

Original Research Article

Morphological spectrum of gall bladder lesions and their correlation with cholelithiasis

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ABSTRACT

Background: Gall stones are known to produce diversified histological changes in the gallbladder such as inflammation, metaplasia, hyperplasia, dysplasia and even precursor lesions of malignancy. The aim of our study was to correlate the various histological changes in the gallbladder with the different types of gallstones (cholesterol, pigmented and mixed).

Methods: This prospective study was conducted in the department of Pathology, Indira Gandhi Medical College, Shimla, Himachal Pradesh from July 2014 to June 2015. The age, sex distribution and the incidence of different types of gallstones was studied. The histological changes in the gallbladders were examined and its correlation with the type of gallstones was evaluated.

Results: In all 1371 specimens of gall bladder were received during this period. The mean age of the patients was 44 years (age range 13-86 years) with Male: Female ratio of 1: 8.3. Out of the total 1371 cholecystectomy specimens, 1259 (91.8 %) had calculi and 112 (8.2%) were acalculus. Of the 1259 cases of calculus disease, mixed stones were observed in 840 (66.7%) patients, cholesterol stones in 289 (22.9%) and pigmented stones in 130 (10.4%) patients. Overall 1291 (94%) cases had chronic cholecystitis followed by acute cholecystitis and chronic active cholecystitis in 2% each, dysplasia in 1.5% and carcinoma cases 0.5%.

Conclusions: Routine cholecystectomy performed for a common condition like gallstone disease can result in detection of diverse and wide spectrum of histopathological lesions ranging from chronic cholecystitis to carcinoma.

Keywords: Gall bladder, Cholelithiasis, Histopathological changes

INTRODUCTION

Cholelithiasis is a chronic recurrent disease of the hepatobiliary system. The impaired metabolism of cholesterol, bile acids and bilirubin are thought to be responsible for gallstone formation.¹

Gall stones are a major cause of morbidity and mortality throughout the world.² The incidence of cholecystitis and cholelithiasis has increased over the past couple of decades throughout the world and is attributed to the

increased intake of fatty, high calorie diet and increased consumption of alcohol.^{3,4}

The prevalence of gall stone disease varies with age, sex, ethnic groups and geographical areas. The female prevalence of gall bladder disease in the American Indians and Pima Indians is as high as 60%-70%, Chile 37% while in UK and in the USA it is 6% to 12% and 11% respectively. China and Japan have a very low prevalence rate of 5%.⁵ The estimated prevalence of gall stone disease in India was 2-29% with marked

differences in North and South India. It is seven times more common in North than in South India.^{6,7}

Gall stones vary in their composition, majority being cholesterol and remaining are either mixed or pigmented stones. Composition and type of gall stones also vary region wise. The cholesterol stones are more common in the Western world while the pigmented stones are more common in the Asian and the African countries. Pigment stones are believed to be associated with infection and parasitic infestation.⁸ Most of the gallstones in North India are cholesterol stones (80%) while in South India they are pigmented (60%).⁹ Morphologically cholesterol stones are single, large, oval, and yellowish. In comparison, pigmented stones are multiple, small, blackish while mixed stones, though multiple, are multifaceted and of variable size.¹⁰ Cholelithiasis is known to produce changes in the gall bladder mucosa ranging from acute cholecystitis, chronic cholecystitis, polyp, granulomatous cholecystitis, empyema, eosinophilic cholecystitis, metaplasia, hyperplasia, dysplasia to carcinoma.¹¹

The study was conducted with the objective to determine the morphologic spectrum of gall bladder lesions and to evaluate its correlation with cholelithiasis in patients undergoing cholecystectomy.

METHODS

This prospective study was conducted in the Department of Pathology, Indira Gandhi Medical College, Shimla, Himachal Pradesh, India from July 2014 to June 2015 after getting approval from institutional ethics committee. All the resected specimens, 1371 by open or laposcopic cholecystectomy, sent to the department of pathology were included in the study.

Gall bladder specimens were subjected to routine pathologic (gross and microscopic) examination. The stones were assessed for their morphological type. Three

sections, one each from the body, fundus and neck were taken. Sections were stained with hematoxylin and eosin in autostainer (Tissue-Tek DRS, Sakura).¹² The histological changes in all the layers of the gallbladder such as hyperplasia, lymphoid follicles, Rokitansky Aschoff sinuses, muscular hypertrophy, pyloric gland metaplasia and intestinal metaplasia were observed and noted. The various histological changes observed in the gallbladder were analysed in correlation with the type of gallstones.

Statistical analysis

Results were summarized in tables and percentages. Quantitative data was summarized using means & standard deviation by using MS Excel 2007. Cross tabulation with outcome variable of interest was done using statistical software Epi-info version 7 (7.1.1.0). A p-value of less than 0.05 was considered statistically significant. Sensitivity, specificity, positive predictive value, negative predictive value of the tests was calculated using standard statistical formulas.

RESULTS

Table 1 presents the age and sex distribution of patients of cholecystectomy cases. Out of the total 1371 cholecystectomy specimens, 1259 (91.8%) had calculi and 112 (8.2%) were acalculus. Of the 1259 cases of calculus cholecystitis, 130 (10.3%) were males and 1129 (89.7%) females. Majority, 872 (69.3%) of these were in the 31 to 60 years' age group.

Out of the 112 acalculus cholecystitis cases, 95 (84.8%) were females and 17 (15.2%) males. Majority of these 80 (71%) were in 31 to 60 years' age group. All cases were subjected to microscopy and were categorized as per their microscopy pattern. Of the total 1259 cases with gall stones, acute cholecystitis was seen in 24 (1.8%) cases, chronic cholecystitis was observed in 1182 (93.8%) cases.

Table 1: Age and sex wise distribution of calculus and acalculus diseases.

Age group (year)	Calculus			Acalculus								
	Male n=130	%	Female n=1129	%	Total n=1259	Male n=17	%	Female n=95	%	Total n=112	%	
11-20	10	7.7	36	3.3	46	3.7	1	5.9	0	0	1	0.9
21-30	11	8.4	166	14.7	177	14.0	1	5.9	14	14.8	15	13.4
31-40	27	20.7	288	25.5	315	25.0	5	29.4	27	28.4	32	28.8
41-50	35	26.9	288	25.5	323	25.6	5	29.4	24	25.3	29	25.7
51-60	22	20.7	212	18.7	234	18.6	1	5.8	18	18.9	19	16.7
61-70	16	12.3	98	8.7	114	9.0	4	23.5	09	9.5	13	11.7
71-80	8	6.15	34	3.0	42	3.4	0	0	03	3.1	3	2.8
81-90	1	0.7	7	0.6	8	0.7	0	0	0	0	0	00
Total	130	100	1129	100	1259	100	17	100	95	100	112	100

Out of 1182 cases of calculus chronic cholecystitis, chronic non-specific cholecystitis was seen in 602 (50.9%), cholesterolosis 180 (15.2%), metaplasia 186 (15.7%) and hyperplasia in 91 (7.7%) specimens. Chronic active cholecystitis was observed in 24 (1.9%) of calculus chronic cholecystitis. Polyps were observed in two (0.2%) cases. Dysplasia was seen in 19 (1.5%) cases. A single case each of adenoma and tuberculosis was found

during the study. Carcinoma comprised 7 cases (0.6%) of calculus disease as presented in Table 2. Of the 112 cases without calculi, chronic cholecystitis was seen in 109 (97.3%) cases. Of these, chronic nonspecific cholecystitis was seen in 54 (49.5%), cholesterolosis 23 (21.1%) and metaplasia in 15 (13.8%) cases. There was only one case of chronic active cholecystitis and two cases of dysplasia in this group as shown in Table 2.

Table 2: Frequency of histopathological alterations in calculus and acalculus disease.

Lesion	Calculus		Acalculus		Total	
	n=1259	%	n=112	%	n=1371	%
Acute cholecystitis	24	1.9	0	0	24	1.8
Chronic cholecystitis	1182	93.8	109	97.3	1291	94.2
Chronic active cholecystitis	24	1.9	1	0.9	25	1.8
Polyp	2	0.2	0	0	2	0.1
Adenoma	1	0.1	0	0	1	0.1
Dysplasia	19	1.5	2	1.8	21	1.5
Carcinoma	7	0.6	0	0	7	0.5
Total	1259	100	112	100	1371	100

Out of 1291 total cases of chronic cholecystitis, chronic nonspecific cholecystitis was observed in 656 cases, chronic cholecystitis with cholesterolosis in 203 cases, chronic cholecystitis with metaplasia in 201 cases,

hyperplasia in 96 cases, cholesterolosis and metaplasia in 24, cholesterolosis and hyperplasia in 64, xanthogranulomatous in 37, follicular cholecystitis in 7 and eosinophilic cholecystitis, tuberculosis and porcelain each in one case as given in Table 3.

Table 3: Epithelial and stromal alterations associated with chronic cholecystitis (n=1291).

Lesion	Calculus diseases		Acalculus diseases		Total	
	n=1182	%	n=109	%	n=1291	%
Chronic nonspecific cholecystitis	602	50.9%	54	49.5%	656	50.8%
Chronic cholecystitis with Cholesterolosis	180	15.2%	23	21.1%	203	15.7%
Chronic cholecystitis with metaplasia	186	15.7%	15	13.8%	201	15.5%
Pyloric metaplasia	160		13			
Intestinal metaplasia	26		2			
Chronic cholecystitis with hyperplasia	91	7.7%	5	4.6%	96	7.4%
Spongoid hyperplasia	72		4			
Adenomatous hyperplasia	13		1			
Primary papillary hyperplasia	6		0			
Chronic cholecystitis with cholesterolosis and metaplasia	23	2.0%	1	0.9%	24	1.9%
Chronic cholecystitis with Cholesterolosis and hyperplasia	57	4.8%	7	6.4%	64	5.0%
Xanthogranulomatous	33	2.8%	4	3.7%	37	2.8%
Eosinophilic cholecystitis	1	0.1%	0	0	1	0.1%
Follicular cholecystitis	7	0.6%	0	0	7	0.5%
Tuberculosis	1	0.1%	0	0	1	0.1%
Porcelain	1	0.1%	0	0	1	0.1%
Total	1182	100	109	100	1291	100

Out of 1259 cases of calculus disease, mixed stones were observed in 840 (66.7%) patients, cholesterol stones in 289 (22.9%) and pigmented stones in 130 (10.4%) patients. From the results shown in Table 4 it was evident that in both genders mixed stones were the common type. Table 5 presents the correlation of histopathological

alterations with types of gall stones. From the results, it was observed that, in the cases of three categories of types of stones (mixed, n=840; cholesterol, n= 289; pigmented, n= 130) majority of the cases were of chronic cholecystitis (787; 279; 116) respectively.

Table 4: Gender wise distribution of types of stones (n =1259).

Type of stone	Male	%	Female	%	Total	(%)
Mixed	85	6.7	755	59.9	840	66.7
Cholesterol	35	2.8	254	20.1	289	22.9
Pigmented	10	0.8	120	9.6	130	10.4

Table 5: Frequency of histopathological alterations in relation to types of stones (n=1259).

Lesion	Mixed (n=840)		Cholesterol (n=289)		Pigmented (n=130)	
		%		%		%
Acute cholecystitis	18	2.1	2	0.7	4	3.1
Chronic cholecystitis	787	93.7	279	96.6	116	89.2
Chronic active cholecystitis	17	2.0	4	1.3	3	2.3
Polyp	1	0.1	1	0.3	0	0
Adenoma	1	0.1	0	0	0	0
Dysplasia	13	1.6	3	1.1	3	2.3
Carcinoma	3	0.4	0	0	4	3.1

Table 6 demonstrates the epithelial and stromal alterations in chronic cholecystitis and its correlation with the types of stones. Chronic nonspecific cholecystitis was commonly observed in majority of cases of mixed stones, 409 (51.9%), cholesterol stones, 138 (49.5%) and with

pigmented stones in 55 (47.4%) cases. Out of the 1371 gall bladders, Rokitansky Aschoff sinuses were seen in 330 (24.0%) specimens. Majority of these, 314 (95%) had cholelithiasis. Rokitansky Aschoff sinuses had significant association with gall stones (odds ratio 1.99) Table 7.

Table 6: Epithelial and stromal alterations in chronic cholecystitis and their correlation with the types of stones.

Lesion	Mixed stone n=787		Cholesterol stone n=279		Pigmented stone n=116	
		(%)		(%)		(%)
Chronic nonspecific cholecystitis	409	51.9%	138	49.5%	55	47.4%
Chronic cholecystitis with Cholesterolosis	108	13.7%	55	19.7%	17	14.6%
Chronic cholecystitis with metaplasia	124	15.7%	37	13.2%	25	21.5%
Pyloric metaplasia	111		31		18	
Intestinal metaplasia	13		6		7	
Chronic cholecystitis with hyperplasia	62	7.8%	23	8.2%	6	5.2%
Spongoid hyperplasia	49		20		3	
Adenomyomatous hyperplasia	8		3		2	
Primary papillary hyperplasia	5		0		1	
Chronic cholecystitis with cholesterolosis and metaplasia	16	2.0%	4	1.6%	3	2.6%
Chronic cholecystitis with cholesterolosis and hyperplasia	35	4.4%	13	4.7%	9	7.8%
Xanthogranulomatous cholecystitis	25	3.2%	7	2.3%	1	0.9%
Follicular cholecystitis	5	0.7%	2	0.9%	0	0
Eosinophilic cholecystitis	1	0.2%	0	0	0	0
Tuberculosis	1	0.2%	0	0	0	0
Porcelain	1	0.2%	0	0	0	0
Total	787	100%	279	100%	116	100%

Table 7: Gender wise distribution of Rokitansky Aschoff Sinuses and their relation to calculi (n=330).

	Sex		Total (%)
	Male	Female	
Calculus	19	295	314 (95.1%)
Acalculus	3	13	16 (4.9%)
Total	22	308	330 (100)

Dysplastic changes were seen in 21 (1.5%) cholecystectomy specimens. Predominant (61.9%) stones

associated with dysplasia were mixed type as given in Table 8.

Table 8: Correlation of dysplasia with types of stone (n=21).

Lesion	Acalculus		Calculus		Cholesterol		Pigmented	
	n=2	%	n=13	%	n=3	%	n=3	%
Dysplasia	2	10	13	62	3	14	3	14

Out of total 1371 cholecystectomy specimens, seven cases of carcinoma were incidentally detected which comprised 0.6% of all gall bladder lesions.

All the patients with carcinoma were females in the age group of 40 to 80 years. Carcinoma was associated with either pigmented or mixed stone comprising of 4 (57.1%) and 3 (42.9%) cases respectively. Cholesterol stones were not associated with carcinoma Table 9.

Table 9: Correlation of gall bladder carcinoma with age and types of stones (n=7).

Lesion	Calculus					
	Mixed		Cholesterol		Pigmented	
	n=3	%	n=0	%	n=4	%
Carcinoma	3	42.9	0	0	4	57.1

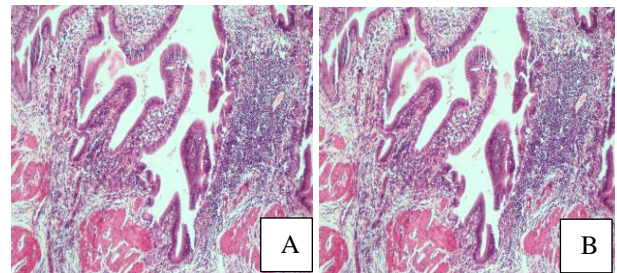


Figure 2: Chronic cholecystitis and chronic cholecystitis with spongoid hyperplasia (H&E staining).

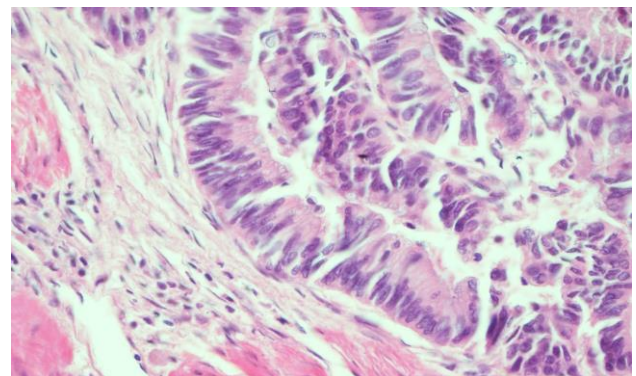


Figure 3: Well differentiated carcinoma (H&E staining 1000X).



Figure 1: Type of stones in gall bladder; (A) cholesterol stone; (B) mixed stone.

DISCUSSION

Gall stone diseases are the major cause of morbidity and mortality throughout the world. The prevalence of the disease varies with age, sex, geographic area and the ethnic groups. The trend of the disease has changed in the

last couple of decades due to the change in the dietary habits, migration of people and environmental insults.¹³

In our study, the mean age of the patients was 44 years. We observed maximum (50.9%) number of patients in 31-50 years age group, equally distributed in each decade. This was in concordance with the studies of Kaur et al in a total of 384 patients in which 196 (52%) were of age group between 31-50 years.¹⁴ However in study by Thamilselvi et al maximum number of patients were in 51 to 60 years of age which is higher in comparison to our study.¹⁰

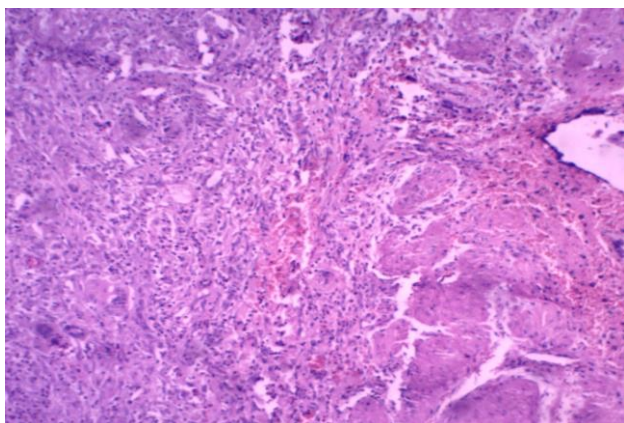


Figure 4: Xanthogranulomatous cholecystitis (H&E staining 400 X).

In this study, we observed female predominance i.e. male: female was 1:8.3 that is similar with the previous studies.^{13,15,16} Inter play of female sex hormones (progesterone and estrogens) and its metabolic effects along with the sedentary lifestyle is said to be responsible for the high incidence of gall stones in females.⁹

Gall stones are generally responsible for various forms of cholecystitis and associated lesions i.e. hyperplasia, metaplasia, dysplasia and carcinoma of the gallbladder.¹⁷ However, in 5 % to 10% of the cases, cholecystitis occurs without calculus.¹⁸ In India, the incidence of calculus disease and its relationship with chronic cholecystitis varies from North to South India. We observed nearly similar frequency of chronic calculus cholecystitis as seen in other North Indian studies by Mohan et al and Goyal et al.^{13,19}

Depending upon the colour, shape and size, the gall stones are classified into mixed (brownish yellow), cholesterol (yellow and white) and pigmented stones (dark brown and black).¹⁵ In the present study, mixed stones were the most common type. This was correlated with the previous studies by Mohan et al, Mathur et al and Gopal Krishnan et al.^{13,16,20}

The various lesions noted with gall stones include acute cholecystitis, chronic cholecystitis with associated epithelial and stromal alterations, chronic active

cholecystitis, dysplasia and carcinoma.¹⁹ In present study, 1.8% of gall bladder lesions were due to acute cholecystitis which is quite close to the figures in the study by Thamilselvi et al.¹⁰

Chronic cholecystitis is associated with cholelithiasis in more than 90 % of the cases. In the present study, we observed chronic cholecystitis in 94.2% cases which is concordant with the observations made by Stanchu et al.²¹ In this study in majority of specimens, cholesterosis was found to be associated with cholesterol stones followed by mixed stones. These observations are similar to those mentioned by Mohan et al.¹³ Incidence of xanthogranulomatous cholecystitis and its association with mixed stones in the present study was similar to those by Mathur et al and Goyal et al.^{16,19}

Frequency of follicular cholecystitis in the present study was found to be 0.5% which is similar to the observation made by Rizvi et al.²² The association of follicular cholecystitis with mixed stones was seen in 71% cases while it was found to be 100% in the study by Goyal et al.¹⁹

Pyloric metaplasia which is frequently seen with cholelithiasis and acts as precursor lesions for dysplasia and carcinoma of gall bladder was seen in 86% cases in the present study and is concordant with Stanchu et al.^{14,21} In the present study, metaplastic changes were commonly associated with mixed stones was seen in 61.1% which is similar to the observations by Mathur et al (66%).¹⁶

Cholelithiasis can induce the epithelium to undergo hyperplastic changes. It could be in the form of primary papillary, spongoid or adenomyomatous hyperplasia. These are the precursor lesions of carcinoma of the gall bladder. In our study, we observed hyperplasia in 7.4% cases which was comparable to the observations made by Mathur et al 8.0% and Stanchu et al 7.8%.^{16,21} On further breakup papillary and spongoid hyperplasia was seen in 73.3% and 26.7% respectively in a study by Stanchu et al while in the present study it was 6% and 79% respectively.¹⁶

Rokitansky Aschoff sinuses which are pseudodiverticula composed of deeply invaginating mucosa without a complete muscle coat were found in 24% cases but were associated with stones commonly (95%). Dysplasia among cholecystectomy specimens varies from 0.40 to 8.5% in various published studies and our observations of 1.5% were in accordance with other studies in literature.^{14,15,21,23.}

Frequency of Incidental detection of gallbladder carcinoma was 0.6% in our study which is lower in comparison to studies by Mathur et al, Mohan et al, Thamilselvi et al, Goyal et al and Rizvi et al.^{16,13,10,19,22} However our observations are closer to Stanchu et al, Kaur et al and Vani et al.^{21,14,23} In concordance with Mohan et al we found pigmented stones to be more

commonly associated with carcinoma. While studies by Vani et al and Rizvi et al had found mixed stones more commonly associated with gall bladder carcinoma.^{22,23}

CONCLUSION

The histopathological spectrum of gallbladder lesions in gallstone disease included chronic cholecystitis and associated variety of mucosal alterations and lesions like cholesterosis, metaplasia, dysplasia and carcinoma. Commonest stones were mixed type and were more frequently associated with premalignant lesions. Frequency of incidental gallbladder carcinoma detection was 0.6% and these were more commonly associated with pigment type of stones. Carefully planned studies to learn the etiopathogenesis of cholelithiasis will go a long way in preventing gallstone formation and its complications.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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