

**Original Article****Histopathological Changes in Gallbladder Mucosa in Relation to Gallstone: A Cross-Sectional Study from a Tertiary Care Center****Niraj Nepal <sup>\*1</sup>, Amit Bhattarai <sup>2</sup>, Dinesh Adhikari <sup>2</sup>**<sup>1</sup> Department of Pathology, Nobel Medical College Teaching Hospital, Biratnagar, Nepal<sup>2</sup> Department of Surgery, Nobel Medical College Teaching Hospital, Biratnagar, NepalArticle Received: 18<sup>th</sup> January, 2026; Accepted: 20<sup>th</sup> April, 2026; Published: 30<sup>th</sup> June, 2026**DOI: <https://doi.org/10.3126/jonmc.v15i1.96386>****Abstract****Background**

Gallstone disease is common in Nepal, yet the relationship between gallstone characteristics and gallbladder mucosal pathology is not well defined.

**Materials and Methods**

This cross-sectional study included 288 consecutive cholecystectomy specimens from a tertiary care center. Clinical data, gallstone number, size and composition (cholesterol, pigment, mixed) were recorded. Gallbladder mucosa was examined histologically and associations between stone variables and inflammatory, premalignant and malignant lesions were analyzed.

**Results**

The mean age was 44 years with a female-to-male ratio of 2.6:1. Chronic cholecystitis was the predominant lesion (72.9%), while epithelial dysplasia and adenocarcinoma were identified in 2.8% and 1.4% cases, respectively. Mixed stones were most frequent (54.2%), followed by cholesterol (30.9%) and pigment stones (14.9%), with no significant association between stone composition and histological severity ( $p=0.125$ ). In contrast, multiple stones (72.9%) and larger stones ( $>20$  mm) were significantly associated with advanced inflammatory and neoplastic changes ( $p<0.001$  and  $p=0.005$ , respectively).

**Conclusion**

Chronic cholecystitis is the predominant histological pattern in gallstone disease, with mixed stones most common and no significant link between stone composition and mucosal severity, whereas multiple and larger stones are clearly associated with advanced inflammatory and neoplastic changes.

**Keywords:** *Cholelithiasis, Gallstones, Histopathology*

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**Citation**

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## Introduction

Gallstone disease is one of the most common biliary tract disorders and a leading indication for cholecystectomy worldwide [1]. Across contemporary South Asian histopathology series, chronic cholecystitis constitutes roughly two-thirds to four-fifths of cholecystectomy specimens, with reported rates of 67.5% and 68.5% in Kathmandu, 64.8% in Nagpur and nearly 79.8% in rural West Bengal [1-4]. Premalignant lesions such as metaplasia or dysplasia occur in roughly 3-6% of specimens, and incidental gallbladder carcinoma is reported in 0.25-5.7% across these studies and a large Brazilian series involving 1,278 cholecystectomies [5]. These findings emphasize the clinical relevance of gallstone related mucosal injury within the inflammation to metaplasia to dysplasia to carcinoma sequence. Stone related factors appear to further influence mucosal alterations. In a composition-based study involving 90 cases of chronic calculous cholecystitis, mixed stones were the most common type at 54 percent [6]. Nearly half of the patients had multiple calculi, and more advanced mucosal changes including hyperplasia, metaplasia, or carcinoma (collectively ~11%) were associated with increasing stone size and weight [7]. A Pakistani series of 87 patients found multiple stones in 77% of cases and demonstrated significant associations between multiple calculi and hemorrhagic or atrophic mucosa, as well as fibrosis, dysplasia, or hyperplasia [6]. A North Indian cohort of 100 cholelithiasis patients reported gallbladder carcinoma in 5% of cholecystectomy specimens, with solitary larger stones and longer symptom duration identified as important correlates on univariate analysis [8].

Most Nepali studies report chronic cholecystitis as the predominant histopathological finding, with associated cholesterosis, adenomyosis, or metaplasia and adenocarcinoma rates ranging from 0.25–1.6%, but correlations with gallstone composition remain poorly explored [1-3]. Given regional variation in stone type and the rising burden of cholelithiasis in Nepal, this study was conducted to evaluate gallbladder mucosal changes in relation to gallstone composition, number, and size in order to identify patterns associated with increased malignant potential.

## Materials and Methods

This observational cross-sectional study was conducted at the department of Pathology, Nobel

Medical College Teaching Hospital, Biratnagar, Nepal, from December 2024 to November 2025. Ethical approval was obtained from the Institutional Review Committee, and written informed consent was taken from all participants. Symptomatic patients aged  $\geq 18$  years with clinically diagnosed cholelithiasis who underwent cholecystectomy during the study period were included. Cases with gallbladder pathologies unrelated to gallstones and poorly preserved or inadequately labelled specimens were excluded. A total of 288 consecutive cholecystectomy specimens were included using consecutive sampling.

All specimens were received in 10% neutral buffered formalin. Gross examination included assessment of gallbladder size, wall thickness, mucosal appearance, and abnormal areas. Gallstones were assessed for number, maximum diameter, and morphological composition as cholesterol, mixed, or pigment stones based on standard gross features including color, texture, and cut-surface appearance. Stone classification was independently verified by two pathologists to improve reproducibility. Representative sections from the fundus, body, neck, and abnormal areas were processed routinely, embedded in paraffin, sectioned at 3–4  $\mu\text{m}$ , and stained with hematoxylin and eosin. Histopathological evaluation included acute and chronic inflammation, cholesterosis, hyperplasia, metaplasia, dysplasia, and carcinoma.

Data were entered into Microsoft Excel and analysed using SPSS version 26. Continuous variables were expressed as mean  $\pm$  standard deviation, while categorical variables were presented as frequencies and percentages. Associations between gallstone characteristics and histopathological findings were analysed using the Chi-square test. Histopathological categories with low frequencies were pooled (acute cholecystitis, acute-on-chronic cholecystitis, and xanthogranulomatous cholecystitis; dysplasia and carcinoma) to satisfy Chi-square test assumptions regarding minimum expected cell counts. A p-value  $< 0.05$  was considered statistically significant.

## Results

A total of 288 cholecystectomy specimens were analyzed. The mean age of the patients was  $43.97 \pm 14.46$  years (range 18–85 years), with a median age of 42 years. Most cases occurred in the 31–40-year age group (29.9%), followed



by those aged 41–50 years (25.0%). Females comprised the majority across all age groups, accounting for 72.2% of the study population, resulting in a female-to-male ratio of approximately 2.6:1 as shown in Table 1.

**Table 1: Age and sex distribution of patients undergoing cholecystectomy**

| Age Group (years) | Male (n)   | Female (n)  | Total (n, %) |
|-------------------|------------|-------------|--------------|
| <30 years         | 12 (27.9%) | 31 (72.1%)  | 43 (14.9%)   |
| 31–40 years       | 24 (27.9%) | 62 (72.1%)  | 86 (29.9%)   |
| 41–50 years       | 20 (27.8%) | 52 (72.2%)  | 72 (25.0%)   |
| 51–60 years       | 14 (26.9%) | 38 (73.1%)  | 52 (18.1%)   |
| >60 years         | 10 (28.6%) | 25 (71.4%)  | 35 (12.2%)   |
| Total             | 80 (27.8%) | 208 (72.2%) | 288 (100%)   |

Chronic cholecystitis was the predominant histopathological finding (72.9%), followed by acute on chronic (9.7%) and acute cholecystitis (3.5%). Xanthogranulomatous cholecystitis (2.1%), epithelial dysplasia (2.8%), and adenocarcinoma (1.4%) were less frequent, while normal or incidental findings accounted for 7.6% of cases. Overall, inflammatory lesions formed the clear majority of gallbladder pathology in this study as shown in Table 2.

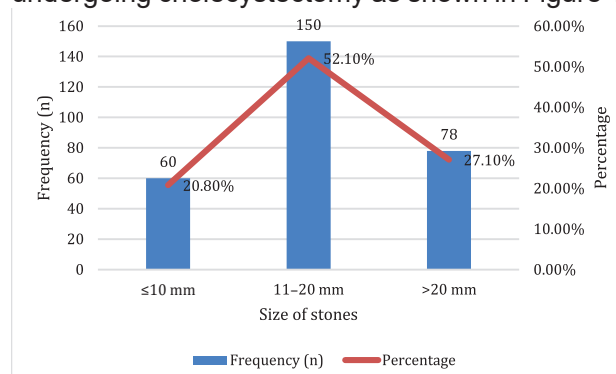
**Table 2: Distribution of histopathological diagnoses among gallbladder specimens**

| Diagnosis                         | Frequency (n) | Percentage |
|-----------------------------------|---------------|------------|
| Chronic cholecystitis             | 210           | 72.9%      |
| Acute on chronic cholecystitis    | 28            | 9.7%       |
| Acute cholecystitis               | 10            | 3.5%       |
| Xanthogranulomatous cholecystitis | 6             | 2.1%       |
| Epithelial dysplasia              | 8             | 2.8%       |
| Adenocarcinoma                    | 4             | 1.4%       |
| Normal / other findings           | 22            | 7.6%       |
| Total                             | 288           | 100.0%     |

Multiple calculi were present in 210 patients (73%), whereas 78 patients (27%) had a single stone. This pattern indicates that most individuals with symptomatic cholelithiasis in the study cohort presented with multi-stone disease as shown in Figure 1. Among the 288 patients included in the study, multiple gallstones were observed in 210 (72.9%) cases, while solitary stones were present in 78 (27.1%) cases, indicating that multiple calculi constituted the predominant pattern of cholelithiasis in the study population.

Stones measuring 11–20 mm were the most frequent, accounting for 52.1% of cases. Stones larger than 20 mm were observed in 27.1%, while

smaller stones measuring 10 mm or less comprised 20.8%. This pattern indicates that medium-sized stones predominated among patients undergoing cholecystectomy as shown in Figure 1.



**Figure 1: Distribution of gallstone size among study population**

Mixed stones were the most frequent type, accounting for 54% of cases, followed by cholesterol stones (31%) and pigment stones (15%) as shown in Figure 3. Mixed gallstones were the most common type, observed in 156 (54.2%) cases, followed by cholesterol stones in 89 (30.9%) and pigment stones in 43 (14.9%) cases, indicating a predominance of mixed stone morphology in the study population.

**Table 3: Association of gallstone composition with histopathological finding of gallbladder**

| Histopathology             | Cholesterol (n, %) | Mixed (n, %) | Pigment (n, %) | Total (n, %) | P-value |
|----------------------------|--------------------|--------------|----------------|--------------|---------|
| Chronic cholecystitis      | 60 (28.6%)         | 120 (57.1%)  | 30 (14.3%)     | 210 (72.9%)  | 0.125   |
| Acute/Acute on Chronic/XGC | 12 (27.3%)         | 22 (50.0%)   | 10 (22.7%)     | 44 (15.3%)   |         |
| Dysplasia/Carcinoma        | 5 (41.7%)          | 5 (41.7%)    | 2 (16.7%)      | 12 (4.2%)    |         |
| Normal/Other               | 12 (54.5%)         | 9 (40.9%)    | 1 (4.5%)       | 22 (7.6%)    |         |
| Total                      | 89 (30.9%)         | 156 (54.2%)  | 43 (14.9%)     | 288 (100%)   |         |

Mixed stones were the most common overall (54.2%), followed by cholesterol (30.9%) and pigment stones (14.9%). Chronic cholecystitis (n = 210) showed a similar pattern, with mixed stones comprising 57.1%. Acute and acute-on-chronic cases (n = 44) also had a predominance of mixed stones (50%). Dysplasia or carcinoma (n = 12) showed equal proportions of cholesterol and mixed stones (41.7% each), while normal or incidental findings (n = 22) were more often associated with cholesterol stones (54.5%). Despite these trends, no significant association was observed between stone composition and histopathology (p = 0.125).



**Table 4: Association between the number of gallstones and histopathological findings in gallbladder**

| Histopathology             | Solitary (n, %) | Multiple (n, %) | Total (n, %) | p-value |
|----------------------------|-----------------|-----------------|--------------|---------|
| Chronic cholecystitis      | 40 (19.0%)      | 170 (81.0%)     | 210 (72.9%)  | <0.001* |
| Acute/Acute on Chronic/XGC | 10 (22.7%)      | 34 (77.3%)      | 44 (15.3%)   |         |
| Dysplasia/Carcinoma        | 6 (50.0%)       | 6 (50.0%)       | 12 (4.2%)    |         |
| Normal/Other               | 22 (100.0%)     | 0 (0.0%)        | 22 (7.6%)    |         |
| Total                      | 78 (27.1%)      | 210 (72.9%)     | 288 (100%)   |         |

\*statistically significant

Multiple stones were most frequently associated with chronic cholecystitis, occurring in 81.0% of these cases, and were also common in acute or acute-on-chronic inflammation (77.3%). In contrast, all cases with normal or incidental findings had solitary stones. Dysplasia or carcinoma showed an equal distribution of solitary and multiple stones (50% each). Overall, multiple stones accounted for 72.9% of the study group. The association between stone number and mucosal pathology was statistically significant ( $p < 0.001$ ) as shown in Table 4.

**Table 5: Association Between Gallstone Size and Histopathological finding of gallbladder**

| Histopathology             | ≤10 mm (n, %) | 11–20 mm (n, %) | >20 mm (n, %) | Total (n, %) | p-value |
|----------------------------|---------------|-----------------|---------------|--------------|---------|
| Chronic cholecystitis      | 35 (16.7%)    | 115 (54.8%)     | 60 (28.6%)    | 210 (72.9%)  | 0.005*  |
| Acute/Acute on Chronic/XGC | 10 (22.7%)    | 22 (50.0%)      | 12 (27.3%)    | 44 (15.3%)   |         |
| Dysplasia/Carcinoma        | 3 (25.0%)     | 5 (41.7%)       | 4 (33.3%)     | 12 (4.2%)    |         |
| Normal/Other               | 12 (54.5%)    | 8 (36.4%)       | 2 (9.1%)      | 22 (7.6%)    |         |
| Total                      | 60 (20.8%)    | 150 (52.1%)     | 78 (27.1%)    | 288 (100%)   |         |

\*statistically significant

Stones measuring 11–20 mm were the most frequent across all categories (52.1%). In chronic cholecystitis ( $n = 210$ ), more than half of the cases (54.8%) had stones in this size range, while 28.6% had stones >20 mm. Acute or acute-on-chronic lesions ( $n = 44$ ) showed a similar pattern, with 50% of stones in the 11–20 mm group. Dysplasia or carcinoma ( $n = 12$ ) also demonstrated a higher proportion of larger stones (>20 mm). In contrast, normal or incidental findings ( $n = 22$ ) more often involved smaller stones (≤10 mm). The association between stone size and histopathology was statistically significant ( $p = 0.005$ ) as shown in Table 5.

## Discussion

In this series of 288 cholecystectomy specimens, gallstone disease chiefly affected mid-

dle-aged adults with a marked female excess (female: male 2.6:1). This mirrors national data from Kathmandu, where Maharjan and Khadka reported a mean age in the mid-forties and a female predominance of 2.2:1 in 400 cases, and Pathak et al. found most patients in the fourth–fifth decades with a female: male ratio of 2.8:1 in 127 cases [1, 2]. Similar middle-aged, female-predominant patterns have also been reported by Awandkar and Wilkinson in Nagpur, Bano et al. in Jamshoro, and Almas T. et al. from a tertiary hospital in Pakistan, further supporting that our cohort reflects the typical South Asian “gallstone belt” demographic [3, 6, 9].

Chronic cholecystitis was the predominant histological diagnosis (72.9%), with acute/acute-on-chronic cholecystitis, xanthogranulomatous cholecystitis and other inflammatory patterns comprising most of the remainder. This closely matches the 67.5% rate of chronic cholecystitis reported by Maharjan and Khadka and its predominance in the series of Pathak et al [1, 2]. Comparable proportions have been reported in India and Pakistan, with Awandkar and Wilkinson documenting chronic inflammatory changes in 54% of 170 specimens, Bano et al. observing nearly half of 87 cases with fibrotic or inflamed mucosa, and Almas T. et al. reporting chronic cholecystitis in 78.7% of their cohort [3, 6, 9]. In a Brazilian series of 1,278 cholecystectomies, Holanda and Lima Júnior reported chronic cholecystitis in 97.8%, underscoring chronic inflammation as the main background lesion [5]. Premalignant and malignant changes, though infrequent, were clinically significant and comparable to other settings. Maharjan and Khadka documented dysplasia in 2.75% and incidental carcinoma in 0.25%, and Pathak et al. reported one adenocarcinoma among 127 gallbladders [1, 2]. Bano et al. observed dysplasia in 1.1% of patients, and Awandkar and Wilkinson reported adenocarcinoma in 5.71% of specimens [3, 6]. Internationally, incidental carcinoma is seen in ~0.5% of Brazilian cholecystectomies and ~0.2% in the Cureus series, again on a background of chronic cholecystitis [5, 9]. Prospective studies by Sharma et al. and Singh et al. show frequent metaplasia, dysplasia and early carcinoma in gallbladders removed for presumed benign disease, supporting the metaplasia–dysplasia–carcinoma sequence and indicating that such lesions are not rare enough to justify selective histology [8, 10].





Mixed stones were the commonest type in our cohort, followed by cholesterol and pigment stones, and composition was not significantly associated with mucosal severity ( $p=0.125$ ). Nayak et al. likewise found mixed stones predominant (54%) and no significant composition-lesion correlation, although carcinoma occurred only with large pigment stones [7]. Singh et al. also reported that stone type and number were not independent predictors of metaplasia or dysplasia after adjustment for stone size and clinical factors, highlighting chemistry as a weak risk discriminator [10]. In contrast, stone burden correlated strongly with mucosal change: multiple stones were present in nearly three-quarters of patients and were significantly associated with chronic and advanced inflammatory changes ( $p<0.001$ ), in keeping with the higher rates of fibrosis, hyperplasia and dysplasia in multiple-stone disease reported by Bano et al. ( $p=0.048$ ) and with multifocal stone disease in Singh et al. and Sharma et al. being linked to metaplastic and dysplastic changes, even when only symptom duration remained independently significant [6-8, 10]. Stone size showed a clear gradient of risk, with larger stones over-represented in acute-on-chronic cholecystitis, xanthogranulomatous cholecystitis and carcinoma ( $p=0.005$ ), echoing Nayak et al., who found progression from chronic cholecystitis to hyperplasia, metaplasia and carcinoma with increasing stone size (up to ~5 cm), and corroborated by prospective data from Singh et al. and other Indian series [7, 8, 10].

These findings, together with similar national and international data, support routine histopathological examination of all gallbladder specimens in high-burden regions. Detection of dysplasia and carcinoma in cholecystectomies performed for benign disease in our series and in those of Awandkar and Wilkinson, Holanda and Lima Júnior, Almas et al., Sharma et al. and Singh et al. reinforces Poller's argument that selective histology, based on gross appearance alone, is unsafe in the "gallbladder cancer belt" [11]. Simple stone-related parameters (number and size) may help refine risk stratification and follow-up, but cannot replace systematic microscopic evaluation. This single-centre, cross-sectional, hospital-based study with semi-quantitative stone assessment and few dysplastic or malignant cases limits generalisability and statistical power for subgroup analysis. Absence of detailed bio-

chemical stone analysis, metabolic profiling and follow-up further restricts causal interpretation.

## Conclusion

Chronic cholecystitis is the predominant histological pattern in gallstone disease, with mixed stones most common and no significant link between stone composition and mucosal severity, whereas multiple and larger stones are clearly associated with advanced inflammatory and neoplastic changes.

## Recommendation

All gallbladders removed for gallstone disease should undergo systematic histopathological evaluation, with particular vigilance in patients with multiple or large stones. Larger, multicenter studies incorporating precise stone chemistry, clinicoradiological correlation and longitudinal follow-up are recommended to refine risk stratification for gallbladder carcinoma.

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**Conflict of interest:** None declared.

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