

Original Article

Cholelithiasis-associated gallbladder mucosal alterations: A histopathological analysis with clinical correlation

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ABSTRACT

Introduction: Cholelithiasis is a common condition with significant histopathological consequences for the gallbladder mucosa, ranging from chronic inflammation to potential malignant transformation. This study aimed to assess the spectrum of microscopic changes in gallbladder specimens from cholelithiasis patients and their correlation with clinical parameters.

Materials and Methods: A cross-sectional, observational study was conducted over two years at the Department of Pathology, Subharti Medical College, Meerut. A total of 100 cholecystectomy specimens from adult patients diagnosed with cholelithiasis were examined. Routine histopathological procedures were employed, and sections were stained with Hematoxylin and Eosin. Additional stains were used when necessary. Findings were correlated with demographic and clinical data, and statistical analysis was performed using SPSS v25.0.

Results: The majority of patients were female (68%), with the highest prevalence in the 41–50 age group. Chronic cholecystitis was observed in 90% of cases, Rokitansky-Aschoff sinuses in 40%, epithelial hyperplasia in 10%, fibrosis/ulceration in 5%, and carcinoma in 2%. Statistically significant associations were found between age and the presence of Rokitansky-Aschoff sinuses ($p < 0.05$). Carcinoma cases occurred exclusively in female patients above 50 years of age.

Conclusions: Chronic inflammatory changes are predominant in gallbladders affected by cholelithiasis. The presence of epithelial hyperplasia and incidental carcinoma emphasizes the necessity for routine histopathological examination of all cholecystectomy specimens to detect early neoplastic changes, particularly in high-risk populations.

Keywords: Cholelithiasis, Gallbladder, Chronic Cholecystitis, Rokitansky-Aschoff Sinuses, Epithelial Hyperplasia, Gallbladder Carcinoma, Histopathology

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INTRODUCTION

Located beneath the liver, the gallbladder is a small, pear-shaped organ responsible for storing and concentrating bile. Anatomically, it is composed of serosal, muscular, and mucosal layers, with the mucosa lined by simple columnar epithelium. Its strategic location and function make it susceptible to a variety of pathological conditions, one of the most common being cholelithiasis, or gallstone disease. Gallstones form primarily due to the precipitation of cholesterol, bile pigments, and calcium salts, and can lead to significant clinical complications, including acute and chronic cholecystitis, mucosal hyperplasia, and even malignancy [1].

Cholelithiasis is a prevalent condition worldwide, with variable prevalence rates depending on geographic, dietary, and genetic factors [2]. In India, the prevalence ranges from 2% to 29%, with higher rates observed in certain Northern states due to specific lifestyle and dietary patterns [3]. The pathogenesis of gallstones is multifactorial, involving alterations in bile composition, gallbladder motility, and the presence of nucleating factors that initiate the crystallization process [4]. Gallstones, especially when asymptomatic, can remain undetected for years; however, when symptomatic, they are often associated with right upper quadrant abdominal pain, nausea, and other gastrointestinal symptoms [5].

Histopathological examination of the gallbladder in patients undergoing cholecystectomy for cholelithiasis has revealed a spectrum of changes, ranging from chronic inflammation (chronic cholecystitis) to more severe alterations, such as Rokitansky-Aschoff sinuses, epithelial hyperplasia, metaplasia, and in rare cases, dysplasia or carcinoma [6]. Chronic irritation caused by the presence of gallstones is a significant factor leading to these changes, as it triggers a cycle of epithelial injury, inflammation, and repair, which over time may contribute to malignant transformation [7].

Chronic cholecystitis, marked by ongoing inflammation of the gallbladder wall, is the most commonly observed histopathological change in gallbladder specimens linked to cholelithiasis. Histologically, It typically shows accumulation of lymphocytes, plasma cells, and sometimes eosinophils into the lamina propria [8]. Rokitansky-Aschoff sinuses, another common feature, are mucosal invaginations extending into the muscular layer, often considered a response to chronic inflammation and increased intraluminal pressure [9]. Epithelial hyperplasia, which may range from simple to adenomatous patterns, is also observed and poses a risk for malignant transformation in a subset of cases [10].

Recent research has emphasized a link between prolonged gallbladder inflammation

and the onset of gallbladder cancer, particularly in areas where cholelithiasis is highly prevalent. Gallbladder cancer continues to be among the most aggressive types of malignancies affecting the biliary tract, with poor prognosis due to its late presentation [4]. Therefore, routine histopathological examination of all cholecystectomy specimens, even in cases of clinically benign disease, is essential for early detection of pre-malignant and malignant changes [2].

This study aimed to examine the microscopic pathological alterations in the gallbladder lining associated with cholelithiasis, to better understand the spectrum of alterations, and to emphasize the importance of routine histological examination in patients undergoing cholecystectomy. By correlating the presence of histopathological changes with clinical parameters, this study seeks to contribute to the understanding of the pathophysiology of cholelithiasis and its complications, providing insights into potential preventive and therapeutic strategies.

MATERIAL AND METHODS

Study Design and Setting: A cross-sectional, observational study was carried out in the Department of Pathology at Subharti Medical College, Meerut, spanning two years from January 2022 to December 2023. During this period, 100 gallbladder specimens were obtained from patients diagnosed with

cholelithiasis who had undergone cholecystectomy which were confirmed via clinical, radiological, and surgical findings. Ethical clearance for the study was granted by the institutional ethics committee, and informed consent was secured from all participants.

Inclusion Criteria:

- Individuals undergoing planned or urgent cholecystectomy with a verified diagnosis of cholelithiasis.
- Both symptomatic and asymptomatic cases were included.
- Specimens from adult patients (age ≥ 18 years) of both genders.

Exclusion Criteria:

- Specimens with a history of primary gallbladder malignancy detected pre-operatively.
- Patients with known chronic liver disease or other biliary tract disorders.
- Specimens showing autolytic changes or improper fixation.

The cholecystectomy specimens were collected immediately after surgery, labeled, and transferred to the pathology department. Each specimen was thoroughly inspected for external and internal features, including the presence of stones, wall thickness, mucosal appearance, and any gross abnormalities. After recording the gross findings, to maintain tissue integrity, the specimens were preserved in 10% neutral buffered formalin for 24 to 48 hours.

Following fixation, the specimens were processed using standard histopathological techniques:

1. **Tissue Sampling:** Tissue samples were selectively obtained from the fundus, body, and neck regions of the gallbladder, along with any visible abnormal areas, such as polyps, nodules, or areas of thickening.
2. **Dehydration and Embedding:** The tissues underwent dehydration through increasing concentrations of ethanol, were then cleared using xylene, and finally embedded in paraffin wax blocks.
3. **Section Cutting and Staining:** Slices measuring 4-5 µm in thickness were prepared with a microtome and stained with Hematoxylin and Eosin (H&E) for microscopic analysis. When needed, extra stains such as Periodic Acid-Schiff and Alcian Blue were applied to highlight particular histopathological characteristics.

Histopathological Examination

The prepared slides were examined under a light microscope by two independent pathologists to ensure accuracy. The histopathological changes observed were categorized into the following:

1. **Chronic Cholecystitis:** Defined by lymphoplasmacytic infiltration, fibrosis, and the presence of lymphoid aggregates.
2. **Rokitansky-Aschoff Sinuses (RAS):** Identified as invaginations of the mucosa extending into the muscular layer.
3. **Epithelial Hyperplasia:** Including both

simple and adenomatous hyperplasia, characterized by the thickening of the mucosal epithelium.

4. **Fibrosis and Ulceration:** Presence of fibroblastic proliferation and loss of mucosal continuity, respectively.
5. **Dysplasia and Carcinoma:** Cases exhibiting cellular atypia, pleomorphism, and mitotic figures were subjected to further immunohistochemical analysis to confirm malignant transformation.

Each histopathological finding was documented, and the frequency of different types of changes was recorded. The presence of specific patterns was noted, and their correlation with clinical parameters (e.g., age, gender, duration of symptoms) was analyzed.

Statistical Analysis

The data were recorded in a Microsoft Excel spreadsheet and analyzed using SPSS software version 25.0. Descriptive statistics were employed to determine the frequency and percentage of the histopathological findings. For continuous variables, the mean and standard deviation (SD) were calculated, whereas categorical variables were presented as percentages. Chi-square tests were used to evaluate the relationship between clinical factors (including age and gender) and particular histopathological findings. A p-value less than 0.05 was regarded as statistically significant.

This study was carried out following the principles of the Declaration of Helsinki. Prior to starting, approval was secured from the Institutional Ethics Committee of Subharti Medical College, Meerut. To ensure confidentiality, all patient information was anonymized, and no personal identifiers were included in the final analysis.

RESULTS

Demographic Profile

A total of 100 cholecystectomy specimens from patients diagnosed with cholelithiasis were analyzed. The patients’ ages varied between 18 and 75 years, with an average age of 45.6 ± 12.4 years. The age group with the highest occurrence was 41-50 years, accounting for 32% of cases. The study population consisted of 68 females (68%) and 32 males (32%), resulting in a female-

to-male ratio of 2.1:1, indicating a higher prevalence of cholelithiasis in females.

Clinical Presentation

The most common clinical presentation was chronic upper abdominal pain, reported in 78% of the patients. Other symptoms included nausea (42%), vomiting (30%), and jaundice (8%). Asymptomatic cases were detected in 12% of the patients during routine health evaluations.

Gross Pathological Findings

On gross examination, all gallbladder specimens had gallstones, with 84% showing multiple stones and 16% showing a single large stone. The stones ranged in diameter from 0.5 cm to 3.5 cm. Thickening of the gallbladder wall (>3 mm) was observed in 68% of the cases, while

Table 1. Demographic Distribution of Patients.

Age Group (Years)	Male	Female	Total (%)
18-30	5	8	13 (13%)
31-40	7	15	22 (22%)
41-50	10	22	32 (32%)
51-60	5	14	19 (19%)
>60	5	9	14 (14%)
Total	32	68	100 (100%)

Table 2. Gross Pathological Features.

Feature	Frequency (%)
Multiple Stones	84
Single Stone	16
Thickened Gallbladder Wall	68
Mucosal Erythema/Irregularity	52

mucosal erythema and irregularity were noted in 52% of the specimens.

Histopathological Findings

The histopathological examination of the 100 gallbladder specimens revealed a variety of changes associated with cholelithiasis. (Fig. 1) Chronic cholecystitis was the predominant finding, present in 90% of cases, followed by Rokitansky-Aschoff sinuses (RAS) in 40% (Fig. 1(b)), epithelial hyperplasia in 10% (Fig. 1(c)), and fibrosis or ulceration in 5% (Fig. 1(d)). Carcinoma was detected in 2% of the specimens (Fig. 1(e & f)).

Correlation of Histopathological Findings with Clinical Parameters

Age and Histopathological Changes:

- Chronic cholecystitis was observed in all age groups, showing a slightly higher

occurrence in patients between 41 and 60 years of age.

- Rokitansky-Aschoff sinuses were more commonly observed in older age groups (>50 years), suggesting a prolonged history of gallstone disease.
- Epithelial hyperplasia and carcinoma were observed primarily in patients above 50 years of age.

Gender and Histopathological Changes:

Both males and females showed similar patterns of chronic cholecystitis and Rokitansky-Aschoff sinuses. However, carcinoma was detected only in female patients.

Statistical Analysis: Chi-square analysis revealed a statistically significant correlation between age and the presence of

Table 3. Histopathological findings in Gallbladder specimens.

Histopathological Finding	Number of Cases	Frequency (%)
Chronic Cholecystitis	90	90%
Rokitansky-Aschoff Sinuses	40	40%
Epithelial Hyperplasia	10	10%
Fibrosis and Ulceration	5	5%
Carcinoma	2	2%

Table 4. Association Between Age and Histopathological Findings.

Age Group (Years)	Chronic Cholecystitis	RAS	Epithelial Hyperplasia	Carcinoma
18-30	12	4	0	0
31-40	21	8	2	0
41-50	30	14	4	0
51-60	17	10	3	1
>60	10	4	1	1

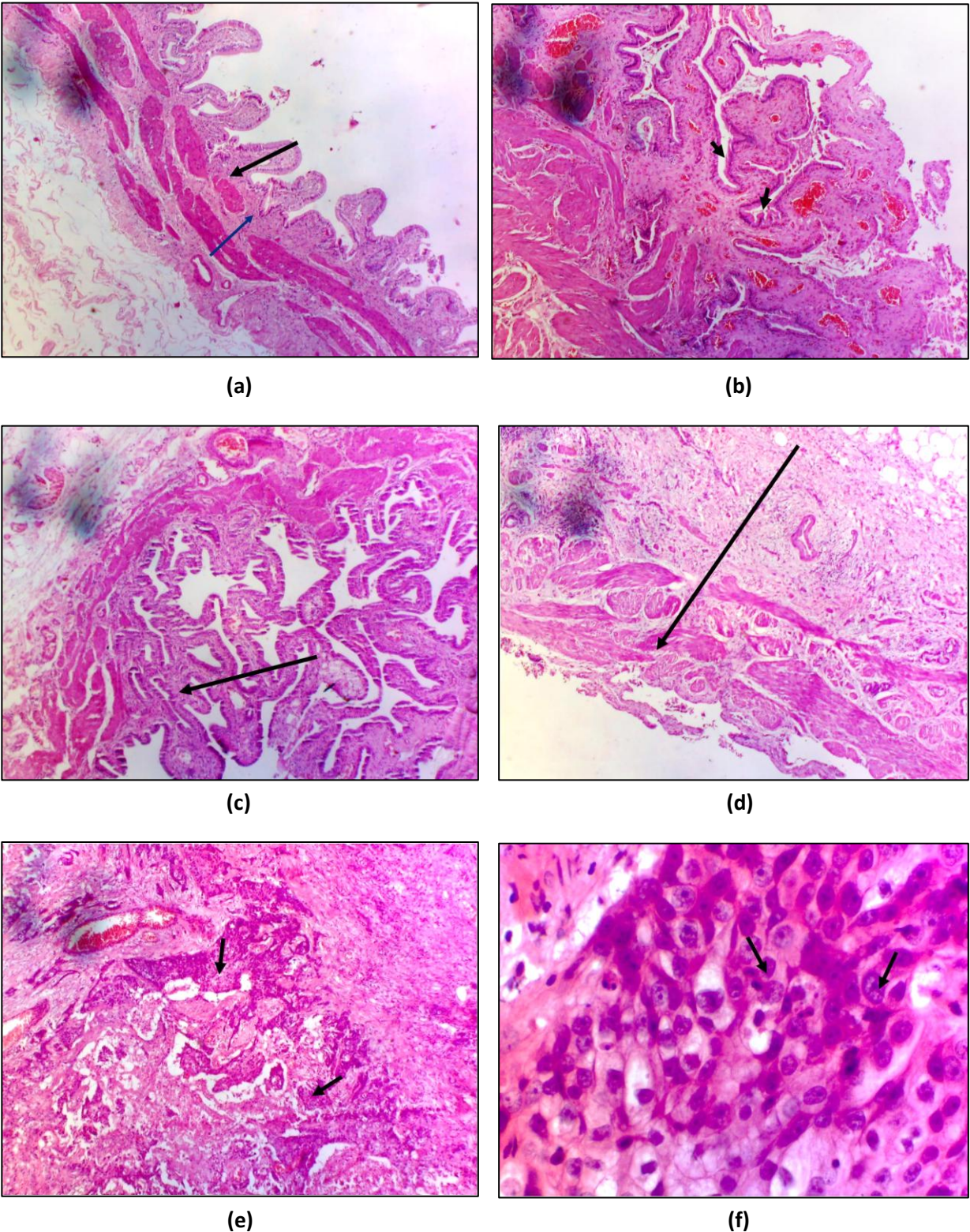


Fig. 1. Transverse section (10x) of human Gall bladder. (a) Normal; black arrow – mucosal layer; blue arrow – fibromuscular layer (b) Chronic cholecystitis with Rokitansky Aschoff sinuses (arrows) (c) Epithelial hyperplasia (d) Transmural fibrosis (e) Carcinoma (10x) (f) Carcinoma (40x)

Rokitansky-Aschoff sinuses ($p<0.05$), indicating a higher likelihood of RAS formation in older patients. The association between gender and carcinoma, though limited by the small number of cases, was also noted ($p<0.05$).

Summary of Key Findings

- 1. Chronic Cholecystitis was the predominant histopathological alteration, found in 90% of the cases, underscoring the chronic inflammatory process associated with gallstone disease.
- 2. Rokitansky-Aschoff Sinuses were seen in 40% of the specimens, suggesting long-standing irritation of the gallbladder wall.
- 3. Epithelial Hyperplasia was identified in 10% of cases, which could represent early precancerous changes.
- 4. Carcinoma was found in 2% of the specimens, highlighting the importance of routine histopathological examination for early detection of malignancy.

These findings indicate a strong association between gallstones and various histopathological alterations in the gallbladder mucosa, highlighting the importance of

thorough examination of all cholecystectomy specimens to detect or exclude early malignant changes.

DISCUSSION

Cholelithiasis, or gallstone disease, remains a significant health concern worldwide, particularly in regions where dietary habits, genetic predisposition, and other risk factors contribute to its high prevalence. The purpose of this study was to examine the histopathological alterations in the gallbladder mucosa linked to cholelithiasis and to understand their correlation with clinical parameters. Our findings are consistent with existing literature, which underscores the variety of histopathological alterations that can occur due to the chronic inflammatory effects of gallstones.

The most frequent histopathological change observed In this study was chronic cholecystitis which was observed in 90% of the cases. This aligns with previous reports indicating that chronic inflammation is the hallmark of gallstone disease, often resulting from prolonged mucosal irritation caused by the physical presence of stones [4,10].

Table 5: Comparison of Histopathological Findings with Previous Studies

Study	Chronic Cholecystitis (%)	RAS (%)	Hyperplasia (%)	Carcinoma (%)
Present Study	90	40	10	2
Zahrani & Mansoor ⁹	97	33.2	8	1.5
Mohan et al. ²⁰	88	38	12	3

Chronic cholecystitis is marked by the presence of lymphocyte and plasma cell infiltration, with varying degrees of fibrosis, which were also observed in our samples [11]. Studies suggest that persistent irritation can lead to a cycle of epithelial damage and repair, predisposing the mucosa to further alterations, such as hyperplasia and metaplasia [12].

Rokitansky-Aschoff sinuses were identified in 40% of the samples. These mucosal outpouchings are considered pseudodiverticula that form as a response to chronic inflammation and increased intraluminal pressure [6,13]. The formation of RAS is often associated with longstanding gallstone disease, and their presence was more common in older patients in our study, indicating a possible correlation with the duration of the disease process. Similar findings have been reported in previous studies, where the prevalence of RAS ranged between 30% and 45% among patients with cholelithiasis [14].

Epithelial hyperplasia was noted in 10% of the specimens, raising concerns about the risk of progression to dysplasia and malignancy. Hyperplasia, marked by a rise in the quantity of epithelial cells, can be an adaptive response to chronic irritation but may also serve as a precursor to neoplastic changes [15]. The transition from hyperplasia to dysplasia and eventually carcinoma has been

documented in the literature, suggesting a continuum of mucosal changes driven by chronic inflammation and cellular stress [16]. In regions with high gallstone prevalence, the incidence of gallbladder cancer has also been noted to be higher, emphasizing the need for vigilance in monitoring histopathological changes in cholecystectomy specimens [2].

The identification of carcinoma in 2% of the specimens underscores the importance of routine histopathological examination of all cholecystectomy specimens, even in the absence of pre-operative suspicion of malignancy. Gallbladder cancer is an uncommon yet highly aggressive tumor with a poor prognosis, frequently diagnosed at a late stage because of its vague symptoms and delayed clinical presentation [5,17]. Our findings underscore the need for early detection of malignancy, which can be facilitated by identifying precursor lesions including dysplasia and carcinoma in situ detected during routine examination [18]. The relationship between gallstone disease and gallbladder cancer has been well-documented, particularly in nations where gallstones are highly prevalent, such as India and Chile [6,7].

Our study found a higher prevalence of gallstone disease and associated histopathological alterations more commonly in females, showing a female-to-male ratio of 2.1:1, which aligns with worldwide data, which

suggests that hormonal factors, particularly estrogen, may play a role in gallstone formation and subsequent mucosal changes [5,19]. The increased risk among females may also be related to pregnancy, use of oral contraceptives, and obesity, all of which are known risk factors for gallstone disease [8,20]. Awareness of these gender-specific risk factors is crucial for early diagnosis and management.

The spectrum of histopathological changes observed in our study is in line with those reported by other researchers. Zahrani and Mansoor reported a similar distribution, with chronic cholecystitis being the predominant finding in their study, followed by RAS and hyperplasia [9]. Another study from North India highlighted a comparable prevalence of epithelial hyperplasia and carcinoma, particularly in older patients, suggesting a link between age and the risk of malignant transformation [20]. However, variations in the incidence of certain lesions, such as carcinoma, may be attributed to differences in sample size, geographic location, and patient demographics.

The primary limitation of this study was its relatively small sample size, which could restrict the applicability of the results. Furthermore, the research was carried out at a single institution, and thus, regional variations in the incidence of histopathological changes may not be fully represented. Further, more extensive multi-

center research with greater sample sizes is required to confirm these results and to examine the molecular processes driving the progression from benign to malignant changes.

CONCLUSION

This study highlights the spectrum of histopathological changes associated with cholelithiasis, with chronic cholecystitis being the most common finding. The identification of precursor lesions like epithelial hyperplasia highlights the crucial role of routine histopathological analysis of cholecystectomy specimens in detecting early neoplastic alterations. The correlation between gallstone disease and gallbladder carcinoma emphasizes the need for preventive strategies and heightened clinical awareness, particularly in high-risk populations.

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