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Gallstone Disease: Demographic Trends and Histopathological Correlations in Cholecystectomy Specimens

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ABSTRACT

OBJECTIVE: To analyze the histopathological spectrum of gallbladder lesions in cholecystectomy specimens associated with gallstone disease.

METHODOLOGY: The study included 270 cholecystectomy specimens received at the Histopathology Section, Pathology Department, Fazaia Ruth Pfau Medical College, Karachi. Patients of all ages and of both genders with clinical history and histology were included in this study between January 2019 and December 2024. Gallbladder samples with incomplete clinical history, insufficient maintenance, or damage to tissues were excluded. Gross and microscopic examinations of the gallbladder stone were performed to determine the number, color, wall thickness, mucosal construction, inflammation, cholesterolosis, lymphoid aggregates, neural fibrosis, polyps, intestinal metaplasia, dysplasia, or malignancy. Data were analyzed using SPSS version 23.0, and the chi-square test was used.

RESULTS: Gallstones were found in 86.3% and 57% of cholecystectomy specimens with multiple stones. Histopathological examination showed a high prevalence of chronic cholecystitis, and in 77% of cases, mild inflammation was observed. In 67.4% of specimens, glandular hyperplasia secondary to sustained pressure and wall thickening caused Rokitansky–Aschoff sinuses. In 12.2% of gallstone cases, intestinal metaplasia was observed, and only 1.1% had dysplasia and malignancy. A significant association was identified between gallstones and mucosal ulceration ($p = 0.029$).

CONCLUSION: Gallstone disease is more common in young females, with an earlier onset, and frequently results in multiple stones. To avoid chronic consequences and minimize the burden of the disease, the study highlights the significance of early detection and prompt action.

KEYWORDS: Gallbladder disease, gallstones, young adults, cholecystectomy, low-income setting

INTRODUCTION

Gallbladder diseases show a wide histopathological spectrum, ranging from benign and inflammatory lesions to pre-malignant and malignant conditions[1]. Worldwide, gallbladder pathology is most often encountered through cholecystectomy specimens, commonly performed for symptomatic gallstone disease. Chronic cholecystitis is the most frequent diagnosis, seen in about 75–82% of cases, followed by cholesterosis, acute cholecystitis, xanthogranulomatous cholecystitis, adenomatous hyperplasia, benign polyps, and a smaller proportion of dysplasia and other pre-malignant lesions. Histopathological examination plays a crucial role in recognizing both expected and incidental findings, which is particularly important in regions with a high burden of gallstone disease and limited access to advanced diagnostic resources [2].

The prevalence of cholelithiasis increases steadily with age, affecting 10–20% of adults in Western populations and more than 30% of older people, with rates in women reaching up to 57% by the age of 70[3]. Gallstone types also change with age: cholesterol stones predominate in younger adults, while pigment stones are more frequent in older individuals. Most patients have multiple stones (60–80%), which are linked to more severe disease and greater surgical risk, while single stones occur less often. Gallstones are significantly more common in women, with a female-to-male ratio between 2:1 and 4:1, especially during reproductive years when hormonal factors increase biliary cholesterol secretion. These demographic and clinical trends demonstrate that gallstones are both highly prevalent and clinically relevant across age groups4,5.

Persistent gallstones cause chronic mechanical and chemical irritation of the gallbladder mucosa. This leads to repeated inflammation, fibrosis, muscular hypertrophy, and thickening of the gallbladder wall, often greater than 3 mm on ultrasound. Histology of chronic cholecystitis almost invariably confirms these fibrotic and hypertrophic changes, which reflect long-standing mucosal injury. These alterations not only mark disease severity but also serve as important predictors of further complications[6].

Chronic inflammation and immune-mediated responses are central to gallbladder carcinogenesis, promoting dysplasia and progression toward malignancy[7]. Dysplasia has been documented in 2–5% of gallbladder specimens with stones, with risk increasing in older patients and in those with long-standing disease. This stepwise sequence from gallstone-related irritation to dysplasia and eventually carcinoma demonstrates a clear etiological link. The presence of large or multiple stones further amplifies the risk, with studies showing nearly a tenfold higher risk of carcinoma in patients with stones larger than 3 cm compared with smaller ones[8].

Gallbladder carcinoma is a very uncommon and highly aggressive cancer of the biliary tract. Many cases are discovered incidentally during histopathological examination of gallbladders removed for presumed benign disease, especially in patients operated on for symptomatic cholelithiasis. Reported frequencies of incidental carcinoma vary globally, ranging from 0.1% to 5.2% of cholecystectomy specimens, with South Asian studies consistently showing rates within this range. Most of these incidental cancers are adenocarcinomas, and a considerable proportion are detected at early stages, where surgical management offers the best outcome[9].

Taken together, these findings establish a strong rationale for routine histopathological examination of all cholecystectomy specimens. In regions with a high prevalence of gallstones, especially among women and older adults, the wide spectrum of pathology from benign to pre-malignant to malignant can only be reliably identified through histology. The rising occurrence of gallstones in younger age groups further strengthens the need for careful evaluation, as early disease may otherwise go unrecognized. This is particularly relevant in low-income settings,

where access to high-end imaging and molecular diagnostics is limited and laboratory-based histology often remains the only dependable diagnostic option. Routine, cost-effective histopathological analysis remains the most reliable strategy to detect dysplasia and early carcinoma, guide timely management, and ultimately reduce the burden of gallbladder cancer.

METHODOLOGY

Study Population

This retrospective study included 270 cholecystectomy specimens from patients aged 15 to 75 years. Data were collected from the Department of Pathology, Histopathology Section, Fazaia Ruth Pfau Medical College, Karachi.

Inclusion and Exclusion Criteria

Patients of all age groups and both genders were included in this study. All archived gallbladder specimens received during the study period (January 2019 to December 2024) were considered, provided they had complete clinical data and well-preserved histology. Specimens with inadequate preservation, autolysis, or damaged tissue, as well as those lacking complete clinical or demographic information, were excluded.

Ethical approval

The study was deemed exempt from full review by the institute's Ethics Review Committee of Fazaia Ruth Pfau Medical College (FRPMC-IRB-2024-62). The committee waived the informed consent requirement.

Tissue Processing and Histopathological Examination

Each sample was fixed in 10% buffered formalin, routinely processed, and embedded in paraffin. Sections of 3 to 5 microns were cut and stained with Hematoxylin and Eosin (H&E).

Gross and Microscopic Examination of Gallbladder Stone

All specimens were processed according to the routine histopathological protocol of the Pathology Department. As this was a retrospective study, the number of tissue sections examined per gallbladder was not (3) consistently available in the archived records.

For gross evaluation, the presence or absence of gallstones was recorded. Stones were further categorized based on number (single or multiple) and color. Microscopic examination was performed to assess gallbladder wall thickness, mucosal architecture (including mucosal depth and ulceration), and degree of inflammation, and the presence of cholesterolosis, lymphoid aggregates, neural fibrosis, Rokitansky-Aschoff Sinuses, polyps, intestinal metaplasia, dysplasia, or malignancy. Whenever suspicious areas were received, they were grossed, which is why we identified cases of dysplasia and malignancy.

Inflammation grades were obtained from the original histopathology reports. As this was a retrospective study, grading was based on the routine assessment of the reporting pathologists, and no additional standardized grading system was applied during data collection. Histopathological findings, including dysplasia, were extracted from the original pathology reports. Dysplasia was recorded as present or absent because grading into low- and high-grade dysplasia was not consistently available in the archived reports.

Statistical analysis

Data were analyzed using SPSS version 23.0 with descriptive statistics and inferential tests (chi-square). Statistical significance was set at $p<0.05$.

RESULTS

Over six years (2019-2024), 270 patients with biliary colic were admitted for cholecystectomy, and their gallbladder specimens were examined. Females constituted 73% (197/270) and males 27% (73/270), with a ratio of 3:1. The age of patients ranged from 15 to 75 years, with a mean age of 41 ± 12.059 years.

Based on physical examination of gallbladder specimens, 86.3% (233/270) of patients had gallstones, indicating cholestasis as a predominant cause of gallbladder pathology. Multiple gallstones were present in 57% (154/270) cases, which indicated repeated irritation, intermittent obstruction and recurrent inflammation. Among specimens with gallstones, yellow-colored stones were most common, accounting for 38.9% (105/270), followed by green-colored stones in 34.8% (94/270) of cases. Stone color was documented based of gross macroscopic examination. No compositional analysis was performed.

Histologically, mild inflammation was observed in 77% of cases, indicating strong evidence of chronic low-grade inflammation that may progress silently and damage the gallbladder wall. A high proportion of specimens showed mucosal ulceration (78.5%; 212/270) and mural fibrosis (78.9%; 213/270), both of which are signs of chronic inflammation and tissue remodeling. Rokitansky-Aschoff sinuses were identified in 67.4% (182/270) of the cholecystectomy specimens. Moreover, intestinal metaplasia was found in 12.2% (33/270) cases, which is commonly associated with adaptive per-neoplastic change due to chronic inflammation or irritation. Although rare, three cases of dysplasia and three cases of malignancy were also identified microscopically in 1.1 % (3/270) of cases (**Table I**).

Table I: Distribution of cases according to histological examination of gallbladder specimens

Variable		Stone Presentation	Percentage (%)
Gross Findings	Gallstones	Present	86.3% (233)
		Absent	13.7% (37)
	Number of Gallstones	Single	29.3% (79)
		Multiple	57.0% (154)
		No Stone	13.7% (37)
	Color of Stones	Yellow	38.9% (105)
		Green	34.8% (94)
		Mixed	12.6% (34)
		No Stones	13.7% (37)
Microscopic Findings	Degree of Inflammation	Mild	77.4% (209)
		Moderate	9.6% (26)
		Severe	13.0% (35)
	Mucosal Ulceration	Present	78.5% (212)
		Absent	21.5% (58)
	Lymphoid Aggregates	Present	53.0% (143)
		Absent	47.0% (127)
	Mural Fibrosis	Present	78.9% (213)
		Absent	21.1% (57)
	Rokitansky Aschoff Sinuses	Present	67.4% (182)
		Absent	32.6% (88)

	Intestinal Metaplasia	Present	12.2% (33)
		Absent	87.8% (237)
	Dysplasia	Present	1.1% (3)
		Absent	98.9% (267)
	Malignancy	Present	1.1% (3)
		Absent	98.9% (267)

A statistically significant association was found between gallstones and mucosal ulceration (χ^2 = 4.739, p = 0.029), indicating that chronic inflammation and constant irritation caused by gallstones may lead to mucosal injury and ulceration. However, no significant association was found between the presence of gallstones and malignancy (p = 0.488) or degree of inflammation (p = 0.492) (**Table II**).

Table II: Association between gallstones and histopathology

Variable		**Gallstones Present (n=233)**	**Gallstones Absent (n=37)**	**χ^2 (df) *p*-value**
Malignancy	Present	3	0	0.482
	Absent	230	37	0.488
Mucosal Ulceration	Present	188	24	4.739
	Absent	45	13	**0.029**
Degree of Inflammation	Mild	181	27	
	Moderate	24	3	1.419
	Severe	28	7	0.492

Although gallstones are common, progression to malignancy is rare and commonly depends on multiple risk factors such as disease duration and genetic predisposition. Similarly, the lack of association with the degree of inflammation may represent individual immune responses or variation in symptom duration. A strong association was observed between the degree of inflammation and mucosal ulceration (χ^2 = 11.688, p = 0.003) (**Table III**). This indicates that severe or prolonged inflammatory responses may directly lead to mucosal damage.

Table II: Association between the degree of inflammation and mucosal ulceration

Degree of Inflammation	**Mucosal Ulceration Present**	**Mucosal Ulceration Absent**	**χ^2 (df) p-value**
Mild	155	53	11.688
Moderate	22	5	**0.003**
Severe	35	0	

DISCUSSION

Gallbladder pathology is the result of complex interactions between chronic inflammation, biliary stasis, and metabolic imbalances[10]. Gallstones, especially those that are frequent or numerous, cause covert damage to the gallbladder wall over time by serving as both mechanical irritants and initiators of a chronic inflammatory cascade[11]. Cholesterol deposition, bacterial infection, and prolonged intraluminal pressure impact the progression of simple cholelithiasis to more complex histological features, such as fibrosis, mucosal ulceration, and even pre-neoplastic changes. These changes' early onset, particularly in younger populations, emphasizes that gallbladder disease is no longer limited to older age groups and can develop covertly in people with underlying risk factors like obesity and metabolic dysfunction.

The gender analysis showed female predominance in our study. This tallies with a population-based study involving 3,541 individuals in 2025, where gallstones were significantly associated with women. The study also indicated that metabolic markers such as LAP and CMI were associated only with females with gallstones, thus underlying a gender-specific metabolic risk[12]. These findings confirm that the higher prevalence in females is due to metabolic factors and hormonal influences, specifically the effect of estrogen on cholesterol metabolism and bile saturation, which increases the tendency toward gallstones. Our findings are in agreement with the regional and global epidemiological trends, where female gender and younger to middle age are significant risk factors for cholelithiasis. Cholelithiasis is diagnosed more frequently now than in previous decades due to the widespread availability and increased use of abdominal ultrasonography as a primary diagnostic modality. Pediatric cholelithiasis, though rare, especially in infants, is increasing in incidence, and its management remains inconsistent in the absence of standardized guidelines and evidence, with most literature focusing on surgical approaches.

The incidence of gallstone disease is increasing among younger populations. Most of the cases in our study were in the 15-35 years' age group, supported by findings of a study done in India where >51.66% of the gallstone cases occurred in the 20-40 years' age group. These studies clearly reflect the emergence of this disease in younger populations, possibly due to sedentary lifestyles, current diet, and growing obesity[13].

Stone characteristics further reflected disease chronicity; in our cohort, 57% of patients presented with multiple stones, suggesting that multifocal gallbladder calculi are a common presentation in this population. In another study, among patients experiencing cholecystitis with multiple gallbladder stones late in the cholecystitis disease process[14], an 8.8% recurrence risk was documented, indicating that multiple gallbladder stones may be implicated in recurrent episodes of inflammation, although recurrence is still relatively uncommon. The data regarding the prevalence of gallbladder stones in our study may be an indication of chronic cholecystitis, having a continuous state of biliary stasis, recurrent irritation of the gallbladder, and a protracted disease course. These findings support the physiologic premise that reactive inflammatory changes with persistent mucosal injury can lead to more stones in this disease state. This supports the importance of early recognition and treatment to reduce the risk of recurrent biliary events and avoid a long-term chronic inflammatory disease state. The high prevalence may be a sign of dietary habits, multi-pregnancy hormonal effects, obesity, a sedentary lifestyle, limited access to preventive health, and an overall lack of awareness or exposure to the disease. This warrants early identification and culturally appropriate, cost-effective preventive strategies to address the effects early.

The study was based on a relatively small sample size and relied on routine histopathological techniques without additional advanced diagnostic methods. Gallstone size was not routinely documented; therefore, its association with histopathological changes, including carcinoma risk, could not be evaluated. Gallstone characterization was based solely on gross macroscopic color, and no chemical compositional analysis was performed. Details regarding the sampling of grossly suspicious areas during histopathological processing were not consistently available in the archived records. The statistical analysis was limited to descriptive statistics and Chi-square tests; therefore, multivariable analysis, odds ratios, confidence intervals, and subgroup analyses by age and gender were not performed. Although a high prevalence of mucosal ulceration was observed, the exact reason for this finding could not be determined because of the study's retrospective nature.

CONCLUSION

Female predominance and an increasing occurrence of multiple gallstone disease in younger individuals suggest prolonged disease progression and recurrent gallbladder irritation, emphasizing the importance of early diagnosis and timely intervention to prevent chronic complications and reduce recurrence, which are implemented in low-income settings to reduce disease burden and prevent chronic complications.

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AUTHOR CONTRIBUTION

Memon S: Data collection, literature search, , manuscript drafting.
Bugti S: Study concept and design, data entry, manuscript editing.
Parween S: Data analysis**,** statistical review, data interpretation, literature search.
Naeem H: Data interpretation, interpretation of results, critical review of manuscript.
Shabana: Supervision of study process, review for intellectual content, final approval of manuscript.
Langhani SK: Study coordination, literature search, manuscript review

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