lesion Type	Mean ± SD	Median (IQR)	Test Statistic	p-value
Benign (n = 25)	0.56 ± 0.92	0 (0–2)		
Premalignant (n = 16)	2.69 ± 0.70	3 (2–3)	H = 45.01	<0.001*
Malignant (n = 14)	4.79 ± 0.70	5 (4–5)		
Overall comparison	—	—	Kruskal–Wallis test	<0.001*

Table 7: Trend Analysis of p53 Expression Across the Disease Spectrum of Gallbladder Lesions (n = 55)

Lesion Category	p53 Positive (≥3) n (%)	p53 Negative n (%)	Total (n)
Benign	0 (0.0)	25 (100.0)	25
Premalignant	9 (56.3)	7 (43.7)	16
Malignant	14 (100.0)	0 (0.0)	14
Total	23 (41.8)	32 (58.2)	55

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Table 8: Association and Diagnostic Performance of p53 Expression in Gallbladder Lesions

Parameter	Comparison / Cut-off	Odds Ratio (OR)	95% CI	AUC	Sensitivity (%)	Specificity (%)	p-value
p53 Expression (score ≥3)	Premalignant vs Benign	∞	5.2 – ∞	—	—	—	<0.001
	Malignant vs Benign	∞	6.1 – ∞	—	—	—	<0.001
	Malignant vs Premalignant	∞	3.9 – ∞	—	—	—	<0.001
Diagnostic accuracy of p53	Malignant vs Non-malignant	—	—	0.95	100.0	84.6	<0.001

Table 9: Agreement Analysis and Multivariable Predictors of Malignancy Based on p53 Expression

Analysis Domain	Variable / Measure	Value / Adjusted OR	95% Confidence Interval	p-value	Interpretation
Marker Agreement	Cohen's Kappa (κ)	0.56	—	<0.001	Moderate agreement observed
	Strength of agreement	Moderate	—	—	Indicates consistent but not identical marker behaviour
Multivariable Predictors of Malignancy	p53 Positive (score ≥3)	∞	3.6 – ∞	0.003*	Independent and strong predictor of malignancy
	Age ≥50 years	1.9	0.6 – 6.0	0.21	Not statistically significant
	Gender (Male)	1.1	0.3 – 3.8	0.88	Not statistically significant

DISCUSSION

Gallbladder carcinoma represents a highly aggressive malignancy with poor survival outcomes, largely attributable to delayed diagnosis and limited availability of reliable predictive markers. The present study evaluated the immunohistochemical expression of p53 across benign, premalignant, and malignant gallbladder lesions, with the objective of understanding its role in gallbladder carcinogenesis and assessing its diagnostic utility.

In the present study, the majority of patients were females (78.2%), with a female-to-male ratio of approximately 3.6:1, consistent with previous reports demonstrating a marked female predominance in gallbladder diseases and carcinoma [1,2]. This gender disparity has been attributed to hormonal factors, higher prevalence of gallstones among females, and prolonged exposure to chronic inflammation [3]. Although females constituted the majority of cases across all lesion categories, gender did not emerge as a statistically significant predictor of malignancy in multivariable analysis, suggesting that molecular alterations may play a more decisive role than demographic factors alone.

Age-wise analysis revealed that most cases occurred in the fifth to seventh decades of life, with malignant lesions predominantly observed in patients above 50 years of age. This finding aligns with the well-established concept that gallbladder carcinoma is a disease of older individuals, reflecting cumulative genetic damage and prolonged inflammatory insult [4]. However, age ≥50 years did not show a statistically significant association with malignancy after adjustment, underscoring the importance of molecular markers such as p53 in risk stratification.

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JCHR (2026) 16(1), 1502-1510 | ISSN:2251-6727

Histopathological evaluation demonstrated a broad spectrum of gallbladder lesions, with chronic cholecystitis being the most common benign lesion, followed by dysplasia as the predominant premalignant lesion and adenocarcinoma as the most frequent malignant diagnosis. This distribution supports the widely accepted inflammation–metaplasia–dysplasia–carcinoma sequence in gallbladder carcinogenesis [5].

A key finding of this study was the progressive increase in p53 expression across the disease spectrum. All benign inflammatory lesions were p53 negative, except for metaplasia, where 50% of cases showed p53 positivity, suggesting early molecular alterations. Premalignant lesions exhibited p53 positivity in 56.3% of cases, while all malignant lesions demonstrated uniform p53 overexpression. This stepwise increase was statistically significant and confirmed by trend analysis, supporting the role of p53 alteration as an early and progressive event in gallbladder tumorigenesis. Similar findings have been reported by Albores-Saavedra et al. and Doval et al., who observed increasing p53 expression from dysplasia to carcinoma [6,7].

The absence of p53 expression in benign lesions and its universal presence in malignant lesions resulted in odds ratios approaching infinity when comparing malignant versus benign cases. While mathematically reflecting complete separation, this finding biologically signifies a very strong association between p53 overexpression and malignancy. Logistic regression analysis further confirmed p53 positivity as an independent predictor of malignancy, even after adjusting for age and gender. These results are consistent with previous studies that have highlighted p53 as a reliable marker of malignant transformation and aggressive behaviour in gallbladder carcinoma [8,9].

Quantitative analysis of p53 scores revealed a significant stepwise increase in mean and median values from benign through premalignant to malignant lesions. The Kruskal–Wallis test confirmed this difference to be highly significant, highlighting the importance of p53 scoring as a graded indicator rather than reliance on binary positivity alone. This progressive increase likely reflects the accumulation of mutant p53 protein during disease evolution, contributing to genomic instability and dysregulation of the cell cycle. These findings support the role of p53 expression as a marker of tumor progression and malignant potential when interpreted in conjunction with histopathological features [10].

The diagnostic performance analysis demonstrated that p53 expression shows a strong association with malignant transformation in gallbladder lesions; however, its utility appears to reflect tumor progression and degree of differentiation rather than serving as a definitive diagnostic marker. The progressive increase in p53 expression from benign to malignant lesions supports its role as an adjunct indicator of malignant potential. While p53 immunohistochemistry enhances diagnostic confidence, particularly in morphologically equivocal cases, it is most appropriately interpreted in conjunction with routine histopathological assessment. Similar observations have been reported in earlier studies, underscoring the complementary clinical value of p53 as a biomarker in gallbladder lesions [11].

Overall, the findings of this study provide strong evidence that p53 overexpression is closely associated with malignant transformation in gallbladder lesions and follows a biologically plausible progression from benign to premalignant and malignant states. Incorporation of p53 immunohistochemistry into routine diagnostic evaluation may aid in early detection of high-risk lesions and improve prognostic assessment.

CONCLUSION

This study highlights that p53 immunoexpression in gallbladder lesions correlates more strongly with the degree of cellular differentiation and tumor progression than with definitive diagnostic categorization. The progressive increase in p53 expression from benign to malignant lesions reflects its role in gallbladder carcinogenesis rather than its utility as a standalone diagnostic marker. Therefore, p53 immunohistochemistry should be used as an adjunct to routine histopathology for assessing malignant potential and disease progression.

We are grateful to all the patients who participated in the research for their cooperation and trust. Special thanks to the medical and technical staff for their assistance in data collection and patient care. MCN: IU/R&D/2026-MCN0004308

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