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Original Research Article

HISTOPATHOLOGICAL LESIONS IN CHOLECYSTECTOMY SPECIMENS AND THEIR CORRELATION WITH CLINICAL PROFILE

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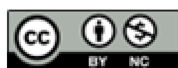


ABSTRACT

Background: The gallbladder is a pear-shaped organ of the biliary system responsible for storage and concentration of bile. Cholelithiasis is one of the most common biliary tract diseases worldwide, with an incidence ranging from 6–20% globally and 2–29% in India. Gallbladder lesions vary from inflammatory and premalignant conditions to carcinoma, and histopathological examination remains the gold standard for definitive diagnosis. **Materials and Methods:** This prospective cross-sectional study was conducted over two years (September 2022–February 2024) in the Department of Pathology, D.Y. Patil Medical College, Kolhapur. A total of 100 cholecystectomy specimens were examined. Specimens were fixed in 10% neutral buffered formalin, processed routinely, and stained with Hematoxylin and Eosin. Clinical and radiological data were recorded. Lesions were categorized as inflammatory, premalignant, or malignant. **Result:** The most affected age group was 41–60 years. Females (58%) outnumbered males (42%) with a male-to-female ratio of 1:1.38. Cholelithiasis was present in 77% of cases. Chronic cholecystitis was the most common lesion (71%), followed by acute cholecystitis (10%), xanthogranulomatouscholecystitis (5%), and cholesterolosis (4%). Metaplasia was observed in 16% and dysplasia in 4% of cases, predominantly in females. Carcinoma gallbladder accounted for 3% of cases, all of which were adenocarcinoma and mostly detected incidentally. **Conclusion:** Benign inflammatory lesions were most common in the fifth and sixth decades, whereas malignant lesions were seen predominantly after the seventh decade, with marked female preponderance. Routine histopathological examination of all cholecystectomy specimens is essential for early detection of incidental gallbladder carcinoma and premalignant changes, thereby improving patient prognosis.

INTRODUCTION

Gall bladder is a pear-shaped hollow organ situated in a shallow depression on the right lobe of liver.[1] It belongs to the biliary system, which is also referred to as the biliary tract or biliary tree.[1] The main function of gall bladder is the storage and concentration of bile and releasing it into the small intestine. After consumption of food, it contracts and releases the bile into intestine.[2]
Gall stones are formed when stored bile gets saturated with cholesterol or bilirubin.
Cholelithiasis is a common condition worldwide and comprises of more than 90% of biliary tract diseases.[3,4]
The geographical distribution of gallbladder diseases varies all over the world with some areas being high risk incidence areas such as Japan, India, South America and Eastern Europe.[5-7] The incidence of gallstone disease in the world is approximately 6-20%.[7,8]
The incidence of gallstone disease in India is 2 to 29%.[9]
The removal of gallbladder or cholecystectomy is one of the most common emergency surgery performed all over the world as gall stones are one of the major cause of morbidity and mortality in the world.[10]

Its indications include inflammation of gall bladder, symptomatic gall stones, risk factor for gall bladder malignancy and pancreatitis caused by gall stones.[3] Due to changes in diets of people with inclusion of high calorie foods and fats and increase in alcohol consumption, the incidence of cholelithiasis and cholecystitis has shown an increasing trend in the last three to four decades.[3,11,12]

Cholelithiasis is known to cause different histopathological changes in gall bladder mucosa like acute or chronic inflammation, cholesterolosis, hyperplasia, metaplasia, dysplasia and carcinoma.[3,13,14]

The various modalities of detection of various gall bladder pathologies are clinical examination, radiological investigations (such as USG, MRI, ERCP, MRCP, etc.),[15] and histopathology. Clinical diagnosis of the gallbladder diseases is made, based on history and clinical examination along with laboratory and radiological findings.[10,15] However, histopathological examination is the gold standard for the diagnosis of gallbladder pathologies. Histopathological examination often times reveals an unusual diagnosis bearing significant implications on the treatment, prognosis and outcome of the patient.[10]

In a study by CD Jokhi et al, they found that clinical presentation of diseases of gall bladder is vague.[10] Even malignancy of gallbladder presents late in the course and with nonspecific symptoms, which can misguide the clinicians.[10,17] Findings of malignancy are subtle even on radiological examinations. Confirmatorydiagnosis of malignancy is made only by histopathological examination.[10]

Gallbladder carcinoma is the one of the most common cancer of the biliary tract and it accounts for the fifth cause of gastro-intestinal or GI cancer5,16. It's geographical distribution is diverse, as its prevalence is high in Japan, as well as some regions of India, Eastern Europe and South America, while it is relatively rare in Northern Europe and America. The disease represents a diagnostic and clinical challenge, since it presents often with non-specific findings and in two-third of the cases, it is found incidentally on histopathologic examination after cholecystectomy – when it is known as incidental gallbladder carcinoma (IGC). Prognosis in this case is poor, as the overall 5-year survival rate is less than 5%.[18,19]

The various lesions observed in gallbladder diseases are: Benign Lesions20, Cholelithiasis, Cholecystitis: Chronic cholecystitis, Acute cholecystitis, Xanthogranulomatouscholecystitis, Follicular cholecystitis, Eosinophilic cholecystitis, etc. Cholesterolosis, Benign Neoplasms20, Polypoidal Lesions, Adenomyomatous hyperplasia, Papillary Hyperplasia, Metaplasia, Mucocele RokitanskyAschoff Sinus, Heterotopia, Adenoma, Malignant Neoplasm: Adenocarcinoma, Squamous cell carcinoma, Adenosquamous carcinoma, Neuroendocrine tumors and neoplasms Mixed neuroendocrine non-neuroendocrine neoplasm.

MATERIALS AND METHODS

An observational prospective cross-sectional study was conducted for 2years subjects were fulfilling all exclusion and inclusion criteria were selected for study. and the materials would comprise of the cholecystectomies received at The Department of Pathology, D. Y.Patil Medical College, Kolhapur, between September 2022 to February 2024. The sample size was54.

Inclusion criteria

All cases of cholecystectomy performed at Dr. D. Y.Patil Medical College Hospital and Research Centre, Kolhapur. Exclusion Criteria Autolysed specimen.

Nvestigations: Radiological investigations- USG.

Methods of Research: Total of 100 specimens of gallbladder were examined which were sent in 10% neutral buffered formalin solution. Relevant clinical data, including age, sex, chief complaints and laboratory and radiological investigations were noted from the requisition forms sent along the specimens.

A detailed study of the specimens was done with respect to size, colour, external surface, mucosa and the presence of gall stones, and the findings were noted down.

The entire organ was opened longitudinally. Stones if present, were washed, an estimate of the number and the size of the largest was made. Lymph nodes were searched along the neck of the gallbladder.

Three sections including the entire wall thickness were taken one each from the fundus, body and neck. Additional sections were taken from any gross pathological lesions such as wall thickening, ulceration, polyp, etc.

Sections weretaken from the cystic duct and/or lymph nodes in case of a visible tumor or suspicion of maliganacy.

Paraffin embedding of the sections was done. The sections were cut and stained with haematoxylin and eosin.

FIXATION68,69:

For morphological examination of the tissue, autolysisis to be prevented. Specifically, bacterial contamination which leads to putrefaction i.e. enzymes are released from within the tissue which cause autolysis, labile substances can be destroyed and soluble substances can be lost into the surrounding medium. The primary aim of fixation is to prevent or arrest these changes.The reagent used for fixation of tissue in this study was neutral buffered formalin. 10% formalin consists of 10 ml of 40% formaldehyde and 90 ml water. The volume of fixative i.e. formalin should be atleastten times the volume of the tissue to be fixed. The tissue is immersed in the fixative for 12-24 hours.

Steps involved in the processing of the sample68,70:

Dehydration, Clearing, Impregnation with wax, Embedding with wax, Dehydration:

This is the process of removal of water from the tissue. The procedure is to treat the tissue bits with increasing gradient of alcohol. For this the tissue bits are placed in a tissue capsule along with theidentification number written on a small piece of paper with pencil and it is then exposed to 70%, 90% and absolute alcohol in a successive pattern (3 changes of 1-2 hours each). Other dehydrating agent can also be used likeacetone.

Clearing: Alcohol and paraffin wax are not miscible; hence, these solutions should be replaced by wax solution. Since most of the wax solutions increase the refractive index, the tissue appears clear. Hence, the process is known as clearing. The substances used are as follows: Chloroform, Xylene, Benzene and Toluene.

Impregnation with wax: After clearing, the tissue is transferred to a bath of molten paraffin wax maintained at a constant temperature of 50-56°C for 2-3 hours. During this process, the clearing agent is eliminated from the tissue by diffusion into surrounding molten wax (infiltration) following which the wax diffuses into the tissue to replace the clearing agent (impregnation). Infiltrated paraffin after cooling gives necessary firmness and support tothe intracellular structures to maintain proper relation to each other when they are cut on microtome.

Embedding: This is also known as blocking. It is a method of placing the infiltrated impregnated tissue in warm liquid paraffin (embedding medium) that solidifies into a firm block when it cools down. The most commonly used mould is called Leuckhardt'sMould which is made from brass. For embedding, the metal mould is filled with appropriate paraffin wax. Then the tissue is rapidly transferred to wax containing mould with a pair of warm forceps. No air bubble should be trapped and the tissue with the surface to be sectioned should be placed down, against the base of the mould. After solidification is complete, the block is removed from the mould.

Preparation of section68: The block is clamped to the head of the Rotary Microtome and thinsections of 4-6 microns are cut. Microscopic slides are prepared byapplying a thin layer of Mayer's Glycerol-Albumin mixture (equalvolume of egg white, glycerine and small crystal of thymol which acts asa preservative). The tissue section is floated on warm water bathmaintained at a temperature 2°C lower than the melting point of wax. Thecoated slide is dipped at 90° angle such that the section touches the edgeof the slide.

Staining and mounting68:

Basic steps are as follows:

Removal of wax with xylene.

Removal of xylene with alcohol.

Hydration-Section is placed under water for 5 minutes.

Staining Routinely employed stain is H&E.

Dehydration Section is treated with alcohol.

Clearing-By treating the section with xylene.

Mounting Coverslip is placed on mounting medium laid over section.

Haematoxylin and Eosin Stain:

Solutions used: Harris's Hematoxylin: Its constituents are as follows- Hematoxylin - 2.5 gm, Absolute alcohol - 25ml, Potassium alum - 50 gm, Distilled water - 500ml, Mercuric oxide - 1.25 gm, Glacial acetic acid - 20 ml

The haematoxylin is dissolved in the absolute alcohol, and is then added to the alum, which has been dissolved in the warm distilled water in a 2-litre flask. The mixture is rapidly brought to the boil and the mercuric oxide is then slowly and carefully added. Plunging theflask into cold water containing chipped ice rapidly cools the stain. When the solution is cold, the acetic acid is added, and the stain is ready for immediate use. Addition of glacial acetic acid gives more precise and selective staining of nuclei.

Acid alcohol: Alcohol 70% - 1000 ml, Hydrochloric acid, conc. - 10 ml

Ammonia water: Tap water - 1000 ml, Ammonium hydroxide 28% - 2-3 ml

Saturated lithium carbonate, Lithium carbonate - 1 gm, Distilled water - 100 ml

1% stock eosin, Eosin Y, water soluble - 1 gm, Distilled water - 20 ml

Dissolve and addAlcohol 95% - 80 ml

Working eosin solution

Eosin stock solution - 1 part

Alcohol 80% - 3 parts

Just before use add 0.5 ml Glacial acetic acid to each 100 ml of stain and stir.

Staining Procedure

Dewax sections, hydrate through graded alcohols to water.

Stain in Harris's hematoxylin for 15 mins.

Wash in running tap water until the sections 'blue' for 5 minutes.

Differentiate in 1% acid alcohol for 5-10 secs.

Wash well in tap water until sections are "blue" again.

Stain in 1% eosin Y for 10 minutes.

Dehydrate through graded alcohols, clear and mount.

Results:

Nuclei - blue/black

Cytoplasm - varying shades of pink.

RESULTS

The following observations were made after analyzing the cholecystectomy specimens received in the Department of Pathology, D.Y. Patil Medical College, Kolhapur from September 2022 to February 2024.

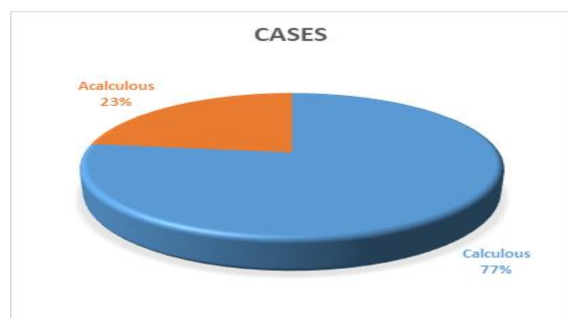
Table 1: Distribution of cases by histopathological diagnosis

Diagnosis	Cases
Chronic cholecystitis	71
Acute cholecystitis	10
Xanthogranulomatouscholecystitis	5
Cholesterolosis	4
Acute on chronic cholecystitis	3
CA Gallbladder	3
Gangrenous Gallbladder	2
Mild Dysplasia	1
Lymphoeosinophiliccholecystitis	1
Total	100

It is evident by this table that: Chronic cholecystitis (71%) is the most common histopathological diagnosis.
Followed by Acute cholecystitis (10%), Xanthogranulomatouscholecystitis (5%) and cholestrolosis (4%).Carcinoma gallbladder and and acute on chronic cholecystitis had incidence of 3% each.Least common diagnosis is Gangrenous gallbladder (2%), Mild dysplasia (1%) and lymphoeosinophiliccholecystitis (1%).

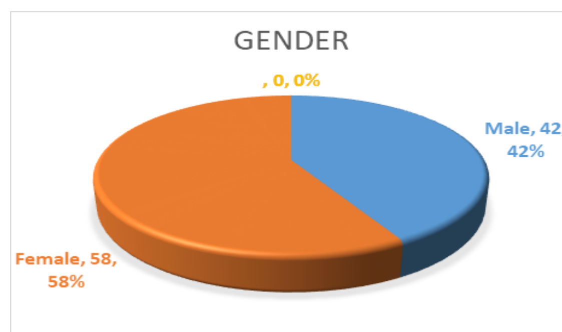
Table 2: Presence of Calculi in cholecystectomy specimens

Calculous	77
Acalculous	23
Total	100



Pie Chart 1. Distribution of Calculous and Acalculous cases

A higher number of cholecystectomy cases were found to have cholelithiasis (77%).
Cholelithiasis showed female preponderance.
Acalculouscholecystitis showed slight male preponderance.



Pie chart 2. Distribution of cases on basis of gender

It is evident by this chart that:
Female preponderance is more than male.
The M:F ratio is 1:1.38
The M:F ratio is in concordance with the study by Kumar H et al71 which was 1:1.33.

Table 3. Distribution of gallbladder lesions on basis of gender

Male	42
Female	58
Total	100

Table 4: Age distribution of cholecystectomy cases

Age Range	No. of Cases
10-20	1
21-30	9
31-40	14
41-50	20
51-60	20
61-70	18
>71	18

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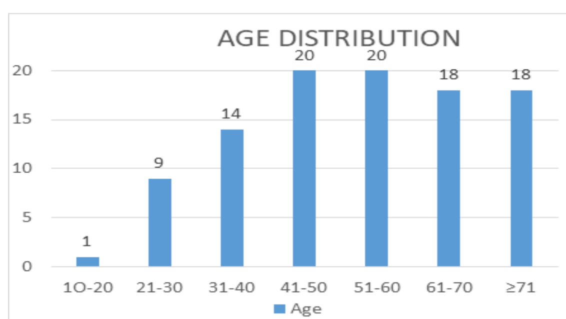

Bar chart 1. Distribution of cases based on age

It is evident based on this chart:
The most commonly affected age groups are fifth and sixth decade of life.
The least common age group is second decade of life.
No cases were found in first decade of life.

Table 5: Distribution of gallbladder lesion according to Age

Histopathological Diagnosis	10-20	21-30	31-40	41-50	51-60	61-70	>70	Total
Chronic Cholecystitis	1	7	11	15	12	13	12	71
Acute Cholecystitis	-	-		2	2	3	3	10
Xanthogranulomatouscholecystitis	-		1	1	2	1	-	5
Cholesterolosis	-	1	1	1	1	-	-	4
Acute on chronic cholecystitis	-	-	-	1	-	1	1	3
CA Gallbladder	-	1	-	-	-	-	2	3
Gangrenous Gallbladder	-	-	-	-	2	-	-	2
Mild Dysplasia	-	-	1	-	-	-	-	1
Lymphoeosinophiliccholecystitis	-	-	-	-	1	-	-	1
Total	1	9	14	20	20	18	18	100

It is evident from this table that:
Most commonly affected age in commonest finding i.e. chronic cholecystitis is fifth decade.

Carcinoma gallbladder is observed most commonly after the seventh decade of life.
Gallbladder lesions are commonly observed from the fifth decade of life.

Table 6: Incidence of gallbladder with and without gallstones

Histopathological Diagnosis	Calculous	Acalculous	Total
Chronic Cholecystitis	64	7	71
Acute Cholecystitis	5	5	10
Xanthogranulomatouscholecystitis	3	2	5
Cholesterolosis	2	2	4
Acute on chronic cholecystitis	1	2	3
CA Gallbladder	1	2	3
Gangrenous Gallbladder	0	2	2
Mild Dysplasia	1	0	1
Lymphoeosinophiliccholecystitis	0	1	1
Total	77	23	100

It is evident from this chart:
Higher number of cholecystectomy cases had calculi.
Calculous chronic cholecystitis was the most common finding.

Acalculouscholecystitis was seen more in cases of acute on chronic cholecystitis, gangrenous gallbladder and lymphoeosinophiliccholecystitis.

Table 7: Incidence of Metaplasia and Dysplasia

Histopathological Finding	Females	Males	No. of cases
Metaplasia	11	5	16
Dysplasia	3	1	4
Total	14	6	20

It is evident from this chart: Metaplasia was found in 16% of cases and the most common type found was intestinal metaplasia. Metaplasia was most commonly seen in females as compared to males. Dysplasia was seen in 4% of cases in the study with a female preponderance. Half of the cases had mild dysplasia whereas the other half had moderate dysplasia. 50% of the cases were associated with adenocarcinoma of gallbladder.

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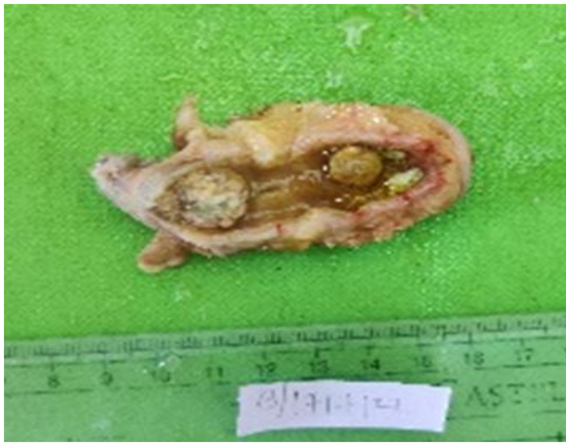

Figure 1: Gross image of gallbladder with thickened wall and cholesterol stones.

Figure 2: Gross image of gallbladder with bile-stained mucosa and single pigmented stones.

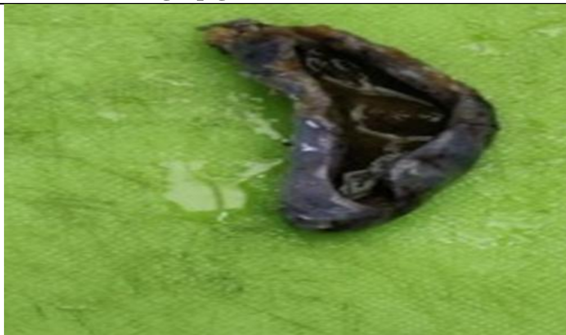

Figure 3: Specimen showing a tumour in the wall of gallbladder

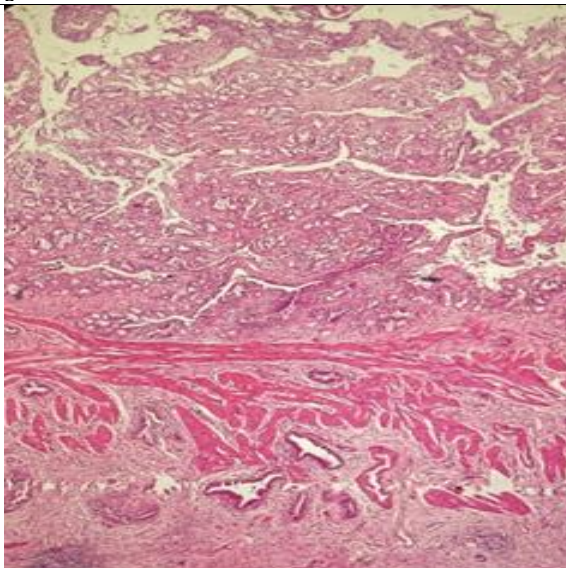

Figure 4: Acute Cholecystitis- Showing epithelial hyperplasia and RokitanskyAschoff Sinuses (100X)

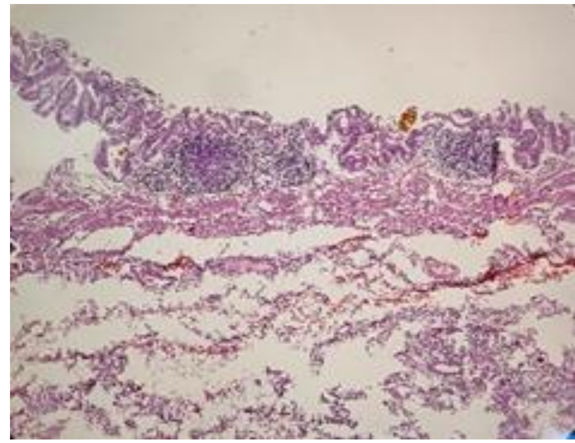

Figure 5. Chronic cholecystitis with follicle formation in muscle layer

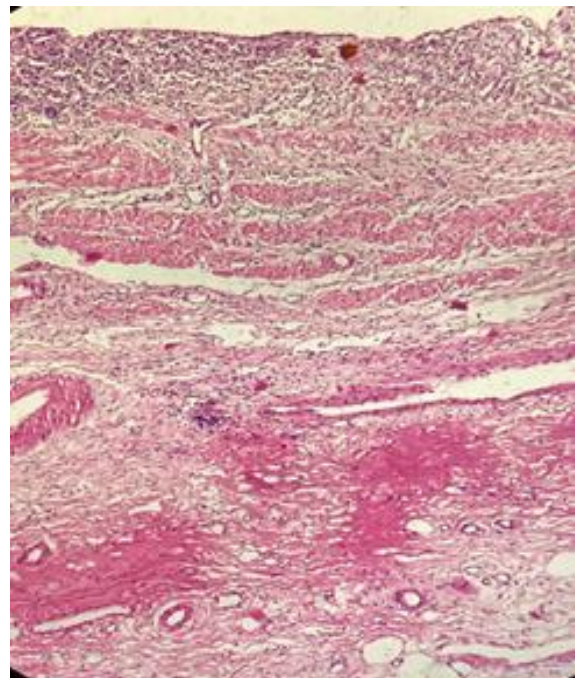

Figure 6: Chronic cholecystitis with marked fibrosis.

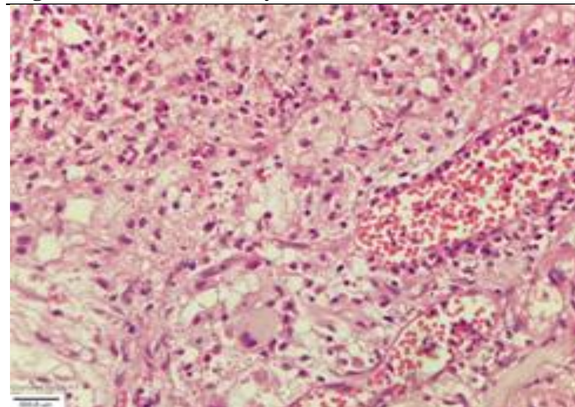

Figure 7: XanthogranulomatousCholecystitis – Foamy macrophages

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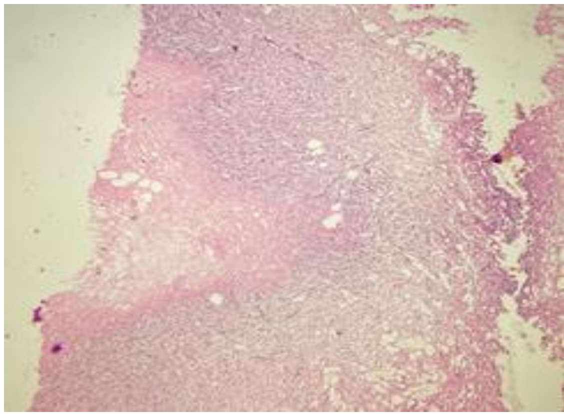

Figure 8: Gangrenous Cholecystitis- Dense inflammation with areas of necrosis

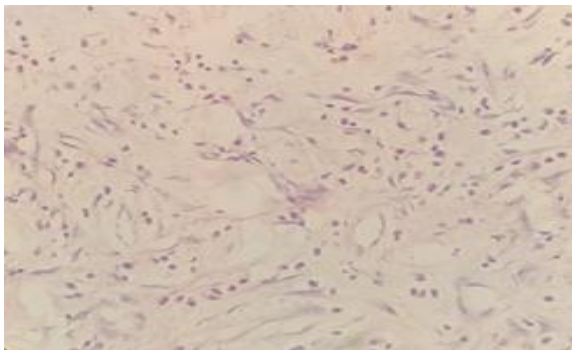

Figure 9: Lymphoeosinophiliccholecystitis

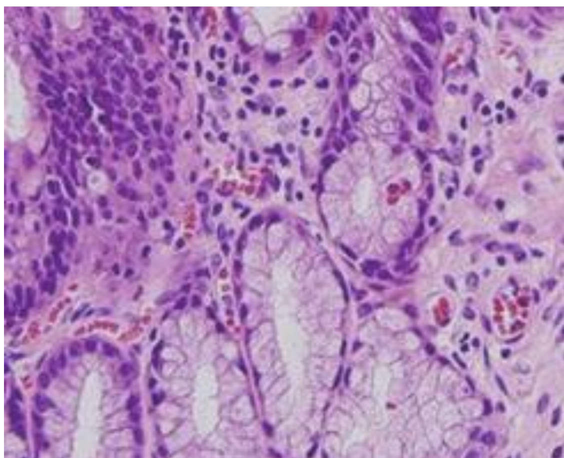

Figure 10: Intestinal Metaplasia

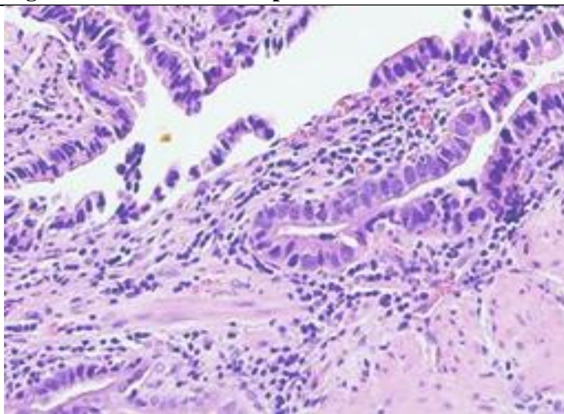

Figure 11. Dysplasia

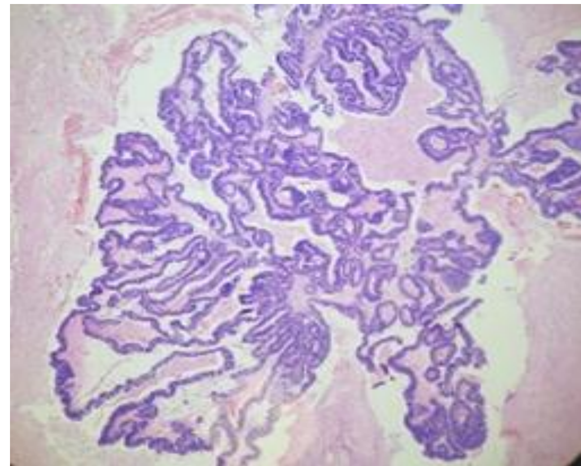

Figure 12: Adenocarcinoma Intracholecystic Papillary Neoplasm(ICPN) type

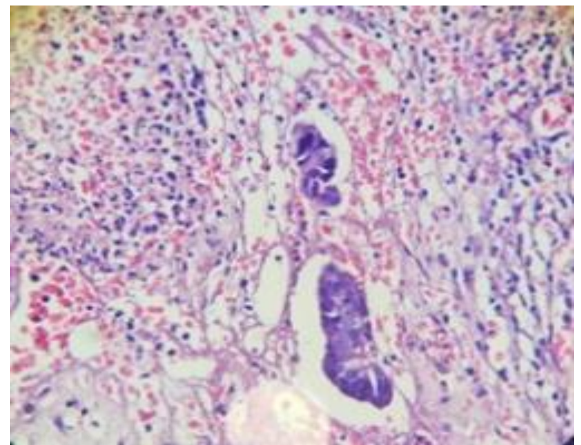

Figure 13: Adenocarcinoma – Lymphovascular invasion

DISCUSSION

The geographical distribution of gallbladder diseases varies all over the world with some areas being high risk incidence areas. The incidence of gallbladder disease in the world is approximately 6-20%.[8]

The known epidemiology of gallstone disease like fatty, fertile, female of forty seems to be shifting nowadays towards thin young female and male patients. The trend is changing with most of the patients presenting in their thirties with basal metabolic index (BMI) in overweight and normal category instead of obese along with narrowing of the male: female ratio.[21]

The various pathological lesions observed in gallbladder diseases are acute cholecystitis, chronic cholecystitis, cholelithiasis, cholesterolosis, xanthogranulomatouscholecystitis, eosinophilic cholecystitis, gangrenous cholecystitis, cholecystitisfollicularis, gallbladder adenoma, dysplasia, and adenocarcinoma of gallbladder.

India is also a high incidence area for Gall Bladder Cancer (GBC) and contributes about 10% of the global GBC burden.

The aim of this study was to quantify various histopathological outcomes of routine

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cholecystectomy specimen examination along with socio-demographic characteristics of patients involved.

In this study the most common age group affected was 41-60 years. This is in concordance with study by Anushree CN et al, Jokhi et al and Kumar et al.[3,10,28]

The second most common age group affected was more than 60 years followed by age group 31-40 years and 21-30 years. Only a single case was observed in the age group of 10-20 years. No cases were seen in the first decade of life.

Majority of the neoplastic cases were seen in eighth decade of life. This is in concordance with study by DiMauro et al while Jokhi et al, Kumar et al reported after seventh decade of life.[5,10,28]

Majority of the cases seen in the age group of 21-30 years (total 9 cases) were of chronic cholecystitis (7 cases) i.e. 77.7%. while one case each was of Cholesterolosis and Adenocarcinoma of gallbladder. Most of the cases (88.8%) in that age group were associated with cholelithiasis.

In this study the total number of females affected was 58% and males affected was 42%.

This showed female preponderance with a M:F ratio of 1:1.38. This was in concordance with study conducted by Kumar et al, Anushree CN et al and Bhosale VR et al.[3,28,29]

Cholelithiasis was observed in higher number of cholecystectomy cases (77%). This is in concordance with all the previous studies.

The most common lesion associated with gallstones was chronic cholecystitis. Acalculous cases were mostly seen in gangrenous gallbladder and lymphoeosinophiliccholecystitis.

Earlier studies show 2-3 times higher risk of gallstone diseases in females.

This study showed M:F ratio of 1:1.06 for gallstone diseases.

In this study the incidence was highest for chronic cholecystitis among all the comorbidities studied which was 71%

The most common clinical presentation observed was pain in abdomen.

Kumar et al,[28] had an incidence of 66.75% with female preponderance. The study by Degloorkar et al,[23] had an incidence rate of 70.8%.

Butti et al,[30] had an incidence rate of 70.86%.

Kotasthane et al,[22] showed an incidence of 75%. All studies showed female preponderance and high association with gallstones as was seen in our study.

Of the 71 cases, 7 cases (9.85%) of chronic cholecystitis were observed in the age group of 21-30 years and a single case (1.4%) was seen in a 17year old.

Majority of the cases seen in the age group of 21-30 years were females (71.42%) and 85.71% cases showed cholelithiasis.

On gross average the wall thickness of gallbladder specimens observed was 2mm to 5mm and mucosa was bile stained with ulceration and presence of gallstones.

In this study, we found 10% incidence of acute cholecystitis with male preponderance after the seventh decade of life. Our study also matches in clinical features such as pain in abdomen, nausea and fever with the literature. Stones were found in 50% of the cases in our study.

Kumar H et al,[28] had an incidence of 6% in their study with female preponderance and high association with gallstones.

Jokhi et al,[10] had and incidence rate of 9.20% in their study with 66.6% association with gallstones.

Study by Degloorkar et al,[23] showed a higher incidence than our study of 27.78%.

Xanthogranulomatouscholecystitis is difficult to distinguish from chronic cholecystitis clinically. Microscopically it shows high cellularity along with presence of foamy macrophages.

In this study the incidence of xanthogranulomatouscholecystitis is 5% with most cases seen in sixth decade of life and slightly higher association with gallstones.

In the study by Srinivasan et al,[9] the incidence was 3%.

Jokhi et al,[10] found an incidence rate of 2.3% in their study with higher association with gallstones.

Kotasthane et al,[22] found an incidence of 2.3% in their study.

The incidence of xanthogranulomatouscholecystitis was slightly higher in our study as compared to others.

In this study we found a 4% incidence of cholesterolosis and the cases ranged between third to sixth decade of life.

The study by Kotasthane et al,[22] showed incidence of 5.81%.

Anushree CN et al,[3] showed higher incidence of 8.42% for cholesterolosis.

In this study 3% cases were observed of acute on chronic cholecystitis with a greater number of cases after seventh decade of life. There is high association with gallstones with female preponderance.

In study by Abeer et al,[31] they found an incidence of 6%.

In our study we found an incidence of 2% with all cases in sixth decade of life and 100% cases were acalculous.

Kumar H et al,[28] had an incidence of 2.25% in their study with slightly higher association with gall stones.

Lymphoeosinophiliccholecystitis is a rare diagnosis observed in acalculouscholecystitis cases. The etiology and pathophysiology is not well understood but is suggested to be association with various conditions such as parasitic infections due to Ascaris species, Clonorchis species or Echinococcus species, gastroenteritis, allergies or hyper-eosinophilic syndromes or drug therapies like cephalosporins and herbal medicines.[32]

The study showed a single case (1% incidence) in a male patient in the sixth decade of life and associated with acalculouscholecystitis.

In this study epithelial changes like metaplasia were observed in 16% of cases with a female preponderance.

Majority of the cases were seen in the seventh decade followed by the eighth decade.

In this study 93.75% cases showed intestinal metaplasia.

In study by Kumar H et al,[28] metaplasia was seen in 23.75% cases.

In study by Anushree et al,[3] epithelial metaplasia was observed in 16.8%.

A study by Mathur et al,[33] showed 18% cases with metaplasia.

The incidence of carcinoma gallbladder is 3% in our study. 100% cases were of Adenocarcinoma of gall bladder which is in concordance with other studies.

100% of the cases were observed in female patients with most of the cases seen after seventh decade of life with more cases being acalculous.

The study by Kim et al also showed lower association of gallstones with gallbladder carcinoma than acute cholecystitis as was seen in our study.

In a study by Jokhi et al,[10] the incidence was observed to be 3% and higher number of acalculous cases.

In a study by Kotasthane et al,[22] the incidence was 2.33% with female preponderance.

Kumar H et al,[28] had an incidence of 1.25% with female preponderance.

A study by Shaffy et al,[34] showed an incidence of 3.5%.

Most of the cases of carcinoma presented as chronic cholecystitis where adenocarcinoma was an incidental finding which is in concordance with previous studies. Peak incidence seen in seventh decade of life is similar to that seen in previous studies.

CONCLUSION

Various histopathological lesions including benign, premalignant and malignant were studied extensively and classified accordingly. This study found benign lesions commonly in the fifth to sixth decade while malignant lesions were seen after eighth decade. The benign as well as malignant lesions showed female preponderance.

Though the incidence of gallbladder carcinoma is less, it is of utmost importance to be detected early, because in the primary stages cholecystectomy is curative and failure to detect could lead to catastrophic consequences. Gallbladder carcinoma in clinical presentation mimics benign entities, and most of the cases are diagnosed incidentally on routine histopathological examination. Thus, histopathology is the gold standard for diagnosis of carcinoma.

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