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Original Article

Pathology Section

Histomorphological Study of Acalculus and Calculus Gall Bladder in a Tertiary Care Hospital of Western Uttar Pradesh

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ABSTRACT

Introduction: Gall bladder is the most common surgically resected specimen as gallstones are one of the major causes of morbidity. There is increasing incidence of gall stones in India and more so in North India. It can be due to changes in life style such as fat rich food and sedentary life style. Stones can produce a wide variety of mucosal changes ranging from inflammatory, metaplastic, dysplastic and neoplastic lesions.

Aim: This study was performed to find out the spectrum of histopathological lesions in gall bladder in cholecystectomy specimens with or without stones.

Materials and Methods: This was a hospital based cross-sectional study conducted on 415 cholecystectomy specimens received between 1st November 2019 to 31st October 2020 in the department of Pathology of NCR Institute of Medical Sciences, Meerut. The specimens were received in 10% formalin, the gross details of specimens and stones were studied. Sections were taken from the representative areas, processed and stained with Haematoxylin and Eosin stain. These sections were studied meticulously. Demographic data was expressed as percentages. The p-value was calculated by chi-square test.

Results: Of the 415 cholecystectomy specimens 337 were from females and 78 were from males with female to male ratio of 4.3:1. The most common histopathological finding was chronic cholecystitis followed by cholesterolosis observed in 206/231 (89.17%) and 28/31 (90.32%) respectively in gall bladders with stones and in 25/231 (10.82%) and 3/31 (9.36%) of gall bladders without stones. Metaplasia and dysplasia was found in 81/83 (97.59%) and 15/16 (93.75%) of cases with stones, and in 2/83 (2.40%) and 1/16 (6.25%) cases without stones.

Conclusion: Cholelithiasis produces diverse mucosal alterations some of which are precancerous and can progress to cancer. So, histopathological examination of all the gall bladders is essential for the detection of premalignant and malignant conditions.

Keywords: Gall bladder, Cholelithiasis, Cholecystitis, Dysplasia, Cancer

INTRODUCTION

The gallbladder is among the most commonly surgically resected organ for stones in majority of cases. The number of cholecystectomies has increased by more than 50% in the last decade [1-3]. Gallstones are a major cause of morbidity and mortality throughout the world. The irritation caused by stones produces changes in cell differentiation, thereby resulting in an adaptive response to this aggression, i.e., the gastric and intestinal metaplasia [4,5].

Cholecystitis associated with cholelithiasis is a common disease found in resected gall bladders which is associated with fertile and obese females in their 4th and 5th decades of life, but can affect male and children also. This condition has increased in the past two decades, both in the Western World and India, due to increased intake of high calorie diet, fatty meals and alcohol [6]. Frequently, chronic cholecystitis presents a large range of associated lesions such as cholesterolosis, muscle hypertrophy, parietal fibrosis, polypoid and adenomatous proliferation of mucous glands [7].

Several morphologic variants of chronic cholecystitis have been described such as Follicular cholecystitis, diffuse lymphoplasmacytic cholecystitis [8], eosinophilic cholecystitis, Xanthogranulomatous cholecystitis, cholecystic granuloma [9,10] and ceroid granuloma.

Gallstones mainly injure the mucosal columnar epithelium and this causes changes like metaplasia, dysplasia and neoplasia. Cholecystectomy is a most common surgical procedure in the western part of Uttar Pradesh but this work has not been published from the Nalpur area of Meerut to the best of our knowledge. So the study was performed with the aim to find out the spectrum of histopathological lesions in gall bladder in cholecystectomy specimens with and without stone. The objectives of the study were age and sex distribution of patients with various lesions in cholecystectomy specimens, study of the histomorphological spectrum in calculus and acalculus cholecystectomy specimens, association of dysplasia and carcinoma with morphological types of stones in gall bladder specimens, prevalence of non neoplastic and neoplastic conditions in gall bladder with or without stones.

MATERIALS AND METHODS

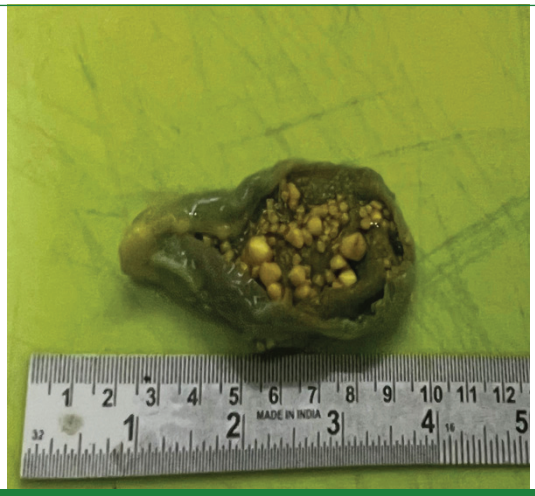
The present hospital based retrospective study was carried out in the Department of Pathology, NCR Institute of Medical Sciences, Nalpur, Meerut. Total 415 cholecystectomy specimens received from 1st November 2019 to 31st October 2020 were studied after taking institutional ethical clearance IEC013/NCRIMS011/Patho001-OP.

Inclusion criteria: All the cholecystectomy specimens received in department of pathology from 1st November 2019 to 31st October 2020.

Exclusion criteria: Cholecystectomy specimens which were inadequate or autolysed and without complete medical records were excluded from the study.

Methodology: The specimens were collected in 10% formalin and fixed for 24 hours. Demographic data was noted from the files of pathology department. Gross features like size of gall bladder, wall thickness, mucosal changes were noted. If stones were present their number, colour and size was noted [Table/Fig-1]. Then full thickness sections were taken from fundus, body and neck of gall bladder after observing the gross morphological details of all specimens and stones, if present. Additional sections were taken from abnormal appearing mucosa. Sections were stained with haematoxylin and

eosin stain after the processing. The stained slides were studied thoroughly to observe the changes in mucosa.



[Table/Fig-1]: Gross specimen showing multiple mixed stones.

STATISTICAL ANALYSIS

Demographic data collected was expressed as percentages. Data was analysed using IBM SPSS trial version software. Chi-square test was applied to find out p-value. Results were considered statistically significant when p<0.05.

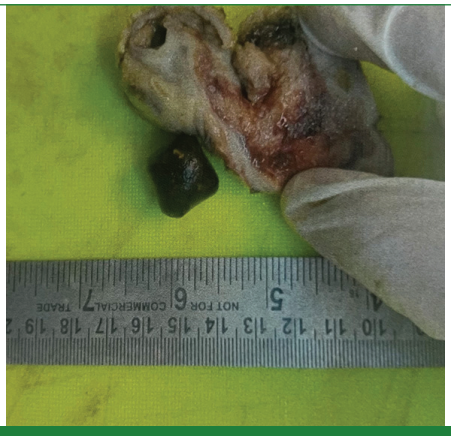
RESULTS

Age and Sex-wise distribution of cases: The age range of these cases was from 14 years to 75 years. Maximum number of cases was in 4th decade followed by 5th decade [Table/Fig-2]. Majority of females were in 4th decade while males were in 7th decade. There was a female preponderance with 337/415 cases (81.20%) while 78/415 cases (18.80%) were male. Thus, female to male ratio was 4.3:1.

	Female		Male		
Age group	No. of cases	%	No. of cases	%	Total
11-20	07	77.77	02	22.22	09
21-30	85	90.42	09	09.57	94
31-40	95	84.07	18	15.92	113
41-50	88	84.61	16	15.38	104
51-60	52	83.70	10	16.12	62
>61	10	30.30	23	69.70	33
Total	337	81.20	78	18.80	415

[Table/Fig-2]: Age and sex-wise distribution of 415 cases.

Gall bladder stone: Gall stones were present in 372/415 (89.63%) cases. Mixed stones were found in 204/372 (54.84%), cholesterol stones in 91/372 (24.46%) and pigment stones 77/372 (20.70%). Stones were multiple in 327/372 (88.02%) [Table/Fig-1] and single in 45/372 (11.98%) cases [Table/Fig-3].

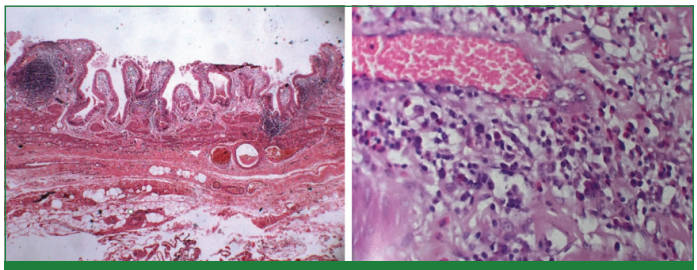


[Table/Fig-3]: Gross specimen showing thickened gall bladder wall with single stone.

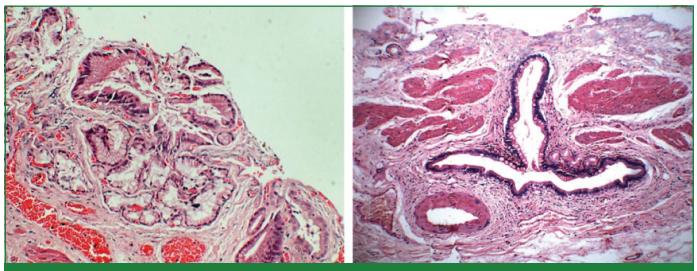
Gross changes: Out of 415 gall bladder, 202 (48.67%) were normal in size (7-10cm), 189 (45.55%) were contracted <7 cm and distended gall bladders >10 cm were 24 (5.78%). On cut stones were present in 372 cases (89.63%) and were absent in 43 cases (10.36%). Wall thickness varied from 0.2 cm to 1.2 cm. Gross mucosal changes in gall bladder with stone were normal mucosa in 59 (90.77%), atrophy 95 (86.36%), hypertrophy 109 (84.50%), cholesterolosis 31 (100%), congestion 67 (89.33%) and ulceroproliferative growth was seen in 3 (60.00%) cases.

Mucosal changes in gall bladder without stone were hypertrophic mucosa in 20 cases (15.50%) followed by atrophic mucosa in 15 (13.64%) and normal mucosa in 6 (9.23%) cases. Ulceroproliferative growth was seen in 2 (40.00%) cases.

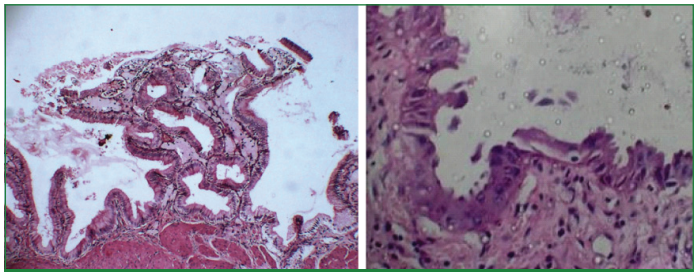
Association of histomorphological lesions with gallstones: Most common histomorphological lesion associated with gall stones was chronic cholecystitis [Table/Fig-4,5] in 206 out of total 231 cases (89.17%)followed by metaplasia [Table/Fig-6,7] in 81/83 cases (97.59%), cholesterolosis [Table/Fig-8] 28/31 cases (90.32%), chronic active cholecystitis 23/28 cases (23%), dysplasia in 15/16 cases (93.75%) cases [Table/Fig-9,10] and carcinoma [Table/Fig-11] 03/05 cases (60%). Chi-square test was applied to find out p-value. It was observed that histopathological changes in gall bladder



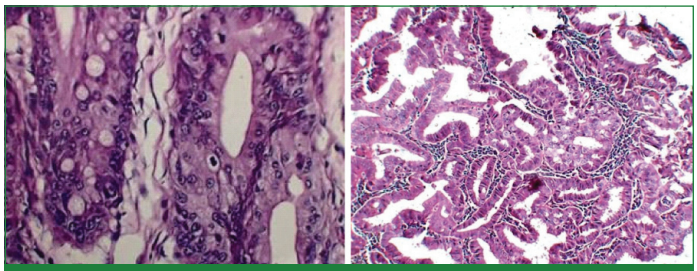
[Table/Fig-4]: Gall bladder showing chronic cholecystitis (40x, H&E). **[Table/Fig-5]:** Gall bladder showing eosinophilic cholecystitis with increased eosinophils (40x, H&E). (Images from left to right)



[Table/Fig-6]: Antral metaplasia (40x, H&E). **[Table/Fig-7]:** Intestinal metaplasia (20x, H&E). (Images from left to right)



[Table/Fig-8]: Cholesterolosis (20x, H&E). **[Table/Fig-9]:** Low grade dysplasia showing stratification and hyperchromatic plump nuclei (40x, H&E). (Images from left to right)



[Table/Fig-10]: High grade dysplasia showing pseudostratification and mitotic figures (40x, H&E). **[Table/Fig-11]:** Well differentiated ad-enocarcinoma (20x, H&E). (Images from left to right)

with stones were highly statistically significant (p-value=0.0007) in comparison to without stone [Table/Fig-12].

Histopathological finding	Total no. of cases	GB with stones	Percentage	GB without stone	Percentage
Chronic active cholecystitis	28	23	82.14%	05	17.85%
Eosinophilic cholecystitis	03	01	33.33%	02	66.66%
Chronic cholecystitis	231	206	89.17%	25	10.82%
Follicular cholecystitis	07	07	100.00%	00	00 %
Cholesterolosis	31	28	90.32%	03	9.36%
Xanthogranulomatous cholecystitis	09	06	66.66%	03	33.33%
Cholegranulomatous cholecystitis	02	02	100%	00	00
Metaplasia	83	81	97.59%	02	2.40%
Dysplasia	16	15	93.75%	01	6.25%
Carcinoma	05	03	60%	02	40%
Total	415	372	89.63%	43	10.36%

[Table/Fig-12]: Histomorphological lesions in gallbladder with and without gallstone.
Chi-square test value: 28.827; Degree of freedom: 9; p-value=0.0007

Distribution of neoplastic and non neoplastic conditions in gall bladder with or without stone: In the 410 cases of non neoplastic conditions, 369 cases (90.00 %) were seen in gall bladder with stone while only 41 cases (10.00 %) were in gall bladder without stone. Out of 5 cases of neoplastic conditions, 3 cases (60%) were in gall bladder with stone while only 2 cases (40%) were seen in gall bladder having no stone. The p-value was applied to find out the difference in association of neoplastic and non neoplastic conditions with or without stones. It was observed that non neoplastic and neoplastic conditions were significantly associated with stones in comparison to without stones (p-value=0.029) [Table/Fig-13].

Lesion	Total	With stones	Without stones	
Non neoplastic	410	369 (90.00%)	41 (10.00%)	p-value=0.029
Neoplastic	5	3 (60%)	2 (40%)	
Total	415	372 (89.63%)	43 (10.36%)	

[Table/Fig-13]: Distribution of neoplastic and non neoplastic conditions in gall bladder with or without stone.
Chi-square value=4.787; Degree of freedom=1

Association of dysplasia and carcinoma with type of stone: Dysplasia was more common with cholesterol stone, seen in 10 cases (66.66%), followed by mixed in 3 cases (20%) and pigment type of stone in 2 cases (13.34%). Carcinoma was seen only with cholesterol stone in 2 cases (66.66%) and with mixed stone in one case (33.34%), respectively. The p-value was 0.741 which was not significant [Table/Fig-14]. There is no statistically significant association of type of stones with dysplasia or carcinoma.

Lesions	Cholesterol stone	Mixed stone	Pigment stone	
Dysplasia	10 (66.66%)	3 (20%)	2 (13.34%)	p-value=0.741
Carcinoma	2 (66.66%)	1 (33.34%)	00	

[Table/Fig-14]: Association of dysplasia and carcinoma with type of stone.
Chi-square value=0.6; Degree of freedom=2

DISCUSSION

Cholelithiasis is the most prevalent biliary tract pathology globally with a prevalence rate of 10% to 15% [11]. In India estimated prevalence has been reported between 2% to 29% and it is seven times more common in North than in South India [12]. Stones are associated with large number of mucosal changes ranging from non neoplastic to neoplastic pathologies [13]. This study was performed to find out the spectrum of changes in gall bladders with or without stones.

Age and sex distribution: Gall bladder disease was found to be more common in females of fourth to fifth decade. These findings were similar to a large number of studies who also reported 4th and 5th decades to be the common age group [9,14-18] and female preponderance with male to female ratio ranging from 1:1.3 to 1:1.66 [Table/Fig-15] [7,10,12-18].

Studies	Male:Female
Present study 2022	1:4.3
Mathur SK et al., [7]	1:1.66
Almas T et al., [10]	1:3
Mohan H et al., [12]	1:6.4
Selvi TR et al., [13]	1:3.2
Khan M R et al., [14]	1:3.8
Tyagi SP et al., [15]	1:6.5
Kaur A et al., [16]	1:3
Banerjee A et al., [17]	1:3.3
Singh A et al., [18]	1:5.7

[Table/Fig-15]: Comparison of male:female ratio in various studies.

Female predominance may be due to increased level of estrogen hormone. As a result of pregnancy, hormonetherapy, use of birth control pills, cholesterol levels in bile are increased and there is decreased gallbladder movement, resulting in gallstone formation [13]. Female sex hormone appears to play a role mainly in between 20-30 years of life [13]. Progesterone may also contribute to gallstone disease by inhibiting gall bladder contraction and promoting hypomotility and bile stasis [19].

Histomorphological Lesion of Gall Bladder

Non neoplastic lesion: Chronic cholecystitis is the most common inflammatory disorder observed in gall bladder. It is more common in females and is associated with stones in majority of cases. How stones lead to inflammation is not exactly understood but it can be possible that intermittent obstruction and retention of bile may lead to chronic irritation and mucosal changes. In the present study, maximum cases were of chronic cholecystitis 231 cases out of 415 (55.66%). In the past also chronic cholecystitis was reported as the commonest lesion associated with stone ranging from 60% to 93.8% [9,10,13,15,16,18,20,21].

Out of 415 cases, only 31 cases (7.2%) of cholesterolosis were seen, of which 28 were associated with stone. These observations were similar to study done by Giri S et al., who reported 7.61% cases of cholesterolosis [19]. In a study by Kaur A et al., 12.25% cases of cholesterolosis were found which was higher than this study [16]. AlmasT et al., reported a significantly higher percentage (32.8%) for cholesterolosis [10].

Follicular cholecystitis was found in 07/415 cases (1.2%). Mathur SK et al., Mohan H et al., Kaur A et al., and Vahini G et al., reported follicular cholecystitis ranging from 1.81% to 5% [7,12,16,20]. It may or may not be associated with stones. In this study it was associated with stone in all the cases.

Eosinophilic cholecystitis was found in 3/415 cases (0.72%). Studies conducted by Jokhi CD et al., Kaur A et al., Vahini G et al., and Dattal DS et al., reported eosinophilic cholecystitis ranging from 0.8% to 2.3% [9,16,20,22]. In these cases history of allergies and drug intake was taken. Parasitic infestation was excluded.

Xanthogranulomatous cholecystitis is an uncommon variant of cholecystitis. In this condition rupture of Rokitansky-Aschoff sinus results in accumulation of bile and mucin which initiate inflammatory response. Ultimately histiocytes phagocytose cholesterol and other lipids resulting in yellow streaks and nodules in the wall of gall bladder. Xanthogranulomatous cholecystitis was found in 9/415 cases (2.16%). Xanthogranulomatous cholecystitis ranging from 1.04% to 3.0% has been reported in the past by a large number of workers [7,9,10,16,20]. Its diagnosis remains elusive and can be confused with carcinoma gall bladder [10].

Preneoplastic lesions: Metaplastic changes are commonly encountered in gall bladder and are associated with stones. Metaplasia was found in 83/415 cases (20.00%) which was in concordance with the study of Dattal DS et al., who observed 15.56% cases of metaplasia [22]. Singh A et al., also reported it in 15% cases [18].

Khanna R et al., observed metaplasia in 32.85% cases [23]. Seretis C et al., revealed an overall prevalence of metaplastic features in the resected gallbladder specimens in 22/86 (25.6%) cases [24]. This can be due to mechanical irritation caused by gall bladder stones as 81/83 (97.59%) cases were associated with stone in the present study.

Dysplastic and neoplastic lesions: Dysplasia was found in 16/415 cases (3.85%). This was almost similar to the study of Kaur A et al., Jain BB et al., who reported it to be 3.64% and 4.0% respectively [16,25]. Adenocarcinoma gall bladder was observed in 5/415 (1.2%) cases. Various studies show incidence varying from 0.20% to 3%for adenocarcinoma gall bladder [9,10,18,26].

Dysplasia and adenocarcinoma was found to be associated with stone in 93.75% and 60% cases respectively in the present study. These observations were in close proximity to study of Jain BB et al., who reported them to be 88.8% and 78.5%, respectively [25]. Thus, gall bladder stones are associated with inflammatory lesions, metaplasia, dysplasia and carcinoma.

Yamamoto M et al., proposed two histogenetic pathways for the development of carcinoma [26]. Once from normal epithelium and other from metaplastic epithelium. Metaplasia can occur due to constant irritation by stones and chronic inflammation resulting in ulceration of mucosa followed by regeneration and metaplasia. Metaplasia can be of antral type or intestinal type. It has been observed that intestinal metaplasia is associated with either low grade or high grade dysplasia which can progress to carcinoma.

Limitation(s)

This is a hospital based study. Number of cases of dysplasia and carcinoma are too less to comment upon the association of stones with these lesions. A multicenter study involving large population is desirable to find out the association of stones with pre neoplastic and neoplastic condition of gall bladder.

CONCLUSION(S)

Gallbladder stones are associated with a wide variety of mucosal alterations including dysplasia and neoplasia. So, all the cholecystectomy specimens should undergo histopathological examination to find out some unusual lesion having implication on treatment and prognosis.

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