Original Article

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Clinicopathological study of chronic calculous cholecystitis with chemical analysis of gallstones

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Abstract:

BACKGROUND: Chronic-calculous-cholecystitis is the most common benign disease affecting the gallbladder occurring in the middle aged female population. Our aim is to study clinicopathological features of gallstone disease with biochemical analysis of calculi. **METHODOLOGY**: This study was conducted between August 2011 –February 2013 at Upgraded department of pathology, Osmania general hospital. We received 100 cholecystectomy specimens with gallstones which were analysed histopathologically and chemical analysis of gall stones was done. **RESULTS**: Maximum number of cases in our study were seen in fourth decade with female preponderance (1:2.1).55% of cases presented with pain in right hypochondrium . 85% were non vegetarians. Obesity was seen in 35% of cases. .80% of cases had multiple stones and 78% had mixed stones on biochemical analysis. Increased thickness of wall of gallbladder seen in 57% of cases on gross examination. On histopathology, 80% of cases with chronic nonspecific cholecystitis were associated with mixed stones.. Follicular-choleystitis and xanthogranulomatous-cholecystitis seen in 8% and 2% of cases and were associated with pigment and cholesterol stones respectively. 4% of cases had acute on chronic cholecystitis and were associated with mixed stones .Carcinoma of the gallbladder was seen in 6% of cases which were associated with mixed and pigment stones. **CONCLUSION**: In our study 80% of cases had chronic nonspecific cholecystitis and mixed stones were commonest. Clinicopathological study of gallstone with biochemical analysis helps us to understand its etiopathogenesis and hence maybe helpful in preventing cholelithiasis.

Key words: Biochemical analysis of gallstones; Chronic calculous Cholecystitis; Mixed stone; Pathogenesis

Introduction:

Gallstone disease is a very common gastrointestinal disorder seen in middle aged female population [1] and they are present mostly in the western world [2]. The incidence of gallstone disease is 10% to 20% of the world population [3]. In India, prevalence of gall stones disease has been reported as 2-29% and seven times more common in the north India [4].10% of the adult patients have asymptomatic gall stones. The prevalence varies with age, sex and ethnic group. The incidence of cholecystitis is higher in females, with a female to male ratio of 3:1 upto the age of 50 and a ratio of approximately 1.5:1 thereafter [1]. Prevalance of this disease increases from 21 to 80 years. Risk factors like female gender, obesity, dietary factors and diabetes play a vital role in the development of calculous cholecystitis. Gallstones can occur anywhere within the biliary tree, including the gallbladder and the common bile duct.

Some patients with cholesterol gallstones were treated with high doses of chenodeoxycholic acid, and this kind of treatment was suitable only for 20% or less of gall stones carriers. Therefore cholecystectomy is presently one of the commonest operation done for this disease. Understanding the epidemiology and pathology of gallstone formation helps in improving therapy and possibly preventing the disease. Varied morphological changes ranging from inflammation to malignancy can be observed with the gallbladder associated with stones.

Increased pathophysiological interest in the formation of gallstones made the knowledge of their exact chemical composition very important especially of cholesterol gallstones, the only stone

which can be treated with cholelitholytic agents. Analysis of chemical composition of gallstones can provide a significant reference to the treatment and prevention of their reoccurrence .To the best of our knowledge there is no published literature available on clinicopathological study of gallstone disease along with biochemical analysis of calculi in our region .

Objective:

Aim of the study is to correlate clinicopathlogical features of chronic calculous cholecystitis with chemical analysis of gallstones and review of literature.

Materials and Methods:

This is a prospective study conducted at Upgraded department of pathology, Osmania general hospital between August 2011 –February 2013(19 months) for 100 cases of gallbladder with cholelithiasis.

Inclusion criteria-

Patients with prior clinical diagnosis of cholelithiasis only were selected for study.

Exclusion criteria-

Specimens sent as acalculous cholecystitis and with prior diagnosis of malignancy were excluded from study.

Cholecystectomy specimens sent were cut open fresh, gallstones removed, labelled with corresponding histopathology number and biochemical analysis of gallstones was done. The specimens were fixed in 10% buffered formalin, minimum of three sections were taken from fundus, body and neck of gall bladder. Tissue was processed by routine histological technique with paraffin embedding and sectioning at 4 micron thickness and stained with hematoxylin and eosin.

Biochemical analysis of gallstones-

Gallstones were powdered using a mortar and dissolved in different solvents depending on the type of chemical constituent to be analysed and analysis was done as per the procedure described by Varley Harold [2]. Different tests done for different constituents are as follows

Tests for cholesterol-

Liberman's buchard reaction-

Dissolve the stone residue in chloroform and add mixture of acetic anhydride (9.9ml) and concentrated sulphuric acid (0.1ml).Presence of grass green colour indicates it is positive for cholesterol.

Tests for calcium-

To stone residue, add ammonium oxalate, KOH, 1% acetic acid ,25% HCL. Presence of white precipitate indicate it is positive for calcium.

Tests for oxalates-

To stone residue, add calcium chloride, KOH, 1% acetic acid, 25% HCL.Presence of white crystals indicate it is positive for oxalates.

Tests for phosphates-

To stone residue, add 25% HCL, ammonium molybdate, vitamin C.Presence of yellow colour is seen first which later turns blue when it is positive for phosphates.

Tests for carbonates -

To the stone residue, add few ml of 25% HCL.Presence of effervescence indicate it is positive for carbonates.

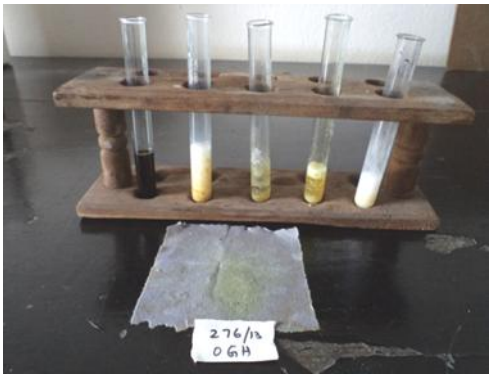


Figure 1: Showing biochemical analysis-mixed stones.

Results:

Total number of specimens studied were 100.

Age wise distribution-

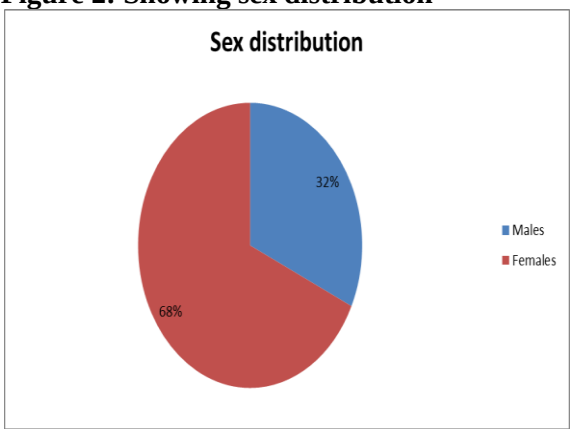
Table 1: Age wise distribution

Age in years	No. of cases	Percentage of cases
18 – 20	6	6%
21 – 30	18	18%
31 – 40	36	36%
41 – 50	21	21%
51 – 60	13	13%
61 – 70	4	4%
71 – 80	1	1%
81 – 90	1	1%

Age range in our study was between 18 years to 90 years. The youngest patient reported was 18 years & the oldest was 90 years. Maximum number of cases were in the 4th decade

Sex Distribution:

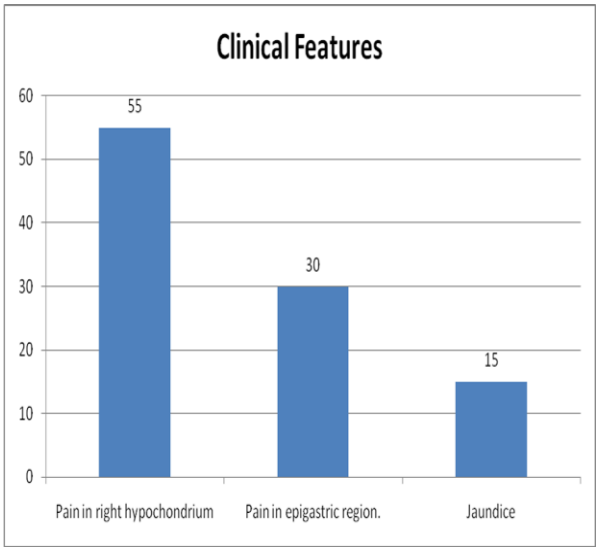
Figure 2: Showing sex distribution



Our study had 68 (68%) females and 32 (32%) males. Females out numbered the males with a male to female ratio of 1:2.1.

Clinical features-

Figure 3: Showing clinical features



Pain in the right hypochondrium was the most common symptom seen in 55 cases(55%) .

Diet and BMI:

Table 2: Showing dietary habits and BMI.

Diet	**No.of cases**	**Percentage of cases.**
Non vegetarian	85	85%
Vegetarian	15	15%
BMI kg/m^2	**No. of cases**	**Percentage of cases.**
>30	35	35%
<30	65	65%

Non vegetarians were affected more than vegetarians with a ratio of 5.6:1. Obesity was seen in 35% of cases in our study with a BMI >30 kg/m^2

Number of gallstones:

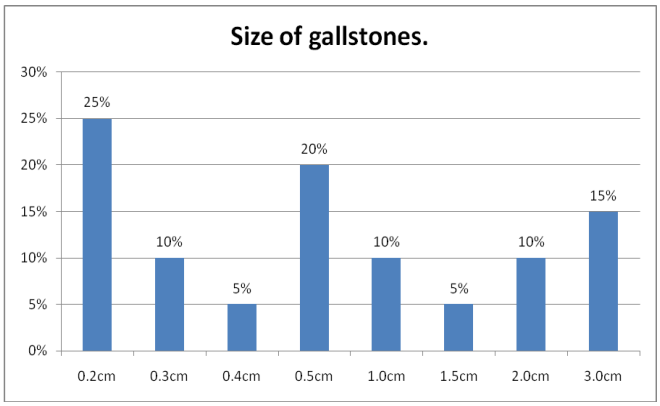
Table 3: Showing number of gallstones

No. of gallstones	**No. of cases**	**Percentage of cases.**
Multiple stones	80	80%
Single stones	20	20%

Multiple stones were the most commonest stones seen in 80% of the cases.

Size of the gallstones:

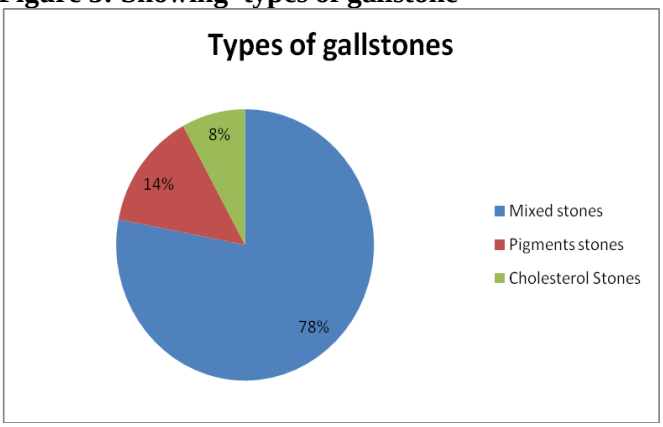
Figure 4: Showing size of gallstones.



Size of the gallstone varied from 0.2 to 3 cm with maximum number of stones measuring 0.2cm (25%) in our study.

Types of gallstones on biochemical analysis:

Figure 5: Showing types of gallstone



Mixed type of stones were the most commonest stone seen in 78% of cases on biochemical analysis followed by pigment stone seen in 14% of the cases and cholesterol stones seen in 8% of cases.

Gross examination of gallbladder:

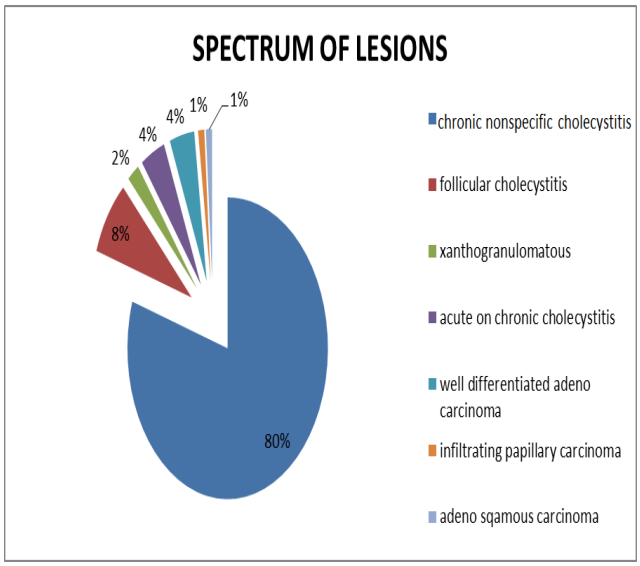
Table 4: showing thickness of gallbladder.

Thickness of gallbladder	No. of cases	Percentage of cases.
>3mm	57	57%
<3mm	43	43%

On gross examination, thickness of gall bladder was increased in 57% of cases.

Spectrum of lesions of the gall bladder:

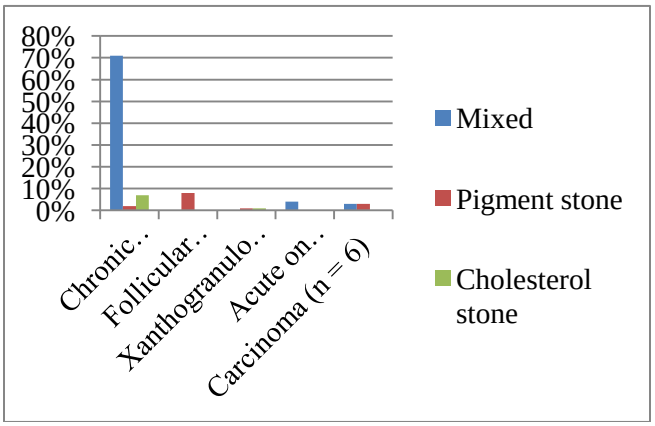
Figure 6: Showing spectrum of lesions of the gallbladder



Chronic nonspecific cholecystitis was the commonest lesion encountered in our study seen in 80% of cases

Correlation of types of gallstones and lesions of gall bladder:

Figure 7: Showing correlation of types of gallstones and lesions of gallbladder.



Mixed stones (71%) were the commonest stones associated with chronic nonspecific cholecystitis. Pigment stones (8%) were seen in follicular cholecystitis . xanthogranulomatous cholecystitis had both pigment (1%) and cholesterol stone(1%).Acute on chronic cholecystitis was associated with mixed stones in 4% of cases. Out of 6 cases of adenocarcinoma , both mixed and pigment stones were seen in 3% of cases each respectively.

Discussion:

Cholelithiasis is the most common biliary pathology. Gall stones has an incidence ranging from 10% to 20% worldwide. The prevalence of gallstone varies widely in different parts of the world. In India it is estimated to be around 4%. An epidemiological study restricted to rail road workers showed that north indians have 7 times higher occurrence of gallstones as compared to south Indians.

Gallstones are categorized as cholesterol, mixed, black pigment, or brown pigment stones[5]. Cholesterol and mixed gallstones are formed from biliary sludge, while pigment stones are composed of calcium salts of unconjugated bilirubin, with varying amounts of cholesterol and protein. Incomplete and infrequent emptying of the gallbladder may cause the bile to become concentrated and contributes to gallstone formation. The second factor is the presence of proteins in the liver and bile that either promote or inhibit cholesterol crystallization into gallstones. Increased levels of the hormone estrogen as a result of pregnancy, hormone therapy, or the use of birth control pills, may increase cholesterol levels in bile and also decrease gallbladder movement resulting in gallstone formation. Other factors are parity, smoking, alcohol, diabetes and obesity. Symptoms of gall stone diseases are pain in the right upper abdomen, fever, jaundice, abdominal fullness, clay-colored stools, nausea and vomiting. Complications include gallstone pancreatitis, gallstone ileus, biliary cirrhosis and gallbladder cancer.

Our study was done to know clinicopathological features of gallstone disease in correlation with chemical analysis of gallstones. Age range of patients in our study was between 18 – 90 years of age with a mean age of 54. The youngest patient who was 18 years old is a known case of sickle cell anemia and had pigment stones. Oldest patient was 90 years old who was asymptomatic for many years. Asymptomatic cholelithiasis seen in >80% of cases has been documented in literature.

Maximum number of cases in our study were in the range of 31 – 40 years (36%) which is similar to Mohan et al [4] in which maximum number of cases were in the 4th decade. Thamil selvi et al [2], SK Mathur et al [6], had a peak incidence in 5th decade.

Pain in the right hypochondrium was the most common symptom in our study which is similar to Thamil selvi et al [2] and Kamran et al [7]. Pain in the epigastric region is seen in 30% of cases in our study whereas Thamil selvi et al [2] had only 15.3% cases.Jaundice was seen in 15% of cases in our study whereas Thamil selvi et al [2] observed jaundice as presenting feature only in 3.8% of cases .In our study patients presented late with symptoms of obstruction thereby presenting with jaundice. In India, 97% cases of cholelithiasis were found in non vegetarians. Most of the patients in our study are non vegetarians (80%) compared to vegetarians (20%) with a ratio of 4:1 similar to Thamil sevi et al [2] in which nonvegetarians were preponderant with a ratio of 6:1.Obesity is defined as body mass index >30 kg/m2 which in our study was seen in 35% of cases as compared to Thamil sevi et al [2], who had 39% of obese cases. cholelithiasis is seen in overweight patients because bile salts in bile is reduced leading to increase in cholesterol.

Multiple stones are more common (80%) than solitary stones (20%) in our study which is similar to Thamil sevi et al [2] and SK mathur et al [6]who also had increased number of multiple stones. This indicates that cholecystitis with multiple stones are more symptomatic than those with solitary stones. Sizes of stones in our study varied from 0.2 to 3.0 cm. Stone of the largest size was a solitary cholesterol stone. In study by Thamil selvi et al [2], size of stone varied from 0.3 to 2.0 cm. On biochemical analysis, mixed stones (78%) were the commonest stones seen in our study similar to Thamil selvi et al [2],Chandran et al [8]which were studies done in south India. However a study by Taher et al [9]which was conducted in Baghdad , found cholesterol stones as the most commonest stones which again emphasizes the regional variation due to ethnicity and dietary habits.

On gross examination,increased thickness of gall bladder (>3 mm) due to chronic inflammation was seen in 57% of cases which is similar to SK Mathur et al[6] . On histopathology,majorityof cases in our study had chronic nonspecific cholecystitis (80%) comprising of lymphocytes, plasma cells, histiocytes and occasional eosinophils which is similar to study by Mustafa mazlum et al [10],SK mathur et al [6]and Thamil selvi et al [2]. In our study,follicular choleystitis and xanthogranulomatous cholecystitis were seen in 8% and 2% of the cases each respectively whereas in study by SK mathur et al [6], follicular and xanthogranulomatous cholecystitis were seen in 5% and 3% of cases respectively. Follicular cholecystitis occurs in gram-negative bacterial infection and may be associated with stones. Xanthogranulomatous cholecystitis occurs due to penetration of bile into the gall bladder wall from mucosal ulcers or ruptured rokitansky aschoff sinuses along with outflow obstruction by calculi and infection. Eosinophilic cholecystitis was not seen in our study since it is usually associated with acalculous cholecystitis which were excluded from our study. Acute on chronic cholecystitis was seen in 4% of the cases in our study whereas SK Mathur et al [6] had 12% of the cases.

Carcinoma of the gall bladder was seen in 6% of cases in our study. All the cases were sent with a prior clinical diagnosis of chronic cholecystitis. In contrast to our study, Thamil selvi et al [2] and Mustafa mazlum et al [10] has a lower incidence of carcinoma.

Chronic nonspecific cholecystitis seen in 80% of cases was most commonly associated with mixed stones which is similar to Mohan et al[4]. Adenocarcinoma seen in 6 % of the cases in our study was associated with mixed and pigment stones equally whereas in study by Mohan et al[4], carcinoma seen in only 1.09% cases is associated only with pigment stones.

Conclusion

Cholelilithiasis has an increased prevalence in females and non vegetarians. Efforts should be taken to reduce all variable risk factors which lead to cholelithiasis, especially among females. Upper abdominal ultrasound helps in early screening and detection. Early cholecystectomy is the treatment of choice.

Studying the morphological spectrum of gallstone disease with chemical analysis of gallstones helps us to understand its etiopathogenesis and hence maybe helpful in preventing cholelithiasis.

Figure 8: Showing Gallbladder and pigment stone

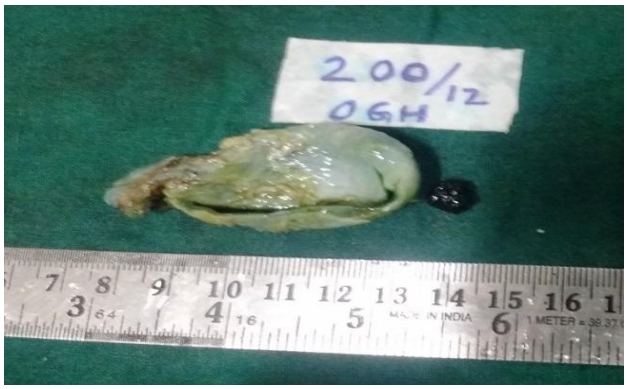


Figure 9: Showing Gallbladder and cholesterol stone

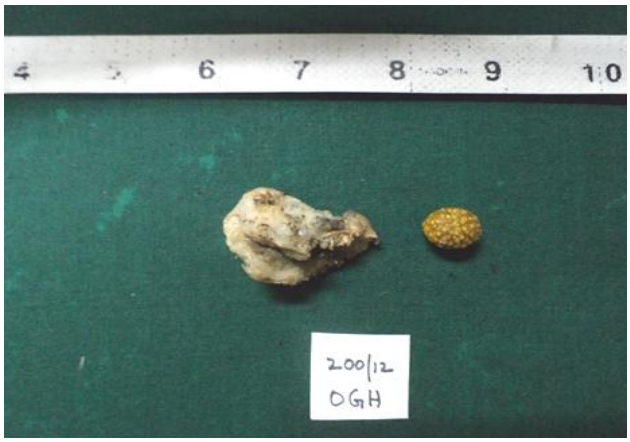


Figure 10: Showing gallbladder with greywhite areas and pigment stones

Figure 11(H&E,4x): Histopathological section of chronic nonspecific cholecystitis

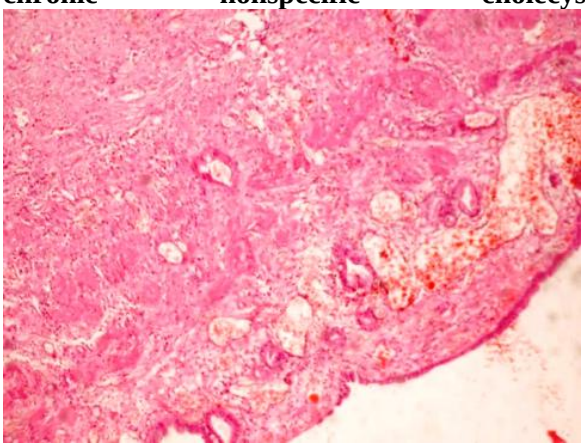


Figure 12(H&E,10x): Histopathological section of chronic nonspecific cholecystitis.

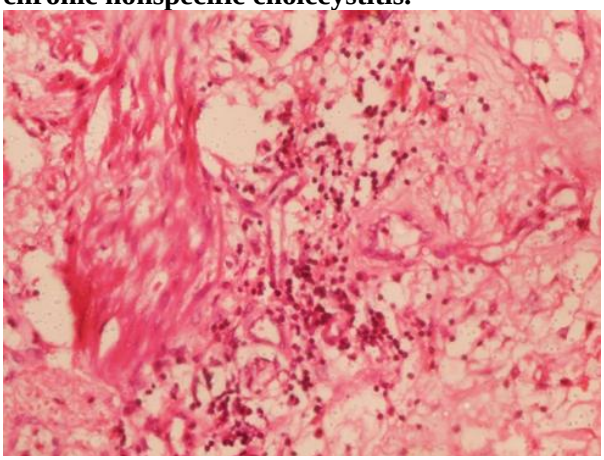


Figure 13(H&E,4x): Histopathological section of xanthogranulomatous cholecystitis

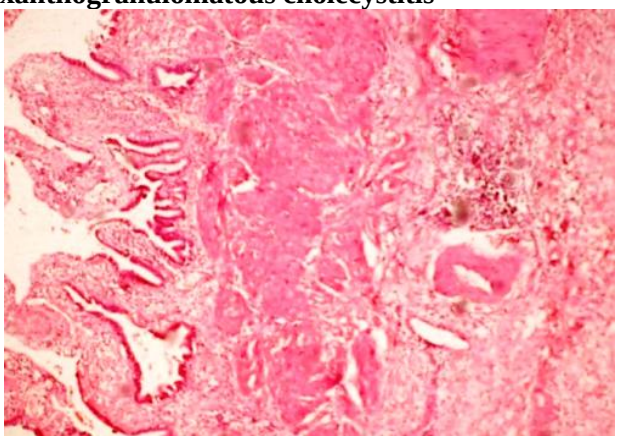


Figure 14(H&E,10x): Histopathological section of xanthogranulomatous cholecystitis

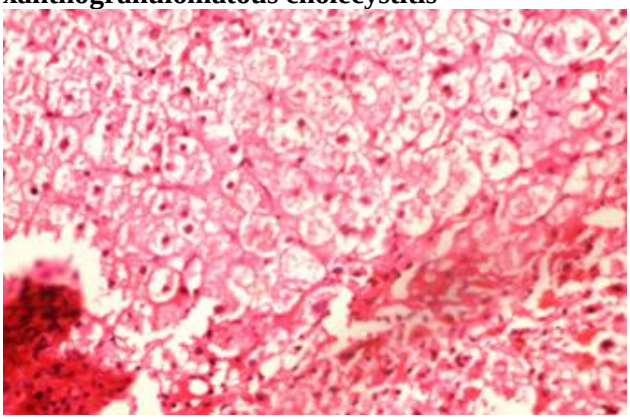


Figure 15(H&E,4x): Histopathological section of adenocarcinoma

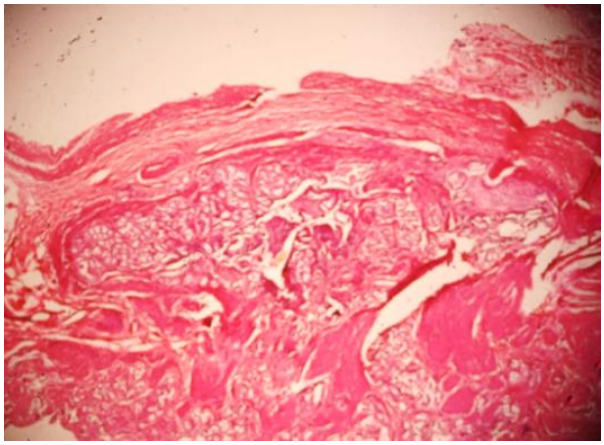
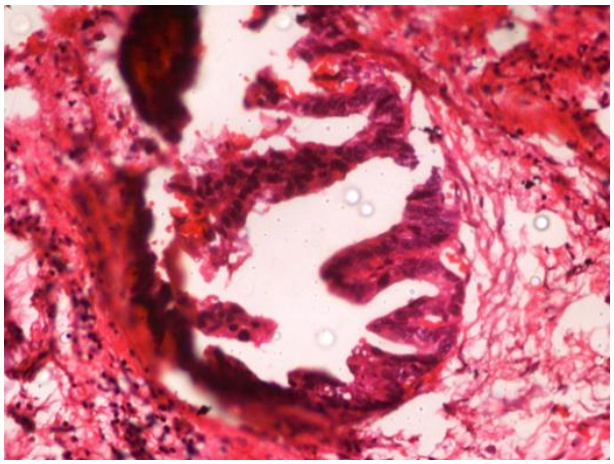


Figure 16(H&E,10x): Histopathological section of adenocarcinoma



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