

Histopathological Changes in Gallbladder Mucosa Associated with Cholelithiasis: A Prospective Study

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ABSTRACT

Background and Aims: Cholelithiasis is known to produce diverse histopathological changes in the gallbladder mucosa. In the present study, we aimed to find the correlation between various gallstone characteristics (i.e., number, size, and morphological type) with the type of mucosal response in gallbladder mucosa (i.e., inflammation, hyperplasia, metaplasia, and carcinoma). **Methods:** The present study was conducted prospectively on 100 patients undergoing cholecystectomy for symptomatic cholecystitis. Gallstones were assessed for various parameters, i.e., number, size, and morphological type. Gallbladder mucosa was subjected to histopathological examination. Sections were taken from body, fundus, and neck of gallbladder. **Results:** Of 100 cases, maximum type was of mixed stones (54%) and was multiple in number (46%). However, gallstone type and number are nonsignificant variables to produce precancerous lesions (i.e., hyperplasia and metaplasia). Statistically significant results were obtained while comparing the mucosal response with gallstone size ($P = 0.012$). **Conclusion:** As the gallstone size increases, the response in gallbladder mucosa changes from cholecystitis, hyperplasia, and metaplasia to carcinoma. Gallstone type and number are nonsignificant variables to produce precancerous lesions.

KEYWORDS: Cholelithiasis, gallbladder mucosa, histopathology

INTRODUCTION

Cholelithiasis has been described as a disease of civilization. It has been observed in Egyptian mummies dating as far back as 3400 B.C. It appears likely that Charaka (two centuries B.C.) and Sushruta (six centuries B.C.) from India were also familiar with this disease of the biliary tract.^[1]

Cholelithiasis is a worldwide medical problem, but the incidence rates show substantial geographical variation, with the lowest rates reported in African populations. In most cases, they do not cause symptoms, and only 10% and 20% will eventually become symptomatic within 5 years and 20 years of diagnosis, respectively.

Cholelithiasis produces diverse histopathological changes in gallbladder mucosa, namely acute inflammation, chronic inflammation, granulomatous

inflammation, hyperplasia, cholesterolosis, dysplasia, and carcinoma.^[2,3] When a patient with cholelithiasis becomes symptomatic, therapeutic intervention is necessary. The present study was planned to correlate various gallstone characteristics (number, size, and morphological type) with the type of mucosal response in gallbladder mucosa (inflammation, hyperplasia, metaplasia, and carcinoma).

METHODS

The present study was conducted on 100 patients undergoing cholecystectomy for symptomatic cholecystitis due to cholelithiasis, irrespective of

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How to cite this article: Singh A, Singh G, Kaur K, Goyal G, Saini G, Sharma D. Histopathological changes in gallbladder mucosa associated with cholelithiasis: A prospective study. *Niger J Surg* 2019;25:21-5.

Access this article online	
Quick Response Code: 	Website: www.nigerianjsurg.com
	DOI: 10.4103/njs.NJS_15_18

age or gender. The following features were noted on gross examination of the gallbladder: dimensions, wall thickness, and mucosa. The gallstones (received either in the specimen or the container) were also subjected to biochemical analysis. The tissue was properly sampled and processed by routine histological techniques for paraffin embedding and sectioning at 4 μ thickness. Histopathological diagnosis was established on hematoxylin and eosin staining of the sections. Four sections including entire wall were obtained: two from body and one each from fundus and neck of the gallbladder. Additional sections if deemed necessary were taken from abnormal mucosa.

Physical characteristics of the stones were noted as per the following parameters: (1) type: based on morphology; (2) number: single/double/multiple; and (3) size: average of two major diameters. In the event of multiple gallstones, the diameter of largest and smallest stone was recorded. Crushed stone crystals were subjected to biochemical analysis for qualitative presence of cholesterol, bile pigments, and mixed components by the method described by Oser.^[4]

The pattern of response in the gallbladder mucosa was studied with regard to number, size, and morphological type of the stone(s). The various morphological responses were then categorized into four broad categories – cholecystitis, hyperplasia, metaplasia, and carcinoma.

The data thus collected were systematically compiled and analyzed using analysis of variance for averages and Chi-square test for contingency tables and proportions. Statistical significance was considered when $P < 0.05$.

RESULTS

In the present study, of 100 cases, majority of the patients (29%) were in age group of 40–49 years [Table 1]. Of the total 100 cases studied, 85% were female and 15% were male patients. Male-to-female ratio was 1:5.7 [Table 2]. Gallbladder size was normal in 59%, enlarged in 27%, and fibrotic in 14% of the specimens [Table 2]. The average gallbladder wall thickness is also shown in Table 2.

Morphological types of gallstones among the 100 cases found were as follows: 54% had mixed stones, 30% combined, 9% pigment stones, and 7% cholesterol stones [Table 2]. Number of stones varied from a single calculus in 40%, double in 14%, and multiple in the remaining 46% cases [Table 2].

Table 1: Age group distribution (years)

Age group	Frequency (%)
<30	15 (15.0)
30-39	22 (22.0)
40-49	29 (29.0)
50-59	19 (19.0)
>60	15 (15.0)
Total	100 (100.0)

Table 2: Gender distribution and gallstones characteristics

Parameter	Observation
Gender distribution (male/female)	15/85
Number of stone (single/two/multiple stones)	40/14/46
Type of stone (mixed/combined/cholesterol/pigment stones)	54/30/7/9
Gallbladder size (normal/enlarged/fibrotic)	59/27/14
Gallbladder wall thickness (<3 mm/>3 mm)	56/44

Table 3: Distribution of subjects according to various mucosal responses

Mucosal response on histological examination	Frequency (%)
Chronic cholecystitis with cholelithiasis	79 (79.0)
Chronic cholecystitis with metaplasia and cholelithiasis	8 (8.0)
Adenomatous hyperplasia with cholelithiasis	3 (3.0)
Adenomyomatous hyperplasia with cholelithiasis	3 (3.0)
Acute on chronic cholecystitis with cholelithiasis	2 (2.0)
Xanthogranulomatous cholecystitis with cholelithiasis	2 (2.0)
Papillary carcinoma	2 (2.0)
Intestinal metaplasia and cholelithiasis	1 (1.0)
Total	100 (100.0)

All 100 cases underwent microscopic evaluation and were categorized histologically. In specimens with more than one mucosal response, the predominant pattern was used for categorization (one condition per case) [Table 3]. The majority of cases had chronic inflammation, of which chronic cholecystitis was predominant comprising 79%, followed by chronic cholecystitis with metaplasia in 8% cases [Table 3]. In 2% of cases, carcinoma was observed, while intestinal metaplasia was observed in 1% of cases. All the patients suffering from carcinoma were female [Table 3].

Association of mucosal response with gallstone type, gallstone number, gallbladder wall thickness, and gallstone size is shown in Tables 4-7.

Table 4: Association of mucosal response with gallstone type

Histological diagnosis	Gallstone type				Total	χ^2	P
	Mixed	Combined	Cholesterol	Pigmented			
Cholecystitis	45	24	7	7	83	2.463	0.982*
Hyperplasia	3	2	0	1	6		
Cholecystitis with metaplasia	5	3	0	1	9		
Carcinoma	1	1	0	0	2		
Total	54	30	7	9	100		

* $P>0.05$; not significant, $P<0.05$; significant**Table 5: Association of mucosal response with gallstone number**

Histological diagnosis	Gallstone number			Total	χ^2	P
	Single	Double	Multiple			
Cholecystitis	34	10	39	83	4.798	0.570*
Hyperplasia	3	2	1	6		
Cholecystitis with metaplasia	2	2	5	9		
Carcinoma	1	0	1	2		
Total	40	14	46	100		

* $P>0.05$; not significant, $P<0.05$; significant**Table 6: Association of mucosal response with gallbladder wall thickness**

Histological diagnosis	Gallbladder wall thickness		Total	χ^2	P
	<3 mm	>3 mm			
Cholecystitis	47	36	83	5.542	0.136*
Hyperplasia	2	4	6		
Cholecystitis with metaplasia	7	2	9		
Carcinoma	0	2	2		
Total	56	44	100		

* $P>0.05$; not significant, $P<0.05$; significant**Table 7: Association of mucosal response with gallstone size**

Histological diagnosis	Mean size of stone \pm SD (cm)	Test statistics	P
Cholecystitis	0.70 \pm 0.33	10.896	0.012*
Hyperplasia	1.42 \pm 1.03		
Cholecystitis with metaplasia	0.88 \pm 0.50		
Carcinoma	4.00 \pm 0.28		

* $P>0.05$; not significant, $P<0.05$; significant. SD: Standard deviation

DISCUSSION

The estimated prevalence of cholelithiasis in India has been reported between 2% and 29%. In India, this disease is seven times more common in North than in South India.^[5] The present study was conducted to evaluate 100 patients with cholelithiasis undergoing cholecystectomy with an aim to correlate various gallstone characteristics with morphological mucosal responses in the gallbladder.

The majority of the patients in this study were between the age group of 40 and 49 years, with a mean age of

44.1 years [Table 1]. Our results were in concordance with the results obtained by Khanna *et al.*,^[6] Tyagi *et al.*,^[7] and Singh *et al.*,^[8] who reported the mean age of 42.5, 43.6, and 45.3 years, respectively. Eighty-five percent of the patients we studied were female [Table 2]. Similar results have been reported in the studies of Mathur *et al.*^[1] and Mohan *et al.*,^[5] who reported that 86.97% and 86.54% of patients were female, respectively. The age and gender distribution of present as well as previous studies indicate that the incidence of cholelithiasis is higher in adult females. This may be due to decrease in activity of cholesterol reductase and increase in activity of HMG-CoA reductase with age, resulting in increased cholesterol secretion and saturation of bile. The female sex hormones may also expose them to factors that possibly promote the formation of gallstones.^[5]

In the present study, we observed that mixed stones were most common (54%) and the others were less frequent [Table 2]. Similar results have reported in the past in the studies of Mohan *et al.*^[5] and Baig *et al.*,^[9] i.e., 70% and 62.3%, respectively.

We also observed that multiple stones (46%) were more common than solitary or two stones, suggesting that symptomatic gallbladder disease is associated more frequently in patients with multiple gallstones in comparison to patients with solitary stones^[7] [Table 2]. The comparatively higher percentage of solitary stones (40%) in the present study might be due to increased incidence of combined stones in our study which occur predominantly solitary.

We found that average wall thickness of the gallbladder was normal (up to 3 mm) in 56% of the cases, while it was thickened in 44% of the cases in the present study [Table 2]. Khanna *et al.*^[6] reported thickened gallbladder wall in 57.5% of the cases.

Cholelithiasis represents one of the most frequent medical situations requiring surgical intervention. Frequently, chronic cholecystitis presents a large range of associated lesions such as cholesterosis, muscle hypertrophy, adenomatous proliferation of the mucous glands, metaplasia, hyperplasia, and dysplasia. The last three lesions are unanimously recognized as precursor lesions with cancerous potential.^[10]

We found chronic changes in the form of chronic cholecystitis and chronic cholecystitis with metaplasia being more common histological finding than other changes [Table 3]. Similar findings have been reported in the past who reported preponderance of chronic cholecystitis in gallstone patients in their studies.^[5,7,9]

Precancerous changes of gallbladder mucosa are of particular importance for both the clinical and pathological standpoints. Improved diagnostic procedures allow recognizing invasive carcinoma more frequently at early or resectable stage. However, precancerous conditions may be overlooked by a pathologist in the lack of vision of any correlation with gallstone disease.

We observed precancerous lesions (which included both hyperplasia and metaplasia) in 15% of cases [Table 3]. Similar results have been reported in the studies of Stancu *et al.*^[11] and Baig *et al.*,^[9] who have reported the prevalence of hyperplasia in 7.8 and 12.5% of cases, respectively. Mechanical irritation by the calculi could be the possible explanation for these changes as proposed by Elfving *et al.*^[12]

While trying to associate mucosal response with the type of gallstone [Table 4], it was found that precancerous lesions (hyperplasia and metaplasia) although clinically seem to be more common in mixed and combined type of stones as compared to cholesterol stone, this association could not reach statistical significance ($P = 0.982$) on

statistical analysis. Khanna *et al.*^[6] and Mathur *et al.*^[11] also reported similar findings in their studies.

Another interesting finding was that cholecystitis, hyperplasia, metaplasia, and carcinoma, i.e., all the lesions, were more common with multiple gallstones [Table 5]. However, on statistical analysis, nonsignificant results were obtained ($P = 0.570$). Mathur *et al.*^[11] also could not demonstrate any significant association between mucosal response and number of gallstones ($P > 0.05$).

Although we could not establish statistical correlation ($P = 0.136$) between precancerous conditions and gallbladder wall thickness, definitely wall thickness was >3 mm in patients with established carcinoma [Table 6]. Jung *et al.*^[13] and Bazoua *et al.*^[14] also reported increased gallbladder wall thickness in carcinoma cases. We also observed that wall thickness was least in cholecystitis cases, gradually increasing in metaplasia and hyperplasia cases, and abruptly increasing in carcinoma cases.

The size of gallstones impacting on the gallbladder wall is a strong indicator for the possible repeated mechanical irritation of gallbladder mucosa, larger size leading to an extreme filling of the gallbladder lumen volume and causing more irritation. This may cause mechanical damage during the normal contraction and relaxation cycles of the gallbladder that could then lead to further dysfunctional changes.^[15]

In this study, the average size of stone (s) was found to be maximum in cases with carcinoma (4.0 cm), followed by hyperplasia (1.42 cm), metaplasia (0.88 cm), and cholecystitis (0.70 cm). This correlation between average size of the stone and type of mucosal response was found to be statistically significant [$P = 0.012$, Table 7]. Thus, it indicates that the average size of gallstones in cases with carcinoma was significantly more as compared to inflammation and premalignant lesions. Lowenfels *et al.*^[16] reported that 40% of the patients with gallbladder carcinoma had stones that were more than 3 cm in size. Vitetta *et al.*^[15] and Hsing *et al.*^[17] have reported similar findings in their studies. However, case-control studies of Roa *et al.*^[18] and Moerman *et al.*^[19] found no relationship between size and gallbladder cancer.

Hence, it can be concluded that gallstones are accompanied by major changes in the gallbladder epithelium. This is mainly due to the large size stones leading to more irritation to the mucosa in addition to the toxic effect of the lithogenic bile which produces chemical injury to the mucosa. While the exact cause-and-effect relationship cannot be substantiated with the present study, but surely, constant erosion

of the gallbladder wall by gallstones over time constitutes an important risk factor for the development of gallbladder malignancy. Gallstone number and type are less important variables. The identification of premalignant modifications in the morphologic background of chronic cholecystitis is an argument in favor of metaplasia-dysplasia-neoplasia sequence. However, being a small population study, conclusions cannot be drawn and large multicenter study involving large population is desirable to confirm the findings of the present study.

CONCLUSION

We conclude that as the gallstone size increases, the reaction in the gallbladder mucosa changes from cholecystitis, hyperplasia, and metaplasia to carcinoma. Gallstone number and type are less important variables associated with these changes.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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