

Study of Association of Cholelithiasis with Pre –Invasive and Invasive Neoplastic Histopathological Changes in Gallbladder in a Tertiary Care Hospital

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ABSTRACT

Introduction: Gallstone disease is a worldwide medical problem, but the incidence and prevalence rates vary with geographical locations. It produces wide range of histopathological changes in gallbladder mucosa, namely- acute inflammation, chronic inflammation, mucosal hyperplasia, cholesterolosis, gastric and intestinal metaplasia, dysplasia and neoplasia. The present study was conducted to see the association of various gallstone characteristics (number and size) with the pre-invasive and invasive neoplastic histopathological changes in the gallbladder. To study the association of Cholelithiasis with pre-invasive and invasive neoplastic histopathological changes in the gallbladder.

Material and methods: 300 cases, which had undergone cholecystectomy in our hospital were included in the study. The specimens were sent to histopathology laboratory for the gross and microscopic assessment. Number and size of stones was noted. The following microscopic diagnostic criteria were used: - cholecystitis, hyperplasia, metaplasia and carcinoma. The hematoxylin and eosin (H & E) stained sections were systematically examined and the pattern of response in the gallbladder mucosa was studied with regard to number and size of stones.

Results: Significant results were obtained while assessing association of mucosal response with gall stone size, with carcinomas presenting in cases with larger gall stones.

Conclusion: The observations from this study indicate that there is a relationship between pathological changes of gall bladder mucosa and gall stones. While a cause and effect relationship cannot be substantiated with the current study, constant irritation of the gall bladder wall by gallstones over time seems to constitute a risk.

Keywords: Gall Stones, Cholecystectomy, Cholecystitis, Dysplasia, Carcinomas.

INTRODUCTION

Gallbladder is an accessory organ of the hepatobiliary system, having the function of storing and concentrating bile between meals which facilitates the absorption of dietary lipids. The gallbladder undergoes structural and functional changes in many pathological settings, including gallstone disease, which is very common.¹ Gallstone disease is a worldwide medical problem, but the incidence and prevalence rates vary with geographical locations. Gallstones are hardened deposits of the digestive fluid bile that can form within the gallbladder. They occur when there is an imbalance in the chemical constituents of bile that results in precipitation of one or more of the components.²

Gallstones are becoming increasingly common; they are seen in all age groups, but the incidence increases with age; and about a quarter of women over 60 years will develop gall stones in due course of time.²

Gallstone disease produces wide range of histopathological changes in gallbladder mucosa- namely, acute inflammation, chronic inflammation, acute on chronic inflammation, xanthogranulomatous inflammation, granulomatous inflammation, mucosal hyperplasia, cholesterolosis, gastric and intestinal metaplasia, dysplasia and neoplasia. The gallbladder mucus promotes nucleation of stones and thus plays a regulatory role in the formation of cholelithiasis. Mucus, calcium and lipids act in concert to form the gallstones.³

Histologically, most GBC are adenocarcinomas (pancreaticobiliary type), showing variable degrees of differentiation. Several other histologic variants of adenocarcinomas like papillary, mucinous, intestinal, signet-ring cell and clear cell are also seen. More than one histological variant may be present in some tumors. Other types of carcinomas like adenosquamous carcinoma, squamous cell carcinoma, small cell carcinoma, and undifferentiated carcinoma may also be seen in gall bladders. Sometimes, it is difficult to differentiate between the various histological subtypes of these tumors. Prognosis is poor due to failure to detect disease in early course.⁴

Baig *et al* did a study that included 40 patients of gallstone disease, and they assessed the correlation between histopathologic picture and biochemical nature of gall stones.⁵ In another study by Kumar *et al*, authors assessed the various mucosal responses of the gallbladder with the different parameters of gall stones. Out of 50 cases studies

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by them, 80% of the patients had mixed or combined type of stones. In patients with hyperplasia and cholecystitis with metaplasia, the size of stone was 1.5 and 0.85 cm respectively whereas, the mean size of stones in patients with carcinoma was 3.98 cm. Size of stones showed significant correlation with the type of mucosal response in gallstone patients.⁶

The present study was conducted on the association of various gallstone characteristics (number and size) with the pre-invasive and invasive neoplastic histopathological changes in the gallbladder.

MATERIAL AND METHODS

The present prospective study was conducted in the Department of General Surgery and Department of Pathology of Adesh Institute of Medical Sciences and Research, Bathinda. This study included 300 cases, who had undergone cholecystectomy in our hospital from 26 January 2018 to 31 January 2019.

Ethical approval: Ethical clearance was taken from the ethical committee of the institution. Written and informed consent was obtained from all the patients after explaining the entire research protocol. (Letter Ref No: AU/EC/FM/14/2018, dated: 25 January 2018)

Inclusion criteria: The patients who were diagnosed with cholelithiasis and had underwent cholecystectomy in AIMSR Hospital, Bathinda, were included in the study.

Exclusion criteria

Patient who did not have cholelithiasis such as acalculous cholecystitis and emphysematous cholecystitis and patients already diagnosed with carcinoma, gall bladder were excluded from the study.

Methodology

- All the patients who presented to the Surgery Out Patient Department (OPD) with symptomatic cholelithiasis (diagnosed sonographically) were admitted and pre-operative investigations were done. After obtaining fitness for surgery, patient underwent cholecystectomy.
- After proper labeling of the specimen, gallbladder was sent to Department of Pathology for histopathological examination.

The following features were noticed on gross examination:

- Dimensions, external/outer surface, wall thickness and mucosa of gall bladder. The gall stones were examined for various parameters: number, size and nature of stones (pure, pigmented or mixed).
- The specimen was properly sampled and processed by routine histological techniques. Four sections including entire wall were obtained: two from body, one each from fundus and neck of the gall bladder. Additional sections were taken from abnormal mucosa. Histopathological diagnosis was established on routine haematoxylin and eosin (H&E) staining of the sections.

The following parameters were used to assess gallstones:-

Physical characteristics of Gall Bladder Stones:

1. Number: single / double / multiple.

2. Size: Average of two major diameters with a Vernier Caliper (accuracy: 0.01 cm) was determined for size. In the event of multiple gallstones, the diameter of largest and smallest stone was recorded.

The following microscopic diagnostic criteria were used:-

1. Cholecystitis: included chronic cholecystitis, acute on chronic cholecystitis, chronic active, follicular and xanthogranulomatous cholecystitis, cholesterosis.
2. Hyperplasia: included both adenomatous and adenomyomatous hyperplasia.
3. Metaplasia: included both intestinal and pyloric metaplasia
4. Carcinoma: included both carcinoma in situ and invasive carcinoma.

The hematoxylin and eosin (H & E) stained sections were systematically examined and the pattern of response in the gallbladder mucosa such as type of inflammation, cholesterosis, mucocele, hyperplasia, metaplasia, dysplasia and malignant changes were studied with regard to number and size of stones. The various morphological responses were then categorised under three broad categories –

- a. Cholecystitis
- b. Pre-invasive neoplastic lesions
- c. Invasive carcinomas.

STATISTICAL ANALYSIS

All the results were analyzed by MS Excel. Assessment of results was done by using Analysis of Variance (ANOVA) for averages and chi-square test for contingency tables and proportions. P- Value ≤ 0.05 was taken as significant.

RESULTS

Out of total 300 cases, 87 cases (29%) in our study belonged to the age group of 40 to 49 years, while 66 cases (22%) and 57 cases (19%) belonged to the age group of 30 to 39 years and 50 to 59 years respectively. 45 cases each (15%) belonged to the age group of less than 30 years and more than 60 years. Majority of patients belong to age group 40-49 years (29%) followed by 30-39years (22%) and least were in age group of <30 and >60 years that is 15% each. 255 patients (85%) were females while the remaining were males, with male to female ratio 5.67:1.

In 142 cases (47.33%), and 120 cases (40%), multiple and solitary gallstones were present, while in 38 cases (12.67%), two stones were present. Among 300 patients majority had multiple stones, only 38 had two stones.

286 cases were diagnosed as chronic cholecystitis, pre invasive lesions were seen in 6 in cases, out of which, 5 (1.67%) belonged to mild to moderate dysplasia and 1 (0.33%) belonged to severe dysplasia, while invasive lesions were seen in 8 cases, out of which 2 (0.67%) were superficial lesions and 6 (2%) were deep lesions. (Table 1)

Mucosal response in cholelithiasis consisted of variety of histomorphologies, including- non- specific chronic cholecystitis, acute on chronic cholecystitis, chronic cholecystitis with gastric/ intestinal metaplasia, cholesterosis, adenomatous hyperplasia, etc. (Table 2 and

Mucosal response		Frequency	Percent
Chronic Cholecystitis		286	95.33
Pre-invasive	Mild to moderate dysplasia	5	1.67
	Severe Dysplasia	1	0.33
Invasive	Superficial	2	0.67
	Deep	6	2
Total		300	100

Table-1: Distribution of subjects according to various mucosal responses

Mucosal response in Cholecystitis	No of cases
1. Chronic cholecystitis with or without other mucosal responses	273
2. Acute on chronic cholecystitis	13
Total	286

Table-2: Mucosal response in Cholecystitis

1.	Non specific chronic cholecystitis	20
2.	Chronic cholecystitis with gastric metaplasia	167
3.	Chronic cholecystitis with gastric and intestinal metaplasia	21
4.	Chronic cholecystitis with intestinal metaplasia	27
5.	Chronic cholecystitis with cholesterlosis and mucosal hyperplasia	28
6.	Chronic cholecystitis with focal adenomatous hyperplasia	10
	Total	273
	Muscle hypertrophy*	20

Table-3: Chronic cholecystitis with or without other mucosal responses Muscle hypertrophy can be seen associated with any of the changes given above

Diagnosis	No of stones			F	Degree of freedom	p-value
	Single	Two	Multiple			
Cholecystitis	117	33	136	1973.74	2	0.000
Pre-invasive lesions (Dysplasias)	1	2	3			
Invasive(Carcinoma)	2	3	3			
Total	120	38	142			

Table-4: Association of mucosal response with number of stones

Diagnosis	Mean size of stone (cm)	SD (cm)	F	p- value
Cholecystitis	0.70	0.33	357.627 at df = 2	0.000(S)
Pre-invasive neoplastic lesions (dysplasias)	1.12	0.88		
Invasive (Carcinoma)	4.00	0.28		

Table-5: Association of mucosal response with gallstone size

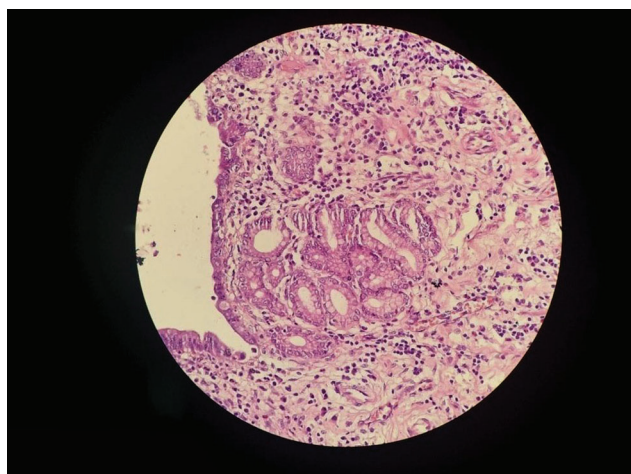


Figure-1: Photomicrograph showing chronic cholecystitis with intestinal metaplasia (H & E, 400 x)

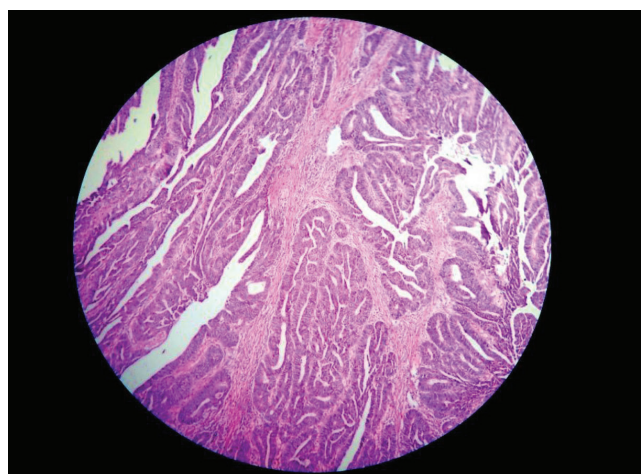


Figure-2: Photomicrograph showing moderately differentiated adenocarcinoma. (H & E, 100 x)

3)

Cholecystitis was more common in 40-49 years of age followed by 30-39 years; pre-invasive dysplasias were also common in 40-49 years, while invasive carcinomas were common in more than 50 years of age group. Using ANOVA statistically significant results were obtained.

Out of 255 female patients in our study 245 were diagnosed as cholecystitis, 4 were pre invasive lesions (dysplasias) and 6 were carcinomas. Out of 45 male patients 41 were diagnosed as chronic cholecystitis, 2 as pre invasive lesions (dysplasias) and 2 carcinomas. Chi-square reveals that ($p > 0.05$) that means the results were statistically not significant.

Out of 286 cases of chronic cholecystitis, 136 and 117 cases had multiple and solitary gallstones respectively, while in 33 cases two stones were present. Out of 6 cases of pre-invasive lesions (dysplasias), multiple and solitary stones were present in 3 and 1 cases respectively, while in 2 cases two stones were present. Out of 8 cases of carcinomas 3 cases had multiple, 2 cases had solitary gallstone, while in 3 cases, two stones were present. Using ANOVA highly statistically significant results were obtained. (Table 4)

Significant results were obtained while assessing association of mucosal response with gallstone size with smaller stones in chronic cholecystitis and larger stones in carcinomas. (Table 5)

In 286 chronic cholecystitis cases, 235 cases had mixed stones, 37 cases had pure cholesterol and 14 had bile pigment stones. Out of 6 pre-invasive lesions (dysplasias), 2 cases had cholesterol, 3 had mixed and 1 had bile pigment stones. Out of 8 cases diagnosed as carcinomas, 4 had mixed stones, while cholesterol and bile pigment stones were present in 2 cases each.

DISCUSSION

Gallstone disease is one of the most common disorders of the Hepatobiliary system, and increasing number of cholecystectomies are being performed each year than any other elective abdominal operation. As such, clinicians need a fundamental knowledge of gallstone disease and the common complications that are associated with this disease.⁷⁻⁹ Overall, the prevalence of gallstones in India is about 2% to 29%, the diseases is 7 times more prevalent in North India than in South India.^{10,11}

In the present study, chronic cholecystitis was found to be present in 286 cases (95.33%) with gastric metaplasia in 167 cases (55.67%), intestinal metaplasia in 27 cases (9%) (Fig 1) and both gastric and intestinal metaplasia 21 cases (7%). Pre-invasive neoplastic changes were found in 6 cases (2%). Among these pre-invasive lesions, mild to moderate dysplasia was seen in 5 cases (1.67%) while severe dysplasia was found to be present in 1 case (0.33%). Invasive carcinomas were diagnosed in 8 patients (2.67 percent) (Fig 2). Among the patients with invasive carcinoma, deep lesions were present in 6 cases (2%), while superficial lesions were seen in 2 cases (0.67%). In another study conducted by Goyal S *et al.*, authors reported that on studying the gall bladder mucosa microscopically, the most common change observed was

chronic cholecystitis (181 cases, 58%). Gastric metaplasia was the second most common finding along with chronic cholecystitis, present in 31 cases (10%), followed by acute on chronic inflammation and intestinal metaplasia with 30 cases each.¹²

In the present study 142 cases (47.33%), and 120 cases (40%), multiple and solitary gallstones occurred, In 38 cases (12.67%), two stones occurred respectively. While studying the association of mucosal response in relation to number of stones, non-significant results were obtained (P value > 0.05). Our results are similar with already reported data by Roa *et al.*¹³ and Mathur *et al.*¹⁴, who also could not determine any significant correlation between mucosal response and number of gallstones (p -value > 0.05).

In present study cholecystitis was more common in 40-49 years of age followed by 30-39 years; pre-invasive dysplasias were common in 40-49 years, while invasive carcinomas were common in more than 50 years of age group. Out of 255 female patients in our study, 245 had cholecystitis, 4 were pre invasive lesions- dysplasias and 6 were carcinomas, while out of 45 male patients 41 were cholecystitis, 2 were pre invasive lesions- dysplasias and 2 were carcinomas. The present study observes significant correlation while assessing the gallbladder mucosal response with age and in significant with gender. Furthermore, a higher incidence of metaplastic alterations would also be expected in females. However; no significant results was obtained in the present study. The increased risk of gallbladder carcinomas in women is partially explained by the higher incidence of gallstones in females as compared to males. Female hormones may play a role in the causation of the disease. Higher and protracted exposure to female sex hormones (such as progesterone and estrogen) may be a predisposing factor. Therefore, younger age at menarche, multiple pregnancies, early age at first pregnancy, and a prolonged reproductive period may increase the development of risk biliary tract cancer.¹⁵

In the present study, we observed significant association of size of gallstones with invasive neoplastic changes in gall bladder (P value < 0.05). The present study indicates that the average size of gallstones was 0.70 cm in cholecystitis cases, 1.12cm in pre-invasive cases and 4cm in invasive cases (P value 0.012). In carcinoma cases average size of stone were significantly more as compared to inflammation and pre-malignant lesions. Lowenfels *et al.* investigated gallstone size, growth, and the relation between stone size and gallbladder cancer. They had used cholecystectomy reports from 1676 female subjects (169 Whites, 531 Blacks, and 976 Native American Indians). They estimated that one-third of all gallbladder cancers in subjects with calculi were associated with large (greater than or equal to 3 cm) stones. They believed that stone size might be used to determine the risk of gallbladder cancer in patients with gallstones.¹⁶ Non-significant results were obtained while correlating the distribution of patients according to composition of gallstones divided on the basis of mucosal response. Similar results obtained by Singh A *et al.*¹⁵ who also reported similar findings in their respective study. Similar results were

also reported by Mathur et al.¹⁴ and Khanna et al.¹⁷ who also failed to demonstrate a significant correlation while trying to correlate the mucosal response with the types of gallstones.^{14,17}

Gallstones are accompanied by major changes in the gallbladder epithelium. This is mainly due to the large size of gallstones leading to more irritation to the mucosa in addition to the lethal effect of the lithogenic bile which harvest the chemical injury to the mucosa. While the exact cause-and-effect relationship cannot be demonstrate 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 preinvasive modifications in the morphologic background of chronic cholecystitis is an argument in favor of metaplasia-dysplasia-neoplasia sequence.^{15,16}

CONCLUSION

The histopathological spectrum of gallbladder lesions in gallstone disease included chronic cholecystitis and associated variety of mucosal alterations and lesions like cholesterolosis, hyperplasias, metaplasia, dysplasias and carcinomas. Commonest stones were mixed type and were more frequently associated with premalignant lesions. The observation from this study indicates that there is a relationship between pathologic changes of gall bladder mucosa and gall stones. While a cause and effect relationship cannot be substantiated with the current study, constant irritation of the gall bladder wall by gallstones over time seems to constitute a risk.

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