

Histomorphological Spectra of Gallbladder Specimens after Cholecystectomy in Benign Disease

Research Article

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Abstract

Background: The gallbladder is a surgically important organ, most often subjected to cholecystectomy for treating cholelithiasis. The presence of gallstones may precipitate the development of cholecystitis which may even lead to gallbladder malignancy, if not treated in time.

Aim: The aims were to study the diverse morphological, histopathological and histochemical features of gallbladder specimens obtained at cholecystectomy done for various benign diseases of the GB.

Material and methods: Included are 132 specimens of the gallbladder obtained at cholecystectomy done for benign diseases at Ganesh Shankar Vidyarthi Memorial Medical College, Kanpur, Uttar Pradesh, India. The specimens thus obtained were studied for the morphological and histopathological changes. Each specimen was subjected to Sudan Black B, Methyl Green-Pyronin Y and Periodic acid-Schiff staining to confirm presence of lipid, plasma cells and glycogen, respectively in GB tissue samples.

Results and Conclusion: Cholelithiasis was predominantly a female disease. The size of the GB varied from < 7 cm (shrunken) to > 10 cm (distended). Cholelithiasis was present in 97.73% of the cases studied. Of these, 89.14 % had mixed stones and 10.85%, cholesterol stones. Of the cases studied, 8.33% had acute cholecystitis alone. It was associated with mucocoele or empyema of the GB in 3.79% and 5.30% cases, respectively. Histopathological changes consistent with chronic cholecystitis and cholesterosis, respectively were seen in 76.52% and 6.06% cases. In the former patients, the presence of the plasma cells in the GB tissues was confirmed using MGPY staining. The presence of lipids in the GB tissue samples of the latter was confirmed using Sudan Black B staining. Cases of chronic cholecystitis with ectopic pancreatic tissue, colonic metaplasia and xanthogranuloma were also seen. Mixed atrophic-hyperplastic mucosa, being the commonest mucosal change was seen in 63.64% cases. Other mucosal changes included atrophic mucosa (in 21.21 % cases) and hyperplasia (in 15.15% cases). The GB tissue samples in 7.5% cases showed PAS-positive mucinous metaplasia.

The tissue samples obtained at cholecystectomy performed for various benign gallbladder diseases showed varieties of histomorphological changes, including metaplasia and precursor changes suggesting the high risk for gallbladder malignancy. This underscores the need for routine histopathological examination of appropriate tissue samples of the gallbladder obtained at cholecystectomy.

Keywords: Cholecystitis; Cholelithiasis; Empyema; Gallstone; Mucosa; Mucocoele.

Introduction

Disorders of the gallbladder are a common surgical condition throughout the world. Cholecystectomy is the mainstay of treatment for cholelithiasis and the laparoscopic cholecystectomy is the gold standard management of symptomatic cholelithiasis [1, 2]. Chronic cholecystitis is associated with cholelithiasis in > 90% of cases [3]. Although symptomatic cholelithiasis per

se is a benign condition, gallstones are a known risk factor for gallbladder malignancy (GBM) which has a very poor prognosis [2-4]. Cholelithiasis produces diverse histopathological changes in the gallbladder. These include acute or chronic inflammation, xanthogranulomatous cholecystitis, glandular hyperplasia, cholesterosis, and metaplasia (gastric or intestinal), dysplasia and carcinoma [3]. The incidence of the gallbladder cancer varies in different races and geographic regions [3, 5]. The incidence

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Received: July 05, 2016

Accepted: August 24, 2016

Published: August 31, 2016

Citation: Gaharwar A, Mishra SR, Kumar V (2016) Histomorphological Spectra of Gallbladder Specimens after Cholecystectomy in Benign Disease. *Int J Anat Appl Physiol*. 2(5), 49-56.
doi: <http://dx.doi.org/10.19070/2572-7451-160008>

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is relatively high in North India, Pakistan, East Asia, Eastern Europe, and South America [6, 7]. In India, there is a distinct female (2.3 versus 1.01) dominance in the incidence (per 100,000 population) of GBM [8, 9].

Untreated cholelithiasis is a potentially serious condition as it may lead to life-threatening complications. The study presented herein aimed at identifying various morphological, histopathological and histochemical changes as might be found in tissue samples obtained at cholecystectomy done for various benign diseases of the GB.

Materials and Methods

The study was the product of a collaborative work involving the Department of Anatomy, the Department of General Surgery and the Department of Pathology, Ganesh Shankar Vidyarthi Memorial Medical College, Kanpur, Uttar Pradesh, India. It was a retrospective, observational study examining 132 tissue samples of the GB obtained at cholecystectomy performed at the Department of Surgery. Included were the tissue samples of the GB obtained from only those patients (121 females and 11 males) diagnosed having benign GB disease. Not included were the tissue samples of the GB obtained from those having GB malignancy

The tissue samples of the GB were fixed in 10 per cent formalin. Prior to this, the gross appearance of the GB, presence or absence of any calculus and the color of any calculus, if present was noted. Then specimens were sectioned serially at the fundus, body and neck. Routine processing of tissues sections and staining with haematoxylin and eosin were done and examined. The histopathological examination of mucosa of the GB was done with special care. Special staining with Sudan Black B, Methyl-Green Pyronin Y stain and Periodic acid - Schiff (PAS) stain was also done in all cases for the demonstration of lipids, plasma cells and glycogen, respectively.

Results

Of the total 132 patients underwent cholecystectomy, 121 were females and 11, males. Hundred and four (78.8%) GBs were 7-10 cm, 21 (15.9%), < 7cm and 7 (5.3%), > 10 cm in size. Calculi were found in 129 of 132 (97.73%) of GBs removed (Figure 1). Concomitant mucocele and empyema of the GB, respectively was found in 5 and 7 cases (Figure 2). No stone was found in 3(2.27%) cases. This subset included two males and one female. Mixed stones and cholesterol type stones (Figure 3) were present

in 106 (89.14%) and 14 (10.85%) specimens, respectively. Pigment stones were not seen in any specimen (Table 1). PorcelainGB with the calcified wall was found in 1 case (Figure 4).

Histopathological features of chronic cholecystitis (Figure 5) with or without associated cholesterosis were seen in 101(76.52%) and 8 (6.06%) specimens, respectively. Acute cholecystitis alone was found in 11 (8.33%) cases (Table 2). Five (3.79%) cases of acute cholecystitis had associated mucocele of the GB. Seven (5.30%) had associated empyema of the GB.

When present, the chronic inflammatory infiltrate was characterized by the presence of scattered lymphocytes, plasma cells and macrophages. The marked plasma cell infiltration in the inflammatory infiltrates of chronic cholecystitis was confirmed using MGPY staining (Figure 6), which showed (Table 3) high (in 5 cases), moderate (in 120 cases), and mild (in 7 cases) degree of positivity for plasma cell infiltration. Sub-epithelial and sub-serosal fibrosis and Rokitansky-Aschoff sinuses were also prominent (Figure 7). A case of xanthogranulomatous cholecystitis was seen in which abundant lipid-laden macrophages and exuberant tissue fibrosis were present (Figure 8). Colonic metaplasia was seen with PAS staining (Figure 9). A rare case of compressed ectopic pancreatic tissue with chronic cholecystitis was also seen in the study (Figure 10). The histochemical confirmation of cholesterosis (Table 3, Figure 11) was based on the presence of sub-mucosal deposition of lipids and cholesterol (Sudan Black B staining).

Hyperplastic GB mucosa was found in 20 (15.15%), atrophic mucosa, in 28 (12.21%) cases and mixed atrophic- hyperplastic mucosa, in 84 (63.64%) cases (Table 2, Figures 12, 13). Mucinous metaplasia (PAS-positive) was found in 10 (7.5%) cases; PAS staining was negative in 122 (92.4%) cases (Table 3, Figure 14). Histological features consistent with malignancy were uniformly absent in all specimens examined.

Discussion

Cholelithiasis (gallstone disease) is the most common surgical condition affecting the biliary tract [10]. In the present study, females outnumbered males by a ratio of 11 to 1, a pattern similar to what has been noted by others [3]. This may not be surprising, considering the lithogenic effects of female sex hormones and the presence of estrogen and progesterone receptors in the GB [9-11].

Figure 1. Cholecystitis with cholelithiasis showing bile stained mucosa & haemorrhagic areas with marked irregular thickening of wall.



Figure 2. Emphyema of gall bladder with adhesions on surface.



Figure 3. Showing multiple multifaceted stones both intact and cut section mixed pigment and cholesterol content.



Table 1. Morphological findings of the gallbladder specimens examined (n=132).

Findings	Number (%) of cases
Length of the GB (in cm)	
< 7	21 (15.9)
7-10	104 (78.8)
> 10	7 (5.3)
Status of cholelithiasis	
Calculi	129 (97.73)
No calculus	3 (2.27)
Types of calculi	
Mixed	106 (89.39)
Cholesterol	14 (10.61)
Pigment	0 (0.0)

Figure 4. Calcified Porcelain gall bladder with multiple multifaceted stone.



Figure 5. Showing Chronic Cholecystitis with marked lymphocytes.

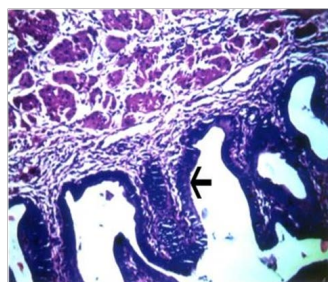


Table 2. Histopathological features of the gallbladder specimens examined (n=132).

Features	Number (%) of cases
Cholecystitis	
Chronic	101(76.52)
Acute	11(8.33)
Acute with mucocoele of the GB	5(3.79)
Acute with empyema of the GB	7(5.30)
Cholesterolosis	8(6.06)
Mucosa	
Hyperplastic	20(15.15)
Atrophic	28(21.21)
Both	84(63.64)

Figure 6. Showing marked plasma cell infiltration in inflammmtory infiltrate of chronic cholecystitis MGPY staining

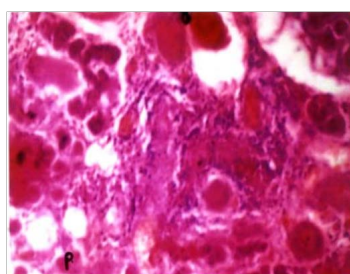


Table 3. Results of histochemical studies of the gallbladders obtained at cholecystectomy (n=132).

Stains used	Target	Number (%) of positive cases	Number (%) of negativcases
Sudan Black B	Lipids	8(6.06)	124(93.93)
MGPY	Plasma cells		
	Mild (1+)	7(5.3)	0(0.0)
	Moderate (2+)	120(90.9)	0(0.0)
	High (3+)	5(3.78)	0(0.0)
PAS	Glycogen	10(7.5)	122(92.4)

Abbreviations: MGPY, Methyl Green -Pyronin Y stain; PAS, Periodic acid-Schiff stain.

The GB was found fully relaxed and distended (measuring > 10 cm in length) in 7 (5.3%) cases. It was normal measuring 7- 10 cm in length [12-15] and shrunken measuring less than 7 cm in length in 104(78.8%) and 21 (15.9%) cases, respectively. Thus the functional status of the GB showed a varying range.

Mixed stones and cholesterol type stones were present in 106 (89.14%) and 14 (10.85%) specimens, respectively. Pigment

stone was not found in any case. Our findings support those of others [11, 16]. Considering the cases in aggregates, stones were found in the GB in 129 of the 132 (97.7%) specimens examined (Figures 1-2). Associated mucocoele and empyema of the GB was found in 5 (3.79%) and 7 (5.33%) cases, respectively (Figure 3). Only 3(2.27%) specimens, taken from two males and one female patient, had no stone. We have found a rare case of porcelain GB with the dystrophic calcification of the GB wall. Of note, many

Figure 7. Rokitansky - Aschoff sinuses (Hematoxylin and eosin, $\times 200$).

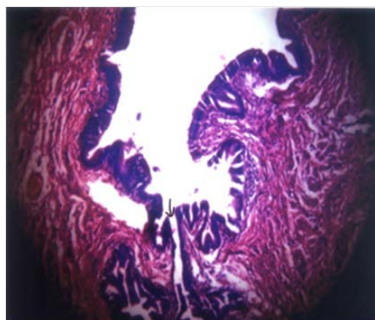


Figure 8. Showing Xanthogranuloma formation in the muscle layer H & E ($\times 450$).

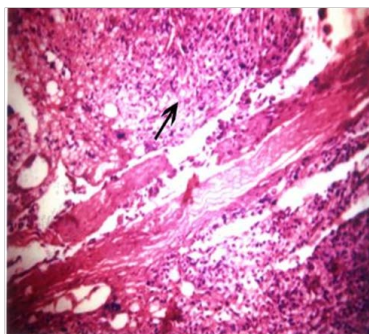


Figure 9. Showing Colonic Metaplasia in chronic cholecystitis – PAS staining ($\times 450$).

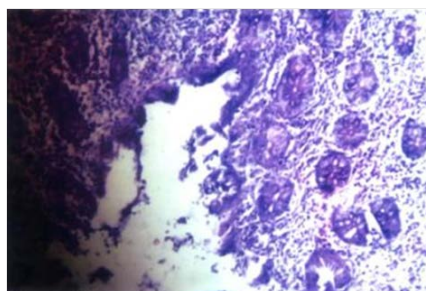


Figure 10. Showing compressed ectopic pancreatic tissue in gall bladder in cholecystitis.

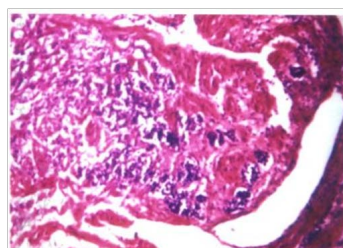


Figure 11. Showing Sudan Black B staining showing diffuse faint positivity in cholesterosis with focal areas of intense positivity ($\times 450$).

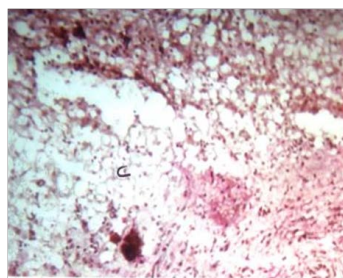


Figure 12. Showing Chronic Cholecystitis with hyperplastic mucosal pattern.

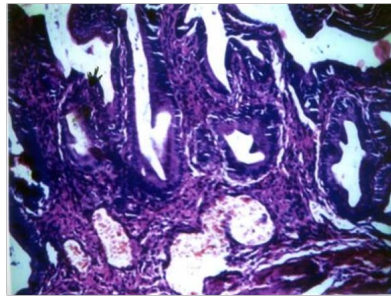


Figure 13. Showing Chronic Cholecystitis with atrophic mucosal pattern (H.E. (x450)).

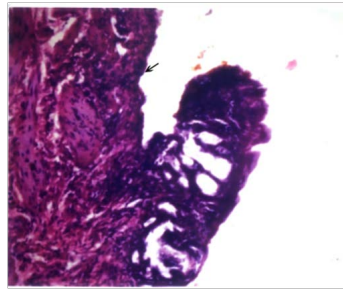
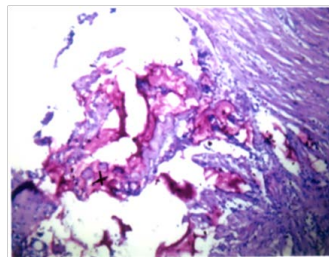


Figure 14. Showing PAS positive areas in mucinous metaplasia.



consider [17] porcelain GB a pre-malignant state. The diversities of the gross appearance of the GB found in the current study bear resemblance to the patterns reported by others [17-19]. In those reports, 49.7% of the GB specimens obtained at cholecystectomy were < 7 cm, 36.46%, 7-10 cm and 9.89%, > 10 cm in length.

We found histologically-proven acute cholecystitis in 18 (13.63%) cases; 5 cases (3.79%) had acute cholecystitis associated with mucocele of the GB. In total, 101 (76.52%) specimens showed histological features of chronic cholecystitis (Figure 5); chronic cholecystitis associated with cholesterosis was found in 8 (6.06%) specimens. In one study [18], the majority (75%) of the post-cholecystectomy GBs examined showed histological evidence of chronic cholecystitis. Relatively fewer cases [18] showed features of empyema (1.04%) and cholesterosis (12.25%) of the GB.

Acute cholecystitis is a rare variant of cholecystitis and is characterized histologically by the presence of edema, hyperemia, extravasations of red blood cells and prominent fibroblastic proliferation. The process may be initiated by chemical irritations or bacteria-induced inflammation [20] probably triggered by the obstruction in the neck of the GB or the cystic duct by stones or edema of the surrounding tissues [2].

Mucocele of the GB has an incidence of 3 per cent [21]. In this study, 5(3.79%) cases of mucocele of the GB presented with features clinically simulating those of acute cholecystitis. All had

an over-distended GB (>10 cm) filled by mucous. Histologic features were remarkable for flattened mucosa of the GB lined by low columnar or cuboidal cells, numerous RASs and inflammatory infiltrates.

In the current study, empyema of the GB was seen in 7(5.33%) cases (Figure 3). The key pathogenic factor is the unresolved cholecystitis induced by gallstones coupled with the bacterial contamination of the bile [22]. The other key factor is the bacterial contamination of the mucous accumulated in the GB [21]. The most frequently isolated bacteria species in such settings include *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus faecalis* and anaerobe (e.g., *Bacteroides* and *Clostridia species*). The resulting suppurative inflammation packs the GB with purulent debris. The incidence of the surgically-proven (i.e., detected at cholecystectomy) empyema of the GB varies widely from 2.4 to 11% [23, 24]. Empyema of gall bladder is often difficult to distinguish from uncomplicated acute cholecystitis [25].

Repeated bouts of mild to severe acute cholecystitis may result in chronic cholecystitis. In nine out of ten cases, it is associated with cholelithiasis [26]. In the current study, none of the GB tissue samples examined showed any microscopic feature that could be considered normal. In one series [27], histologically normal GBs were found in less than one per cent of cases, when cholecystectomy was done for cholelithiasis. These data suggest that as claimed before, cholelithiasis was the commonest

indication for cholecystectomy [11] (Figure 1). In one study, histologic evidence of chronic cholecystitis was found in more than half (50.8%) of the cholecystectomy specimens examined [28]. In another study [18], microscopic features of chronic cholecystitis were found in 64.8% of the GBs examined.

A distinctive feature of the GB is the presence of RASs, deep out-pouchings of mucosa which may extend through muscular layer [29]. The number of RASs is closely related to the degree of inflammation of the GB [27] as in chronic cholecystitis (Figure 5). Included in our series is a rare case of xanthogranulomatous cholecystitis in which gall bladder was shrunken, nodular and chronically inflamed with foci of necrosis and haemorrhages. The condition arises due to the rupture of RASs. This is a benign condition, though may be confused macroscopically with GBM because it infiltrates surrounding structures like the liver, the small and large intestine and the omentum [19, 30].

Cholesterosis of the GB is the deposition of lipids and cholesterol esters in sub-epithelial cells, which project into lumen as polyps [26]. It affects mostly multiparous females [31]. In the current study, cholesterosis was seen in 8 (6.06%) cases. All were females. Others found cholesterosis in 2.7-12.3% of the GBs removed for benign diseases affecting the organ [18, 27].

The lumen of the GB is lined by columnar epithelium with microvilli [32]. Chronic irritations of the GB mucosa due to stones may lead to atypical cellular changes and hyperplasia, which may eventually progress to metaplasia and carcinoma-in-situ [33]. In the current study, the mucosal changes seen in the GB include mucosal hyperplasia in 20 (15.15%), mucosal atrophy in 28 (21.21%) and mixed atrophic variety in 84(63.3%) cases. Others found mucosal hyperplasia in as much as 25.3 % of the GBs examined [18]. One study reported epithelial hyperplasia having been found in 69% and antral metaplasia, in 16.5% of cases [30].

In the current study, mucinous metaplasia (i.e., PAS-positive colonic metaplasia with intra-cytoplasmic mucin) which many regard suggestive of borderline malignancy of the GB was in seen in 10 (7.5%) cases. We, as well as others [35], have found that ectopic pancreatic tissues in the GB associated with chronic cholecystitis due to cholelithiasis a rare occurrence. Metaplasia of the GB mucosa induced by chronic cholecystitis is a pre-malignant condition. Although lacking confirmation, the pathogenesis of the mucosal dysplasia of the GB seen in chronic inflammation may involve loss of the heterozygosity of *p53* gene and excessive expression of the gene [35].

Conclusion

Within the constraints of small sample size, we conclude that cholelithiasis was the commonest underlying reason for cholecystectomy in majority of cases of benign diseases of the gallbladder. The morphological appearance of the gallbladder varied from a shrunken, normal or distended organ with focal haemorrhages, sometimes filled with mucus or pus. The histopathological changes were equally diverse, showing features of acute and chronic cholecystitis, cholesterosis, xanthogranuloma, dystrophic calcification, hyperplasia and metaplasia. Irrespective of the gross appearance and seemingly benign nature of the disease affecting the organ, every

gallbladder removed by cholecystectomy should be subjected to histopathological examinations.

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