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Original Research Article

Biochemical Fingerprints of Gallstones and Their Mucosal Impact: A Prospective Study among Laparoscopic Cholecystectomy Patients

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Conflict of interest: Nil

Abstract:

Background: Gallstone disease is a common biliary disorder requiring laparoscopic cholecystectomy. Gallstones vary in biochemical composition, and prolonged mucosal irritation may produce inflammatory, metaplastic, dysplastic, or malignant changes in the gallbladder.

Aim: To assess the biochemical composition of gallstones and correlate it with gallbladder mucosal changes in patients undergoing laparoscopic cholecystectomy.

Methods: This prospective observational study was conducted at Vels Medical College, from November 2025 to April 2026. A total of 120 patients with ultrasonographically confirmed cholelithiasis undergoing laparoscopic cholecystectomy were included. Gallstones were analysed biochemically and classified as cholesterol, pigment, or mixed stones. Gallbladder specimens were subjected to histopathological examination. Associations were analysed using chi-square test.

Results: The mean age was 45.82 ± 12.64 years, and females constituted 68.3%. Multiple stones were observed in 71.7%. Mixed stones were commonest (50%), followed by cholesterol (29.2%) and pigment stones (20.8%). Chronic cholecystitis was the commonest mucosal change (51.7%). A significant association was found between stone composition and mucosal changes (χ^2=12.486, p=0.014).

Conclusion: Gallstone composition was significantly associated with gallbladder mucosal pathology, supporting combined biochemical and histopathological evaluation.

Keywords: Gallstones; Biochemical Composition; Gallbladder Mucosa; Chronic Cholecystitis; Laparoscopic Cholecystectomy.

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Introduction

Cholelithiasis is a common biliary disorder and remains one of the major indications for laparoscopic cholecystectomy (LC). Gallstones are broadly classified as cholesterol, pigment, and mixed stones, and their biochemical composition reflects underlying disturbances in cholesterol metabolism, bile acids, bilirubin, calcium salts, mucin, and gallbladder microbial activity [1, 2]. In addition to stone formation, prolonged contact between calculi and gallbladder mucosa may produce chronic inflammation, epithelial hyperplasia, cholesterolosis, metaplasia, dysplasia, or rarely carcinoma [3]. Therefore, analysis of stone composition along with histopathological evaluation of the gallbladder provides useful information on the pathogenesis, duration, and biological behaviour of gallstone disease. Recent studies also suggest that regional variation, bile composition, microbiota, and metabolic factors may influence whether stones are cholesterol-dominant, pigment-dominant, or mixed [1, 4]. The present study aimed to assess the biochemical composition of gallstones and to correlate it with gallbladder mucosal changes in patients undergoing LC.

Methods

This prospective observational study was conducted in the department of General Surgery, Vels Medical College from November 2025 to April 2026. Patients diagnosed with gallstones and scheduled for elective LC during the study period were included after obtaining written informed consent. The study population consisted of adult patients of either gender who had ultrasonographic evidence of cholelithiasis with or without features of chronic cholecystitis. Patients with common bile duct stones, obstructive jaundice, acute cholangitis, empyema gallbladder, gallbladder mass, previous biliary surgery, severe systemic illness unfit for surgery, or unwillingness to participate were excluded.

Relevant demographic details such as age, gender, residence, dietary pattern, body mass index, parity in female patients, comorbidities, clinical symptoms, duration of illness, and ultrasonographic findings were recorded using a predesigned proforma. Preoperative investigations including complete blood count, liver function tests, renal function tests, blood sugar, coagulation profile, viral markers, and anaesthetic fitness evaluation were performed as per institutional protocol before surgery.

All patients underwent standard four-port LC under general anaesthesia. Intraoperative findings such as gallbladder wall thickening, adhesions, mucocele, contracted gallbladder, number of stones, size of stones, colour of stones, and bile characteristics were noted. After removal of the gallbladder, the specimen was opened under aseptic precautions. Gallstones were carefully collected, washed with normal saline, dried, labelled, and preserved for biochemical analysis. The stones were classified grossly based on number, size, colour, surface appearance, consistency, and morphology. For biochemical analysis, stones were powdered using a sterile mortar and pestle. The powdered material was subjected to qualitative and/or semi-quantitative analysis for cholesterol, bilirubin, calcium, phosphate, oxalate, carbonate, and bile pigments using standard chemical methods available in the institutional laboratory. Based on the dominant biochemical component, stones were grouped as cholesterol stones, pigment stones, or mixed stones. The findings were entered in the study proforma and linked with the corresponding histopathological report.

The gallbladder tissue was fixed in 10% formalin and sent for histopathological examination. Representative sections were taken from the fundus, body, neck, and any grossly abnormal area. The sections were processed routinely, embedded in paraffin, cut into thin sections, stained with haematoxylin and eosin, and examined under light microscopy. Mucosal changes such as chronic cholecystitis, acute-on-chronic inflammation, cholesterolosis, epithelial hyperplasia, metaplasia, dysplasia, ulceration, fibrosis, Rokitansky–Aschoff sinuses, and incidental malignancy were recorded. Data were entered into Microsoft Excel and analysed using SPSS software. Categorical variables were expressed as frequencies and percentages, while continuous variables were expressed as mean ± standard deviation. The association between gallstone biochemical composition and gallbladder mucosal changes was assessed using chi-square test or Fisher's exact test where appropriate. $P<0.05$ was considered statistically significant.

Results:

Among 120 patients, the mean age was 45.82 ± 12.64 years, and female constituted 68.3% (Table 1). The highest number of cases was observed in the 41–50 years age group (Table 1). Multiple gallstones were more common 71.7% (86) (Table 2). On biochemical analysis, mixed stones were the commonest type (50%; 60), followed by cholesterol stones (29.2%; 35) and pigment stones in 20.8% (Table 2). Histopathological examination showed that chronic cholecystitis was the most frequent gallbladder mucosal change, observed in 51.7% of specimens (Table 3). Acute-on-chronic cholecystitis was noted in 15%, cholesterolosis in 11.7%, epithelial hyperplasia in 10%, intestinal metaplasia in 6.7%, dysplasia in 3.3%, and incidental carcinoma in 1.6% (Table 3). Mixed stones showed a higher association with chronic cholecystitis, whereas cholesterol stones were more frequently associated with cholesterolosis (Table 4). Pigment stones showed relatively higher proportions of metaplasia, dysplasia, and carcinoma (Table 4). The association between biochemical composition of gallstones and gallbladder mucosal changes was statistically significant ($\chi^2=12.486$, $P=0.014$) (Table 4).

Table 1: Distribution of study participants according to age and gender

Variable	Category	Number	%
Age group	21–30	14	11.7
	31–40	28	23.3
	41–50	36	30
	51–60	26	21.7
	>60	16	13.3
Gender	Male	38	31.7
	Female	82	68.3
Mean age	45.82 ± 12.64 years		

Table 2: Gross and biochemical characteristics of gallstones			
Variable	**Category**	**Number**	**%**
Number of stones	Single	34	28.3
	Multiple	86	71.7
Size of stone	<1 cm	42	35
	1–2 cm	56	46.7
	>2 cm	22	18.3
Gross colour	Yellowish	30	25
	Brownish	38	31.7
	Black	18	15
	Mixed colour	34	28.3
Biochemical type	Cholesterol stone	35	29.2
	Pigment stone	25	20.8
	Mixed stone	60	50

Table 3: Histopathological mucosal changes in gallbladder specimens		
Mucosal change	**Number**	**%**
Chronic cholecystitis	62	51.7
Acute-on-chronic cholecystitis	18	15
Cholesterolosis	14	11.7
Epithelial hyperplasia	12	10
Intestinal metaplasia	8	6.7
Dysplasia	4	3.3
Incidental carcinoma	2	1.6

Table 4: Association between gallstone composition and gallbladder mucosal changes			
Mucosal change	**Cholesterol stone (n=35)**	**Pigment stone (n=25)**	**Mixed stone (n=60)**
Chronic cholecystitis	14	10	38
Acute-on-chronic cholecystitis	4	5	9
Cholesterolosis	9	1	4
Epithelial hyperplasia	4	2	6
Intestinal metaplasia	2	4	2
Dysplasia / carcinoma	2	3	1

Discussion

In the present prospective study of 120 patients undergoing LC, gallstone disease showed a clear female predominance, with females constituting 68.3% of the study population, and the peak distribution was observed in the 41–50 years age group. This pattern supports the well-established epidemiological observation that cholelithiasis is more frequent among middle-aged women, probably due to the influence of oestrogen on hepatic cholesterol secretion, bile supersaturation, gallbladder hypomotility, and metabolic factors. In the current study, multiple stones were more common than single stones, accounting for 71.7% of cases. This finding is clinically important because multiple stones may reflect prolonged lithogenic bile exposure, repeated nucleation, gallbladder stasis, and chronic mucosal irritation. Previous studies have shown that gallstones differ not only in number and size but also in biochemical composition, and these differences may be related to age, sex, metabolic status, infection, bile composition, and regional dietary patterns [5, 6]. Therefore, gallstone disease should not be viewed merely as a mechanical disorder but as a clinicopathological condition involving complex interaction between bile chemistry, gallbladder motility, mucosal inflammation, microbiota, and host metabolic factors [2].

Mixed stones were the commonest biochemical type in the present study, accounting for 50% of cases, followed by cholesterol stones in 29.2% and pigment stones in 20.8%. The predominance of mixed stones is consistent with several studies from Asian and Indian populations, where gallstones frequently contain variable combinations of cholesterol, bilirubin, calcium salts, carbonate, phosphate, protein, and mucin rather than a single pure component [7]. Mixed stones may represent a transitional or composite form of lithogenesis in which cholesterol supersaturation, pigment precipitation, calcium deposition, and bacterial activity act together. Lee et al. detected bacterial DNA more commonly in mixed cholesterol and pigment stones than in pure cholesterol stones, supporting the concept that bacterial colonisation

and beta-glucuronidase activity may contribute to pigment precipitation and mixed stone formation [8]. Recent microbiome-based research also demonstrated differences in microbial species and host bile acid biosynthesis between cholesterol and pigment gallstone groups, indicating that gallstone composition may reflect both biochemical and microbial influences within the gallbladder environment [1]. Thus, the predominance of mixed stones in the present study suggests that gallstone formation in this population was probably multifactorial rather than due to isolated cholesterol supersaturation alone.

Histopathological examination in this study revealed chronic cholecystitis as the most common mucosal change, observed in 51.7% of specimens. This finding was expected because persistent gallstones produce repeated mechanical irritation, intermittent cystic duct obstruction, bile stasis, pressure changes, and inflammatory injury to the gallbladder mucosa. Similar prospective histopathological studies have reported chronic cholecystitis as the dominant gallbladder mucosal response in patients with cholelithiasis [3]. In the present study, acute-on-chronic cholecystitis was observed in 15% of cases, suggesting that some patients had superadded active inflammation over a background of chronic disease. Cholesterolosis was noted in 11.7% of cases and was more frequent among cholesterol stone patients. This may be explained by abnormal cholesterol handling in the gallbladder mucosa, lipid-laden macrophage deposition, and increased cholesterol ester accumulation in the lamina propria. Earlier biochemical and mucosal studies have shown that cholesterol stone disease is associated with cholesterol-rich bile, rapid nucleation, and altered gallbladder mucosal lipid metabolism [9, 10]. Hence, the occurrence of cholesterolosis along with cholesterol stones in the present study supports the link between bile cholesterol abnormalities and gallbladder mucosal lipid deposition.

Premalignant epithelial alterations were also observed in the present study, including epithelial hyperplasia in 10%, intestinal metaplasia in 6.7%, dysplasia in 3.3%, and incidental carcinoma in 1.6% of cases. Although these lesions were less common than chronic cholecystitis, they are clinically significant because they represent progressive mucosal adaptation to chronic irritation. Long-standing cholelithiasis may induce a sequence of chronic inflammation, epithelial hyperplasia, metaplasia, dysplasia, and carcinoma in susceptible patients [11]. Stancu et al. reported hyperplasia, metaplasia, dysplasia, and carcinoma in chronic cholecystitis specimens and supported the concept of a metaplasia–dysplasia–neoplasia sequence in gallbladder carcinogenesis [11]. Similarly, Segovia Lohse and Cuenca Torres described a stepwise association between metaplasia, dysplasia, and gallbladder carcinoma, particularly in chronically inflamed gallbladders [12]. In the current study, pigment stones showed relatively higher proportions of metaplasia, dysplasia, and carcinoma compared with cholesterol stones. This may be because pigment stones are often associated with calcium bilirubinate deposition, bacterial enzymatic activity, chronic infection, and prolonged mucosal exposure to irritant bile constituents [13]. Therefore, even when gallstones appear clinically benign, routine histopathological examination remains important to detect unsuspected premalignant or malignant lesions.

The association between gallstone biochemical composition and gallbladder mucosal changes was statistically significant in the present study (χ^2=12.486, p=0.014), indicating that stone composition may influence the type of mucosal response. Mixed stones showed a higher association with chronic cholecystitis, cholesterol stones were more often associated with cholesterolosis, and pigment stones showed relatively greater association with metaplasia, dysplasia, and carcinoma. These findings suggest that different biochemical components may produce different patterns of mucosal injury. Cholesterol-rich stones may mainly reflect metabolic lithogenesis and mucosal lipid deposition, whereas pigment-rich stones may indicate infective or inflammatory lithogenesis with greater epithelial stress. The detection of two incidental carcinoma cases also reinforces the value of careful grossing and histopathological evaluation of all cholecystectomy specimens. Studies on incidental gallbladder carcinoma have shown that malignancy may be missed clinically, radiologically, or intraoperatively and may be diagnosed only after microscopic examination [14, 15]. Therefore, combined biochemical analysis of stones and histopathological assessment of gallbladder mucosa provides a more complete understanding of gallstone disease. The present study highlights that gallstones are not uniform entities; their composition may help explain the severity and nature of gallbladder mucosal pathology and may support more detailed pathological reporting after LC.

Conclusion

The present study demonstrated that gallstone disease was more common among middle-aged females, with multiple stones being the predominant presentation. Mixed stones were the most frequent biochemical type, followed by cholesterol and pigment stones. Chronic cholecystitis was the commonest gallbladder mucosal change, while cholesterolosis, epithelial hyperplasia, intestinal metaplasia, dysplasia, and incidental carcinoma were also identified. A statistically significant association was observed between gallstone biochemical composition and mucosal pathology.

These findings suggest that biochemical analysis of gallstones, along with routine histopathological examination of gallbladder specimens, provides valuable insight into disease pathogenesis and helps detect clinically unsuspected premalignant or malignant changes.

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