

The Study on the Clinico-pathological Spectrum of Non-neoplastic and Neoplastic Lesions in Cholecystectomy Specimens at a Tertiary Care Centre

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ABSTRACT

Background: Most commonly used surgical procedure for gall-bladder pathology is cholecystectomy. Gall-bladder diseases incidence has increased over the past decade and are cause for a number of histopathological changes like pre-neoplastic conditions and ultimately culminating into the neoplasia. Histopathologic examination of the specimen serves as the absolute diagnosis of the non-neoplastic and neoplastic lesions as pre-operative imaging techniques fail to identify the lesions

Aim: To analyse the clinico-pathological spectrum of various non- neoplastic and neoplastic lesions occurring in cholecystectomy specimens with and without gall bladder calculus.

Materials & Method: An observational, cross-sectional study was conducted between 2019 to 2020 at a tertiary care centre in Safedabad, Barabanki, UP. A total of 135 cholecystectomy specimens were evaluated for histopathological examination. After histopathological reporting all cases were clinicopathologically related. Data was analysed using SPSS software, the demographic data were presented as frequency, percentage; continuous variables with mean and standard deviation.

Results: 74.8% were females (n=101) and 25.2% were males (n=34) with mean age of 42.96 years. Gall-bladder stone was present in 85.2% of patients (n=115) and absent in 14.8% patients (n=20). Neoplastic lesion in 7 of the patients, 5 patients with adenocarcinoma, 1 patient with adenosquamous carcinoma and 1 patient with papillary adenocarcinoma. Gall-bladder carcinoma were diagnosed in our study; the poorly differentiated GBC revealed high Ki67 Score.

Conclusion: Gall-bladder diseases can have a varied presentation both clinically and histopathologically. Hence, a complete and meticulous macroscopic and microscopic examination of all cholecystectomy specimens should be mandatory for better patient care.

Keywords: Cholecystectomy, Gall Stones, Non-Neoplastic Lesions, Neoplastic Lesions, Histopathology

Introduction

Cholecystectomy is the most commonly performed surgical procedure for gall bladder pathology. Majority of the cholecystectomies are done for cholelithiasis.¹ Gallstones are found to be the major etiologic factor of gall bladder lesions, the spectrum of which ranges from various types of cholecystitis, cholesterolosis to hyperplasia, metaplasia, dysplasia and fatal malignant transformation and hence, are a major cause of morbidity and mortality throughout the world. Its incidence has increased over the past couple of decades throughout the world and prevalence of gall stone disease also varies with age, gender and ethnic group.²

Gall stone disease might result in gall bladder carcinoma (GBC) in long standing cases. Gall bladder carcinoma carries the worst prognosis, and is 5th most common gastrointestinal malignancy in India following colon, pancreas, stomach and oesophagus.³

Gall stones are responsible for the irritation of the columnar epithelium and is the main underlying cause for a number

of histopathological changes like acute and chronic cholecystitis and patients with cholelithiasis need proper surveillance as most of the carcinoma of gall bladder are found in association of the stones.⁴ Carcinoma gall bladder accounts for only 3% of all gastrointestinal malignancies. It is seen in patients of age more than 50 years and also more prevalent in females like other gall bladder pathologies. As it is diagnosed frequently in late stage, it has 5year survival rate of only 1- 5%.⁵

As pre-operative imaging techniques fail to identify the lesions; thus, it is important to study the clinico-histopathological pattern in order to determine the prevalence and distribution of the lesions, diagnosis of premalignant and malignant conditions and in prognosticating the cases.

Aim

To study the clinico-pathological spectrum of various non-neoplastic and neoplastic lesions occurring in cholecystectomy specimens with and without gall bladder calculus.

Materials and Method

An observational, cross-sectional clinical correlation study was conducted at the Department of Pathology at Hind Institute of Medical Sciences, Safedabad, Barabanki Uttar Pradesh, India from 2019 – 2020 after getting approval from institutional ethics committee. A total of 135 resected gall bladder specimens by open or laparoscopic cholecystectomy were evaluated. Congenital anomalies of Gall bladder and autolysed specimens were excluded from the study. The data of patients age, gender, chief complaints, examination findings and other clinical data were obtained from case sheets of the patients after taking an informed consent.

Received Cholecystectomy specimens were then examined grossly for presence of pus, fibrin haemorrhage, hyperaemia, perforation and presence of any localized lesion. Measurement of the specimen were noted. The specimen were fixed in 10% buffered formalin for overnight. The specimen were then carefully sectioned of 4-5µ thickness were cut and stained with Haematoxylin & Eosin.

After fixation the specimen were sectioned, if gall stones were present. Then, gross findings of the mucosa and any other gross lesions were noted along with wall thickness of the gall bladder in order to determine whether it was diffused or localized. If lymph node (cystic lymph node) was present then any abnormality in the mucosa were noted for example ulceration evidence of cholesterolosis, neoplasm etc., also a gross organ photograph was taken. Three sections, one each from the body, fundus and neck was taken, additional sections were taken if required followed by paraffin embedding and sectioning at 5-micron thickness. The tissue sections were placed on a slide warmer for deparaffinisation marker.

This was followed by processing with routine histopathological techniques for xylene and stained with hematoxylin and eosin staining. Histopathological reporting was done by notifying the epithelial, muscular and serosal changes. Presence and type of inflammation was noted for cases of carcinoma, adenocarcinoma differentiation, grading and lympho-vascular, perineural invasion and depth of tumour invasion was also noted.

After histopathological reporting all cases will be clinicopathologically related. Histologic grade- The following grading system, based on the extent of glandular formation in the tumour, as suggested. Grade X- Grade cannot be assessed, grade 1- Well differentiated (Greater than 95% of tumour composed of glands), grade 2- Moderately differentiated (50 to 95% of tumour composed of glands), grade 3-Poorly differentiated (49% or less of

tumour composed of glands) and grade 4-Tumors with no squamous or glandular differentiation (undifferentiated carcinoma by WHO classification)

Sections were then examined under microscope & distinct nuclear staining to be taken as positive. The percentage of cells positive for Ki-67 were scored semi-quantitatively, according to the number of labelled cells. Ki-67 Labelling index was expressed as percentage of positively stained cells, per 100 epithelial cells after counting at least 1000 cells using high power (40x10) in an area with maximum proliferation

All the collected data was entered in excel sheet after obtaining in the proforma. The demographic data are presented as frequency, percentage, continuous variables with mean and standard deviation. The data are graphically represented using the pie chart and bar diagrams. Ap- value of <0.05 was statistically significant. All the data were analysed using SPSS v23 operating on windows 10.

Results

The present study included 135 cholecystectomy specimens aged between 18-60 years with a mean age of 42.96 ± 13.45 years. Among these, 25.2% were male and 74.8% were female with female preponderance and the male to female ratio was 1:2.97. The common clinical presentation of patients was pain in 96.2% of patients, 83.7% with vomiting, 34% with fever, 8.8% with dyspepsia, 6.7% with the decreased appetite, heaviness and 5.1% with jaundice.

Gall stones could be classified on the basis of morphology in 115 cases. Out of 80 cases with mixed stones, 70 were seen in chronic cholecystitis, 01 in gangrenous cholecystitis, 01 in follicular cholecystitis, 01 in xanthogranulomatus cholecystitis, 4 in adenocarcinoma, 01 in adenosquamous carcinoma, 2 in partially autolysed gallbladder. Pigmented stones were seen in 28 cases, out of which 27 were if chronic cholecystitis, 01 in xanthogranulomatus cholecystitis. Cholesterol stones (7 cases) were seen most commonly in chronic cholecystitis (7 cases).

Amongst 135 cases, the mucosa was focally or diffusely denuded in 9 (6.67%) cases. Focal ulceration was seen in 13(9.63%) cases and normal epithelium was seen in 12(8.89%) cases. Mild to moderate fibrosis was found in 47 cases (34.81%), with lesions including chronic cholecystitis, xanthogranulomatus cholecystitis, follicular cholecystitis and adenocarcinoma. Rokitansky- Aschoff sinuses were seen in 42 cases (31.11%) of chronic cholecystitis.

Further on histopathological examination of the 135 gallbladders, it was found that chronic cholecystitis was the most common diagnosis in 119(88.15%) cases, affecting

more female 92(77.31%) than male 27 (22.69%). The mean age of chronic cholecystitis was 41.87 years. It was characterized by varying degree of lymphoplasmacytic, histiocytic and eosinophilic infiltration along with fibrosis of the gallbladder wall, presence of Rokitansky – Aschoff sinuses (PMG:14), hyperplasia, metaplasia, and reactive epithelial changes. In 12 cases, the epithelium showed hyperplastic changes, out of which papillary hyperplasia was most common 8 cases followed by spongiform type 4 cases. Out of these 12 cases, 9 cases were female and 3 were male. Thus, epithelial hyperplasia was more common in females. Hyperplastic changes were seen most commonly in chronic cholecystitis (12 cases). The mean age of hyperplasia was 39.00 years. Metaplastic changes were seen in 11 cases, out of which 7 cases were of pyloric type followed by 4 cases of intestinal metaplasia. Pyloric metaplasia was more commonly seen in female (5 cases) than in male (2 cases). Out of 4 cases of intestinal metaplasia, 3 cases were female and 1 was male. Metaplasia was seen most common in chronic cholecystitis (11 cases). The mean age for pyloric metaplasia was 48.00 years. The mean age for intestinal metaplasia was 43.75 years. There

were 3 cases of adenomyosis, out of which 2 case were female and 1 male. Out of 3 cases of adenomyomatosis, 2 cases were female and 1 male. Gangrenous cholecystitis was seen in 2 (1.48%) cases, with equal distribution of the cases in male and female. Xanthogranulomatus cholecystitis, characterized by proliferation of foamy macrophages, was seen in 2 female and 1 male. Follicular cholecystitis (PMG: 19), characterized by numerous prominent lymphoid follicles in lamina propria through the gallbladder, was seen in only 1 male.

Malignancy was seen in 7 (5.18%) of 135 cases. 1 (.74%) case was papillary type (male), 5 cases (3.70%) were Adenocarcinoma NOS (male: female = 1:4). One (74%) case was of adenosquamous carcinoma (female). In total 5 cases of gallbladder carcinoma were female with a mean age of 55.4 years and 2 cases were male with mean age of 68 years. Out of these seven cases of carcinoma, six cases had associated gallstones.

In the present study gall stones, either single or multiple, were seen in 85.19% of cases. Gall stones were present in 28 (82.35%) out of 34 males, and 87 (86.14%) out of 101 females.

Table 1: Histopathological Findings

		Frequency	Percentage (%)
Mucosal surface	Congested	22	16.3
	Growth	7	5.2
	Normal	100	74.1
	Partially autolysed	3	2.2
	Ulcerated	3	2.2
	Total	135	100
Serosa	Congested	27	20
	Normal	108	80
	Total	135	100
Gall Bladder wall thickness	Normal	88	65.2
	Thickened	47	34.8
	Total	135	100
Presence of Gall Bladder Calculi	Absent	20	14.8
	Present	115	85.2
	Total	135	100
Distribution of types of Gall Bladder Calculi	Absent	20	14.8
	Cholesterol	7	5.1
	Mixed	80	59.3
	Pigmented	28	20.7
	Total	135	100

Table 2: Distribution of microscopic features.

S. no.	Microscopic feature		No of cases	Percentage (%)
1	Normal epithelium		12	8.89
2	Ulcerated		13	9.63
3	Denudated		9	6.67
4	Epithelial hyperplasia	Papillary	8	5.93
		Spongiform	4	2.96
	Metaplasia	Pyloric	7	5.19
		Intestinal	4	2.96
6	Fibrosis		47	34.81
7	Rokitansky-Aschoff sinus		42	31.11
8	Adenomyoma		3	2.22
9	Adenomyomatosis		3	2.22
10	Malignancy	Papillary type	1	0.74
		Adenocarcinoma	5	3.70
		Adenosquamous carcinoma	1	0.74
		Total	7	5.18

Table 3: Occurrence of neoplastic changes in the gall bladder specimen.

		Frequency	Percentage (%)
NEOPLASTIC	Absent	128	94.9
	Adenocarcinoma	5	3.7
	Adenosquamous carcinoma	1	.7
	Papillary adenocarcinoma	1	.7
	Total	135	100

Table 4: Distribution of Adenocarcinoma cases and Ki 67 Scoring.

		No. of Cases	Percentage of positive Stains (%)
Differentiation of Adenocarcinoma	Well Differentiated	3 (50%)	30
	Moderately Differentiated	2 (33.4%)	32
	Poorly Differentiated	1 (16.6%)	50
	Total	6 (100%)	

Table 5: Gall bladder diseases with or without stone.

Gall Bladder Diseases	With Stones	Without Stones	Total
	Present	Absent	
Chronic cholecystitis	103 86.55%	16 13.45%	119 100%
Gangrenous cholecystitis	1 50%	1 50%	2 100%
Xanthogranulomatous cholecystitis	2 66.70%	1 33.30%	3 100%
Follicular cholecystitis	1 100%	0 0%	1 100%

Gall Bladder Diseases	With Stones	Without Stones	Total
	Present	Absent	
Adenocarcinoma	5 83.33%	1 16.67%	6 100%
Adenosquamous carcinoma	1 100%	0 0%	1 100%
Partially autolysed gallbladder	2 66.70%	1 33.30%	3 100%
Total	115 85.19%	20 14.81%	135 100%

Discussion

Cholecystectomy is the most commonly used surgical procedure for gall bladder diseases. There is a long list of the lesions that are associated with the gall stones which can be either inflammatory namely cholecystitis, cholesteriolosis, adenomyomatosis, polyps or can be premalignant like metaplasia, hyperplasia, dysplasia or even the frank malignancy. They may present initially as gall bladder thickening or small mass, picked up by an expert radiologist but all cases are not diagnosed ultrasonographically. Hence, it has been a traditional practice to send all cholecystectomy specimens for histopathological examination.⁶

In the present study, 135 patients underwent the gallbladder removal for various reasons as discussed below. The mean age of the patients in present study was found to be 42.96 years, with maximum patients in the range of 21-40 years of age (45.9%) and 41-60 years of age (41.5%). 4 patients were below the age of 20yrs and 13 patients were above the age of 60 years. The results of our study are in accordance with the studies conducted by Awasthi et al⁷, Memom et al⁸ and Nigam et al⁹. Among these 135 patients, 74.8% were females (n=101) and 25.2% were males (n=34) include in present study, with female preponderance (female to male ratio was 2.9:1). In study by Awasthi et al.⁷, also documented a female preponderance (95.2%) and ratio of 2.6:1 was recorded (female to male) and various other studies.¹⁰

Pain was the predominant symptoms in the present study involving- 96% patients. The commonest site of pain was in the Rt. Hypochondrium, & the next commonest site was Epigastrium patients complained of pain radiating to the back. pain was colicky in nature.

The mucosal surface on examination was found to be normal in 74.1% of patients, however the congested finding was present in 16.3% (n=22), growth was present in 7 patients; 3 patients were found to have partially autolysed mucosal membrane and in 3 cases it was ulcerated. In concordance to present work, study by Beena et al¹¹, found

normal wall and mucosa among 52.5% of patients and 65.5% respectively.

Serosa of the gallbladder was congested in majority of the patients 16.3% (n=22) which was in contrast with other studies, in Beena et al¹¹ documented in 64% of patients (n=128). Thickness of the wall was found to be normal in 65.2% of patients and 34.8% of patients (n=47) presented with the thickened surface. Similar to present study, Awasthi et al.⁷, documented the normal wall thickness in 72.8% of patients and increased thickness of the wall in 27.2% of cases.

Gallstones are a major cause for mortality and morbidity throughout the world. They are associated with various lesions like acute cholecystitis, chronic cholecystitis with its variants and gallbladder carcinoma. On assessment, the gall bladder stone was present in 85.2% of patients (n=115) and in 14.8% of patients there was absence of stone (n=20). Among the calculi, mixed type of stone was found to be predominant with 59.3% (n=80) followed with pigmented type of calculi (n=28) and in 5.1% showed cholesterol calculi in them (n=7). Similar to present study, study done by Goyal et al.¹², with 346 cholecystectomy specimens, got predominantly mixed stones in 213 cases (68%).(92) Beena et al¹¹ documented 65% of cases with the presence of stone while 35% with no stones. In their study in contrast to our, found pigmented stones as predominant (58.5%) followed with cholesterol stones (4.5%) and mixed stones (2%).

The presence of the inflammatory changes was seen in majority of the patients. 6.5 percent of the patient's gall bladder showed ulceration with chronic inflammatory changes. In 2.2% of the patients, we found the xanthogranulomatous cholecystitis. Xanthogranulomatous cholecystitis is known to present with increased wall thickness and mimic carcinoma on gross examination.¹³ A prospective study by Srikanth et al. showed gall bladder carcinoma in only 2 out of 60 (3.3%) cases of patients presenting with thickened gall bladder wall (>4 mm). They found no statistically significant difference between the incidence of malignancy in patients with or without thick-walled gall bladder.¹⁴

Among all the cases, we found neoplastic lesion in 7 of the patients, 5 patients with adenocarcinoma, 1 patient with adenosquamous carcinoma and 1 patient with papillary adenocarcinoma. On gender distribution of the neoplastic lesion was present in 5 females and 2 males. We found 4/5 cases of adenocarcinoma was present in female, 1 female with Adenosquamous carcinoma and 1 male with papillary adenocarcinoma. The increased risk of GB cancer in women is partially explained by the higher incidence of cholecystolithiasis in women as compared with men. Female hormones may play a role in the aetiology of this disease. The results are in accordance with Beena et al¹¹ and Khan et al.¹³

In the present study we found that the number of Ki67 positive cells increase with decreasing differentiation of the neoplastic cells i.e., they are less in well differentiated GBC and much more in poorly differentiated GBC. The poorly differentiated GBC show large number of proliferating cells with areas of haemorrhage and necrosis with poor prognosis.

Several studies in past have accounted focusing on whether or not gall bladder histopathology must be done routinely or selectively. Some studies such as by Butti et al. concluded that all cholecystectomy specimens must be examined by histopathologists who must decide whether processing for microscopy is needed. Microscopic examination may be reserved for the specimen with a macroscopic lesion. This will result in a reduction of costs and pathology workload without compromising patient management¹⁴. In another study by Nagarajan et al. they concluded that in a country where some parts are highly endemic for gall bladder cancer, we can ill afford to miss incidental Gall bladder carcinoma. It would be dangerous to suggest doing away with histopathology examination of cholecystectomy specimens¹⁵. However, results of the present study suggests that, as Gall bladder carcinoma presents with poor prognosis and is asymptomatic in most patients. Hence, selective histopathology serves as gold standard in saving time and cost.

References

1. Srinivasan G, Sekar ASI. Study of histopathological spectrum of gallbladder in cholecystectomy specimens. *Int J Res Med Sci.* 2019;7(2):593–7.
2. Patil M, Londhe MM, Margam S, Iqbal J. Spectrum of Lesions of Gall Bladder Experience of Five Years at Tertiary Care Hospital. *Ann Pathol Lab Med.* 2018;5(9):779–84.
3. Zaki, Al-Refeidi. Histological Changes in the Human Gallbladder Epithelium associated with Gallstones. *Oman Med J.* 2009;24:269–73.
4. Noel K, Al-hamdany M, Raoof R. Histological Changes of Gall Bladder Mucosa: Correlation with Various Types of Cholelithiasis. 2011.
5. Dowling GP, Kelly JK. The histogenesis of adenocarcinoma of the gallbladder. *Cancer.* 1986;58(8):1702–8.
6. Mathur S, Duhan A, Singh S. Correlation of gallstone characteristics with mucosal changes in gall bladder. *Trop Gastroenterol.* 2012;33(1):39–44.
7. Awasthi N. A retrospective histopathological study of cholecystectomies. *Int J Heal Allied Sci.* 2015;4(3):203–6.
8. Siddiqui FG, Memon AA, Abro AH, Sasoli NA, Ahmad L. Routine histopathology of gallbladder after elective cholecystectomy for gallstones: waste of resources or a justified act? *BMC Surg.* 2013;13:26.
9. Nigam M, Ranwaka R, Nigam B, Singh M, Devpura TP. Prevalence of carcinoma in symptomatic gall stone disease- a study following cholecystectomy. *J Evol Med Dent Sci.* 2013;2:4554+.
10. Ozgur T, Toprak S, Koyuncuer A, Guldur M, Bayraktar G, Yaldiz M. Do histopathologic findings improve by increasing the sample size in cholecystectomies? *World J Surg Oncol.* 2013/10/01. 2013;11:245.
11. Beena D, Shetty J, Jose V. Histopathological Spectrum of Diseases in Gallbladder. *Natl J Lab Med.* 2017;6(4):06–9.
12. Goyal S, Singla S, Duhan A. Correlation between gallstones characteristics and gallbladder mucosal changes: A retrospective study of 313 patients. *Clin Cancer Investig J.* 2014;3(2):157–61.
13. Khan DM, Abilsh S. Histopathological spectrum of gallbladder diseases in a tertiary care centre. *IP Arch Cytol Histopathol Res.* 2018;3(4):206–9.
14. Butti AK, Yadav SK, Verma A, Das A, Naem R, Chopra R, Singh S, Sarin N. Chronic calculus cholecystitis: Is histopathology essential post-cholecystectomy?. *Indian journal of cancer.* 2020 Jan 1;57(1):89
15. Nagarajan G, Kundalia K. Should every cholecystectomy specimen be sent for histopathology to identify incidental gall bladder cancer?. *Indian journal of cancer.* 2020 Jan 1;57(1):2

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