ORIGINAL RESEARCH PAPER

Pathology

CLINICO-PATHOLOGICAL SPECTRUM OF GALLBLADDER DISEASES WITH STUDY OF HISTOPATHOLOGICAL CHANGES ASSOCIATED WITH GALLSTONES

KEY WORDS: Gall bladder diseases, Histopathological changes, Gall stones.

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ABSTRACT

Background : Cholecystectomy specimen (gall bladder) is the most frequently encountered surgical specimen in histopathology laboratory principally indicated in patients with symptomatic gallstones associated diseases.The different histopathological spectrum ranges from inflammation to premalignant changes and carcinoma. Chronic cholecystitis being the most frequently diagnosed disease in all the cholecystectomy cases .

Aims : To study the clinico-pathological spectrum of gall bladder diseases in cholecystectomy specimens and to observe the histopathological changes associated with gallstones .

Methods : A retrospective study based on the histopathological analysis of 1630 cholecystectomy specimens from 1st January, 2017 to 31 st July, 2019 in the department of Pathology, Jawaharlal Nehru Institute of Medical Sciences Imphal, Manipur.

Results: Of the total 1630 cases studied it included 236 males and 1394 females with male to female ratio of 1:5.9.The most commonly affected age group by gall bladder diseases was 40 to 49 years. Chronic cholecystitis (89.7%) was the most commonly diagnosed lesion.The commonest presenting complaint was right hypochondrial pain .There was a strong association of gallstones with chronic cholecystitis (92.8%), dysplasia (92%) , metaplasia(80%), cholesterolosis (50.8%), carcinoma(50%).The incidence of dysplasia was 1.7 % and of incidental carcinoma was 0.13%.

Conclusion: Study of the clinico-pathological spectrum of gall bladder diseases and its association with gallstones would help in understanding the diseases and its complications. We found that chronic cholecystitis is the most common gall bladder disease which is more in female. Gallstones are found to be strongly associated with the development of pre-malignant lesions, hence every resected specimen of gall bladder should be subjected for histopathological examination.

Introduction

Cholecystectomy specimen (gall bladder) is the most frequently encountered surgical specimen in the histopathology laboratory [1] principally indicated in patients with symptomatic gallstones associated diseases. The histopathological finding in most of the cholecystectomy specimen is chronic cholecystitis. However chronic cholecystitis specimen are often associated with other lesions such as cholelithiasis, cholesterolosis, muscle hypertrophy, polypoid and adenomatous proliferation of mucous glands and changes such as metaplasia, hyperplasia and dysplasia [(2,3,4)] . Dysplasia and carcinoma are relatively uncommon, with reported incidence of 3.3 % and 0.3 % [5] respectively .Two schools of thought have been observed as to the pathogenesis of the malignant lesions of the gall bladder due to gall stone diseases: (a) Epithelial hyperplasia > through dysplasia > neoplasia. (b) Epithelial metaplasia leading to dysplasia, carcinoma –in situ and invasive cancer [6].

Aim of the study

1. To study the clinicopathological spectrum of gall bladder diseases in cholecystectomy specimen.
2. To study the association of gallstones with metaplasia, dysplasia and carcinoma.

Material and methods

A retrospective study of all the cholecystectomy specimens , a total of 1630 received at histopathology section in Jawaharlal Nehru Institute of medical sciences,Imphal, was carried out for the period of two and half years from 1stJanuary 2017 to 31stJuly 2019.The study was carried according to the institutional guidelines for such studies. The clinical findings were noted and gross examination was done according to standard protocol. 3 sections were taken, one from the fundus, one from the body and one from the neck. Sections were prepared and stained with H & E and evaluated for histomorphological changes.Whenever dysplasia was found , additional multiple sections were taken for microscopic examination.Immunostaining of p53 and Ki-67 was performed in carcinoma cases .

Statistical analysis: Statistical correlation was done using chi-square test and p-value was calculated. Value of $p<0.05$ was considered statistically significant.

Results:

The study included 1630 cholecystectomy specimens for histopathological evaluation.The age of the patient ranged from 9 to 87 years. Majority of the patients were in the age group of 40-49 years (Table -1). Out of 1630 cases ,1394 (85.5%) were female and 236 (14.4%) were male with a M:F of 1:5.9 (Fig 1)

Table 1:Age and sex distribution of the study cases

Age group(yrs)	Male	Female	Total
9-19	4	33	37
20-29	41	181	222
30-39	40	357	397
40-49	83	438	521
50-59	39	210	249
60-69	21	161	182
70-79	8	12	20
80-89	0	2	2
Total	236(14%)	1394(85.5%)	1630

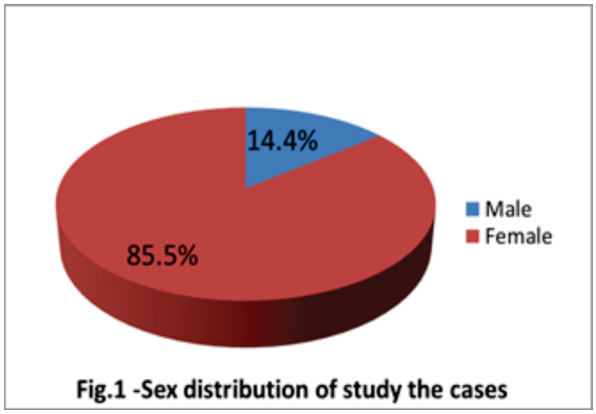

Fig.1 -Sex distribution of study the cases

The most common presenting complaints among patients suffering from gall bladder diseases were right hypochondrial pain (92%), followed by nausea (3.3%), epigastric pain (3%), vomiting (2.6%).(Table 2)

SYMPTOMS	FREQUENCY	%
Right hypochondrial pain	1500	92
Nausea	55	3.43
Epigastric pain	50	3
Vomiting	44	2.6

Table 2:Distribuion of cases according to presenting complaints

Out of 1630 of cholecystectomy specimens, on histopathological interpretation, 1625(99.6%) specimens showed non neoplastic pathology and 6 (0.3%) specimens showed neoplastic pathology(Fig.2)

Fig:2- Neoplastic and non neoplastic distribution of study cases

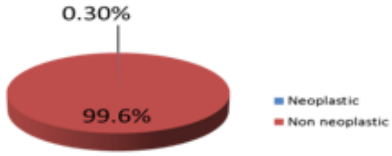

Table 3:Histopathological spectrum of cholecystectomy specimens

Histopatholgical Diagnosis		Number & %
Non –Neoplastic (N=1624)(99.6%)	Chronic cholecystitis with cholelithiasis	1465(89.7%)
	Chronic cholecystitis with cholesterolosis	118(7.85%)
	Follicular cholecystitis	2(0.12%)
	Acute cholecystitis	1(0.06%)
	Chronic cholecystitis with dysplasia	28(1.7%)
	Chronic cholecystitis with metaplasia	10(0.674%)
Neoplastic (N=6)(0.3%)	Benign (Adenoma)	4(0.24%)
	Malignant(well differentiated adenocarcinoma)	2(0.13%)

Amongst non neoplastic conditions, we got predominantly inflammatory lesions mostly chronic chelecystitis followed by cholelithiasis and cholesterolosis .We got 2 cases of follicular cholecystitis(Fig.3) and 1 case of acute cholecystitis. There were 28 cases of chronic cholecystitis with dysplasia(Fig.4) and 10 case of metaplasia, which can be considered as pre malignant lesions but not true neoplastic lesions. We got 6 neoplastic lesions, 4 were benign tumours(Adenomas) and 2 were malignant tumour (Table 3) . Both the malignant tumours were incidental well differentiated adenocarcinoma (Fig.5). The malignant tumours also displayed positivity for p53 and Ki-67 immunostaining (Fig.6 & 7 respectively).

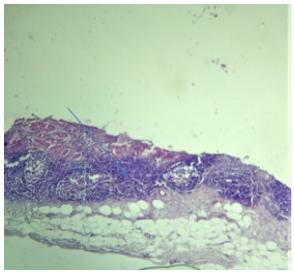

Fig3.Gall bladder mucosa with features follicular cholecystitis (H&E,10x)

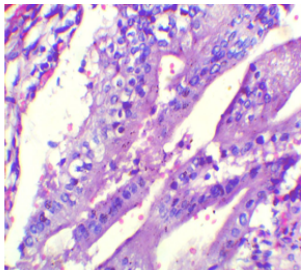

Fig4.Gall bladder mucosa displaying moderate dysplasia (H&E,40x)

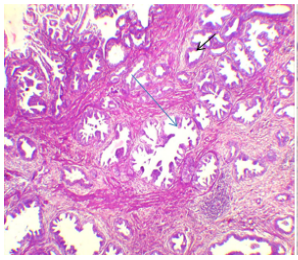

Fig 5.Well differentiated adenocarcinoma(H&E,10x)

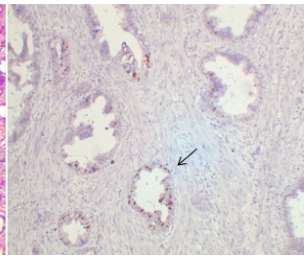

Fig 6. p53 immunostaining

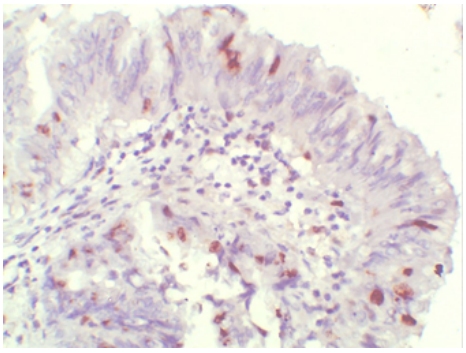

Fig7.Ki67 immunostaining

Out of 10 metaplasia and 28 dysplasia cases, gallstones were present in 80% and and 92% respectively and it was statistically significant, showing the strong association of gallstones in developing metaplasia and dysplasia. Out of 2 incidental carcinoma 50% was associated with gallstones however it was not statistically significant which may be due to small sample size(Table 4)

Table 4: Association of metaplasia, dysplasia and carcinoma

STONES	PRESENT	ABSENT	
METAPLASIA	8(80%)	2(20%)	X^2-28.847, p<0.01(significant)

DYSPLASIA	**26(92%)**	**2(8%)**	**X^2-97.106, p<0.01(significant)**
CARCINOMA	**1(50%)**	**1(50%)**	**X^2-0.403, p=0.693(not significant)**

DISCUSSION:

Histopathological examination of every resected gall bladder is of utmost importance. While the diagnosis in most cases is chronic cholecystitis, a spectrum of other morphoplogical changes are commonly seen which include cholelithiasis, acute inflammation ,cholesterolosis, metaplasia and dysplasia. Uncommonly cholecystectomy specimen may reveal an unexpected gall bladder carcinoma.Therefore a detailed knowledge of the architectural variations of the gall bladder is essential so that none of these changes are misdiagnosed as malignant and no cases of malignancy is missed.

In our study the male to female ratio was calculated to be 1: 5.9. In a study by Selvi et al, gall stone diseases was predominantly seen in female (61.5%) as compared to males (38.4%)[3]. Male to female ratio observed was 1: 4 as also reported by Schirmer et al (2005). The result of the present study exhibits a female preponderance thus holding true the saying that a fatty, fertile, flatulent, female of forty is the classical sufferer from symptomatic gallstones. Female hormones and sedentary habits of most woman predisposed them to factors that possibly promote the formation of gallstones[7].

The majority of the age group of the patient in our study ranged from 40-49 years. Similar results were observed by other studies like Mittal et al 2010, Bawasaheb et al 2013, Arathi NA et al and Awasthi N 2016[8,9,10,11] who had maximum numbers of patients in the 41 to 50 years age group. Deranged cholesterol mechanism which increase with age probably leads to increase in prevalence of gallstone formation and bile saturation.

In the present study the commonest presenting complaint among patients suffering from gallbladder diseases was right hypochondrial pain (92%) . This observation was consistent with findings of Agrawal et al[12].

Our present study showed that the majority cases were inflammatory lesions followed by pre-malignant and malignant conditions. Kumbhakar D [13] found 395(98.75%) specimen of gall bladder showing non- neoplastic conditions and 5 (1.25%) showed neoplastic pathology which was similar to our study. Chronic cholecystitis is the most commonly encountered disease of the gall bladder; therefore majority of the cholecystectomies are performed for this condition [14,15,16]. The degree of chronic inflammation may vary and comprise of predominantly lymphocyte with few plasma cell, histiocytes, and occasional eosinophils. Acute cholecystitis on the other hand is mainly a clinical entity caused by abrupt injury of the gall bladder. It is an acute destructive process typically associated with ischemia, congestion, edema, epithelial denudation, vascular leakage, haemorrhage and fibrin deposition. In our study there were 1465 (89.7%) cases of chronic cholecystitis with cholelithiasis and 118 (7.85%) cholesterolosis, only 1 case of acute cholecystitis and 2 cases of follicular cholecystitis. There were premalignant conditions such as dysplasia in 1.7% cases , metaplasia in 0.674% cases . Neoplastic lesions recorded were 4 benign cases (adenoma) and 2 malignant conditions as well differentiated adenocarcinoma (0.13%) , both of which were incidental findings occurring in older age. Selvi et al had reported 85.8% cases with chronic cholecystitis, 2.5% with acute cholecystitis, 2.5% polyp, 1.2% empyema, 5.1% eosinophilic cholecystitis and 1.2% carcinoma.

It is well known that gallstones is an important risk factor for the development of carcinoma gallbladder but casual relationship is still unproven. Martinez et al[17] have also illustrated that low and high grade dysplasia, tubular adenomas, carcinoma in situ and invasive carcinoma were frequent when cholelithiasis was present (p<0.05) than in case without lithiasis. In present study gallstones were present in over 50% of cases of gall bladder carcinoma. Piehler JM, Crichlow RW et al[18] have also found the incidence of 65-90% of gallstones in the patients with gall bladder carcinoma that is in concordance with present study. Gallstones were present in 92% of gall bladders showing dysplasia and in 80% of cases showing metaplasia. Yamigawa H[19] have found gallstones in 86.9% of cases with dysplasia ; very similar to our study of 92%. They have found intestinal metaplasia and pseudopyrolic gland metaplasia in 80.4% and 100% of patient with gallstones.

The pathogenesis, by which gallstones lead to gall bladder carcinoma is a matter of debate, but it has been suggested that the frequency of various epithelial changes is more common in calculous cholecystitis than in normal gall bladders. It is due to constant chronic irritation and inflammation leading to such epithelial changes that the gall stones may be involved in the pathogenesis of gall bladder cancer [20].

Both the incidental adenocarcinomas in the present study were subjected for immunostaining of p53 and Ki-67 and we could observe that there was overexpression of p53 and positivity of Ki-67 (> 20% of tumour cells were positive for the stain). Several studies of p53 protein overexpression in gallbladder carcinoma and its precursor lesions have suggested an important role in the pathogenesis of the tumour[21]. Shrestha [22] found that patients with gall bladder carcinoma that expressed high Ki-67 immunoreactivity had a worse post operative prognosis than those that did not, but there was no relationship between p53 positive tumours, Ki-67 immunoreactivity and patient survival.

CONCLUSION:

The present study gives the different histopathological pattern of cholecystectomy specimens. Thorough histopathological analysis is important to detect incidental carcinoma. There is strong correlation of gallstones with metaplasia ,dysplasia and carcinomas. Presumably, gall bladder containing stones develop cancer as a result of constant irritation, trauma, and chronic inflammation. High level of ki-67 index predicts early recurrences after surgical ressection.

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