

Association of types of gallstones with biochemical analysis and mucosal changes in gallbladder

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Abstract

Introduction: Carcinoma of the gallbladder is the commonest malignancy of the biliary tract. The incidence of the disease in India is 2.3 and 1.01 per 100,000 in female and male population respectively. It is 10 times more frequent in North compared to South India. Gallstones appear to be the most important risk factor, being reported in 70-98% cases of gallbladder cancer, a far higher prevalence than that in age-matched general population. This study, aims to look at the demography, clinical presentation and risk factors and also analyse the association of morphological characteristics and biochemical nature of the gallstones, and correlates the effect of gallstones on the mucosal changes in wall of the resected gall bladder specimens and its association with malignant changes.

Methods: This was a prospective study in which 50 patients presenting with clinical features of chronic

calculous cholecystitis in Vydehi Institute of Medical Sciences, Bangalore with diagnosis of chronic calculous cholecystitis were included in the study. Their demographic data, symptomatology and associated illness were documented. All were subjected to cholecystectomy, the gall bladder was sent for histopathological examination

Results: The highest age incidence of gallstone disease was 41-55 years of age, more common in females and North Indian people. Pain abdomen was the most common symptom. 90% of the patients consumed mixed diet. 56% of subjects presented with increased BMI. 26% of patients underwent open cholecystectomy, 70% of patients underwent laparoscopic cholecystectomy and of which 4% of laparoscopic cases were converted to open. Multiple calculi was seen in 56% of the subjects and single calculi were seen 44%. mixed stones 44%, pure cholesterol stones 38%, pure pigmented stones 18%.

Calcium oxalate was seen in 38% ,60% of bilirubin,56% of patients had no bilirubin. 10% patients had magnesium Biliverdin, hematin and fibrin where seen in variable amounts. Histopathological changes in the gall bladder mucosa showed features of chronic cholecystitis such as Rokitansky Asc off sinuses 66%,1 patient had features of benign papillary hyperplasia. One patient with B-thalassemia showed xantho granulo matous changes, two patients showed features suggestive of malignancy, one patient had well differentiated adenocarcinoma with focal areas of Rokitansky - Asc off sinuses, one patient had well differentiated adenocarcinoma (papillary variant). Average size of gallstones was found to be 0.762cm in cases with inflammation and benign conditions, whereas in cases with carcinoma was greater (0.902 cm). Average weight of the stone was 0.688 gm in cases of chronic inflammatory changes, whereas in carcinoma average weight was found to be 0.889gm. Mean duration of the hospital stay was more in open cholecystectomy 12.27 days when compared with laparo scopic cholecystecto my7.43 days.

Conclusion: We noted that gallstones contributed to various mucosal changes, of which the mixed and cholesterol stones were found with carcinomatous change in the gall bladder. Were commend that cholecystectomy should be offered to all asymptomatic gallstone patients, as they are a precursor for gallbladder carcinoma.

Keywords: Chronic calculous cholecystitis, Chole cystecto my, Mucosal changes in gall bladder, Bio chemical analysis of gallstones

Introduction

Gallstone disease is common problems affecting the digestive tract. The prevalence of gallbladder stone varies widely in different parts of the world. In India, it is around 4%. An epidemio logical study in rail road workers showed that North Indians have 7 times higher

occurrence of gallstone as compared to South Indians.¹In the United States, the third National Health and Nutrition Examination Survey (NHANES III) has revealed prevalence of gallstones of 7.9% in men and 16.6% in women.²There are several risk factors for development of cholelithiasis such as female gender, obesity, dietary factors and diabetes. The incidence of cholecystitis is higher in females, with a female to male ratio 3:1 upto about the age of 50 and a ratio of approximately 1.5:1 thereafter.⁵ In India, according to study by Wani N. A , the female preponderance was 4.4:1.Cholelithiasis is also found to be the most important risk factor for gallbladder carcinoma and was first described by Maximillian Stoll in 1777 and even after 200 years, it is still considered to be an extremely malignant disease with a poor prognosis.⁶Gallstones are found to be associated in 70% to 90% of the cases of gall bladder cancer and approx. imately 0.4% of all patients affected with gallstone disease develop carcinoma of the gallbladder.^{6,7,8}The incidence of gall bladder carcinoma parallels the prevalence of gall stone disease; large and longstanding gallstones to about four to seven times.⁸Gallstones injure the mucosal columnar epithelium of gallbladder and thus result in various changes like metaplasia, dysplasia and neoplasia in gallbladder epithelium.⁶

Methods

Prospective study of fifty patients between 10-80 years of both gender with gallstone disease admitted operated and consenting for the study at Vydehi Institute of Medical Sciences and Research Centre, Bangalore during January 2016 - July 2017 were included. Assuming the gall bladder carcinoma as 3%, with an absolute precision of 5%, at an alpha level of 5%, a sample size of 45 was calculated, and rounded up to 50. Patients diagnosed with acalculous gall bladder carcinoma, gall bladder polyps and patients not consenting for surgery were excluded.

All patients were followed up for six months. The data with clinical presentation was recorded as per the proforma and subjected to USG abdomen / CT scan, blood investigations and were planned for open or laparoscopic cholecystectomy on elective/emergency. The stones were retrieved from the resected gallbladder specimen and studied for number of stones, Size – diameter measured using Vernier caliper. Weight – using electronic analytical and precision balance and volume water displacement method. The gallstones were powdered using a pestle and mortar and dissolved in different solvents depending upon the type of chemical constituent to be analyzed. To determine total cholesterol and total bilirubin, 30mg stone powder was dissolved in 3 ml chloroform in a test tube and kept in boiling water bath for 2 minutes and the solution was analysed by CHOD-PAP and diazo method. To determine calcium, oxalate, inorganic phosphate, magnesium (calmagite method), chloride, soluble protein, triglycerides (GPO – PAP method), sodium and potassium, 30 mg stone powder was dissolved in 3 ml IN HCl in graduated 10 ml tube and its final volume made up to 10 ml with distilled water and the tube was kept in boiling water bath for 1 hr and the solution used for analysis. To analyze phospholipids, stone powder (20 mg) was dissolved in 15 ml $\text{CHCl}_3 + \text{CH}_3\text{OH}$ in 2:1 ratio, containing 1N HCl. Total cholesterol and triglycerides by enzymatic colorimetric method of Bayer Diagnostic India Ltd, total bilirubin by colorimetric method of Accurex Biomedical Pvt. Ltd. Soluble protein by colorimetric method of Lowry et al, oxalate as described by Satyapal and Pundir based on enzymatic colorimetric method, calcium by OCPC kit method of Miles India Ltd, phospholipid & inorganic phosphate by colorimetric method of Fiske and Subba Rao, fatty acids by colorimetric method of Stern and Shapiro and bile acids by colorimetric method of Carey.

Sections from the entire wall thickness of the gall bladder specimen – 2 from body, one each from fundus and neck, was processed for paraffin embedding. Additional sections were taken from abnormally appearing mucosa; stained with Hematoxylin and Eosin. Study of gall bladder mucosa for the type of inflammation, cholesterol losis, mucocele, hyperplasia, metaplasia, dysplasia and malignant changes was studied.

Fig 1: HPE micro photograph showing features of acute cholecystitis

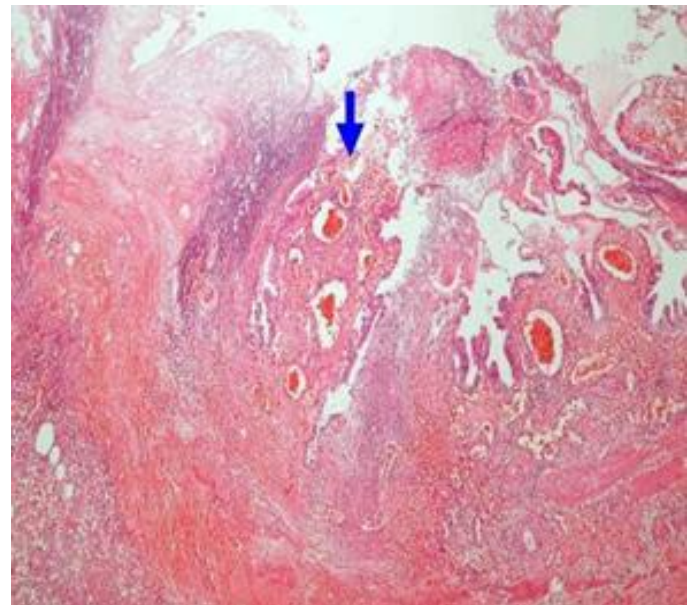


Fig 2: HPE micro photo graph showing chronic cholecystitis

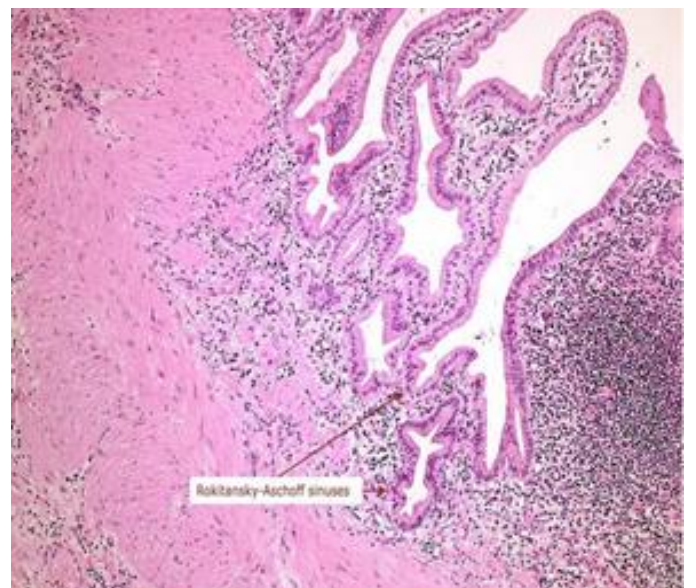


Fig 3: cholecystectomy specimen showing gall stones with carcinoma of gall bladder



Results

Table 1: Distribution of subject according to age group

Age group	Frequency	Percent
10-25yrs	7	14
26-40yrs	18	36
41-55yrs	20	40
56-70yrs	5	10
Total	50	100.0

Table 2: Distribution of subject according to sex

Sex	Frequency	Percent
Female	31	62.0
Male	19	38.0
Total	50	100.0

Table 3: Demographic distribution of patients

Place	Frequency	Percent
North India	38	76.0
South India	12	24.0
Total	50	100.0

Table 4: Distribution of subjects according to BMI

BMI	Frequency	%
18.5-24.9	20	40.0
25-29.9	22	44.0

<18.5	8	16.0
Total	50	100.0

Table 5: Showing association of BMI with the type of stone

BMI	Type of stone			Total	P value
	Cholesterol	Mixed	Pigmented		
18.5-24.9	5	11	4	20	0.028 significant
25-29.9	12	9	1	22	
<18.5	2	2	4	8	
Total	19	22	9	50	

Table 6: Table showing association of diet and type of stone

Diet	Type of stone			Total	P value
	Cholesterol	Mixed	Pigmented		
Mixed	16	20	9	45	0.422 not significant
Veg	3	2	0	5	
Total	19	22	9	50	

Figure 1: Bar Graph showing distribution of subject according to presenting sign tenderness were present in 62% of the subjects, icterus in 20% of the subjects, fever were present in only 4% of the subject.

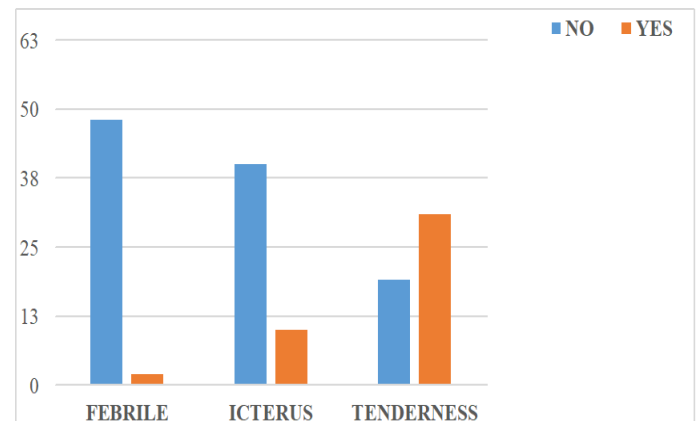


Table 7: Distribution of subject according to type of stones

Type of Stone	Frequency	Percent
Cholesterol	19	38.0
Mixed	22	44.0
Pigmented	9	18.0
Total	50	100.0

Table 8: Based on biochemical analysis of the stones

Cholesterol	Frequency	Percent
Nil	9	18.0
20%	1	2.0
30%	7	14.0
40%	3	6.0
50%	2	4.0
60%	1	2.0
70%	3	6.0
80%	4	8.0
90%	1	2.0
100%	19	38.0
Total	50	100.0

Table 9: Showing distribution of calcium oxalate in gallstones

Calcium oxalate	Frequency	Percent
Nil	31	62.0
10%	9	18.0
20%	10	20.0
Total	50	100.0

Table 10: Showing distribution of bilirubin in gallstones

Bilirubin	Frequency	Percent
Nil	28	56.0

20%	1	2.0
30%	4	8.0
40%	10	20.0
50%	3	6.0
60%	4	8.0
Total	50	100.0

Table 11: Table showing distribution of subject according type of stones

Magnesium	Frequency	Percent
Nil	45	90.0
10%	5	10.0
Total	50	100.0

Table 12: Showing distribution of other components in gallstones

	Frequency	Percent
Nil	27	54.0
Biliverdin 10% hematin 10%	2	4.0
Biliverdin 10% hematin 20%	1	2.0
Biliverdin 20%	2	4.0
Biliverdin 20% fibrin 10%	1	2.0
Biliverdin 30% hematin 10%	1	2.0
Biliverdin 40% hematin 20%	2	4.0
Biliverdin 10% fibrin 20%	1	2.0
Biliverdin 40% fibrin 10%	1	2.0
Calcium carbonate 10%	1	2.0
Calcium carbonate 10% cysteine 10%	1	2.0
Fibrin 10%	1	2.0
Fibrin 10% hematin 10%	1	2.0
Fibrin 20% mucin 30%	1	2.0

hematin 20%		
Fibrin 30% mucin 10%	1	2.0
Fibrin 40% hematin 20%	1	2.0
Hematin 20%	1	2.0
Mucin 20%	1	2.0
Phosphate 10%	1	2.0
Phosphate 10% calcium carbonate 10%	1	2.0
Phosphates 20%	1	2.0
Total	50	100.0

Table 13: Distribution of subject according to type of stones and other changes

Others	Type of stone			Total
	Cholesterol	Mixed	Pigmented	
Nil	1	0	0	1
Acute Cholecystitis	0	1	0	1
CIC	1	0	0	1
Rokitansky-aschoff Sinuses	7	2	1	10
Well Differentiated Adenocarcinoma with Rokitansky sinuses	0	1	0	1
Rokitansky-aschoff Sinuses+ Reactive lymph nodes	0	2	1	3
Rokitansky-aschoff Sinuses + Wall Congestion	9	15	5	29
Papillary Hyperplasia	0	0	1	1
Cholesterosis	1	0	0	1

Xanthogranulomatous Cholecystitis	0	0	1	1
Well Differentiated Adenocarcinoma (Papillary variant)	0	1	0	1
Total	19	22	9	50

Table 14: Showing correlation of type of stones with different mucosal changes

	Type of stone			Total
	Cholesterol	Mixed	Pigmented	
Cholesterosis	1	0	0	1
	5.3%	.0%	.0%	2.0%
Chronic cholecystitis	18	20	7	45
	94.7%	90.9%	77.8%	90.0%
Papillary Hyperplasia	0	0	1	1
	.0%	.0%	11.1%	2.0%
Well Differentiated Adenocarcinoma	0	2	0	2
	.0%	9.1%	.0%	4.0%
Xanthogranulomatous	0	0	1	1
	.0%	.0%	11.1%	2.0%
Total	19	22	9	50
	100.0%	100.0%	100.0%	100.0%

Table 15: Showing correlation of number of stones with the different mucosal changes

	Multiple calculi	Single calculi	Total
Cholesterosis	0	1	1
	0%	4.5%	2.0%
Chronic cholecystitis	25	20	45

	89.3%	90.9%	90.0%
Papillary Hyperplasia	1	0	1
	3.6%	0%	2.0%
Well Differentiated Adenocarcinoma	1	1	2
	3.6%	4.5%	4.0%
Xanthogranulomatous	1	0	1
	3.6%	.0%	2.0%
Total	28	22	50
	100.0%	100.0%	100.0%

Table 16: Table showing average stone weight and size

	Average	Standard deviation
Stone Size	0.762 cm	0.36 cm
Weight of stone	0.688 gm	0.56 gm

Results

The age of patients were 10 to 80 years. Most in 41-55yrs age group 40% [Table 1]. This is similar to the study done by Shveta Narang et al 2014.⁸ there were patients between 71-80 years of age.

female 31 (62%) and male patients 19 (38%) [Table 2], male: female ratio being 1:1.63, other previous studies showed cholelithiasis is higher in older age group and in females. Incidence of gallstones were found to be high in Northern population 38(76%) than the Southern part of India 12(24%) [Table 3]. Pain 44 (88%), vomiting 15 (30%), fever 2 (4%), jaundice 9 (18%). 29 patients (58%) presented with recurrence of symptoms. 8 out of 50 of the subjects had other comorbidities. Ulcerative colitis, sickle cell anemia, primary infertility, acute kidney injury each were present in 2% of total subjects. B-thalassemia and coronary arterial disease, each had in 4% of the subjects. In our study, 20 patients had a BMI 18.5-24.9 (40%), of which cholesterol, mixed and pigmented were 5, 11, 4 patients respectively, 22 patients (44%) had a

BMI of 25-29.9 of which 12, 9 1 had cholesterol, mixed and pigmented stones respectively, and 8 patients (16%) had a BMI less than 18.5 had 2, 2, 4 cholesterols, mixed and pigmented stones respectively. Majority of patients were overweight 22 (44%) of which 12 patients had cholesterol stones with a P value of 0.028, was found to be statistically significant [Table 6]. Mixed diet contributed to the increased frequency of gallstones [Table 7]. Tenderness in right hypochondriac was the predominant sign 31(62%), followed by icterus 10(20%), and fever in 2 (4%) was observed [Table 8]. Ultrasound abdomen showed 56% of patients had multiple calculi with features suggestive of chronic cholecystitis such as thickened gallbladder wall, 2% had features of acute cholecystitis, 16% had CBD calculi, 2% had associated splenomegaly whereas, 44% of patients had single calculi. 26% of patients underwent open cholecystectomy, 70% of patients underwent laparoscopic cholecystectomy, 4% of laparoscopic cases were converted to open due to frozen Calot’s triangle and dense adhesions. 8 patients had cholelithiasis with choledocholithiasis and required ERCP. 2 patients with choledocholithiasis had failed ERCP, and required open CBD exploration with cholecystectomy as treatment. Majority of the patients had multiple calculi 28(56%) and single calculi seen in 22 (44%) [Table 15]. mixed stones 22 cases (44%), pure cholesterol stones in 19 cases (38%), pure pigmented stones in 9 cases (18%) On Gross the mixed stones were multifaceted, greyish-white in colour, cut surface showed yellow-coloured radiating crystals with a black Centre. Cholesterol stones were mostly solitary, round to oval and yellow in colour, Pigmented stones were brown black in colour and were mostly multifaceted. Combined gall stone were single and multiple, greyish white in colour. Its cut surface showed central nucleus and outer shell.

Total of 19 patients [38%] had 100% concentration of cholesterol, the rest had variable amounts of cholesterol as content, whereas 9 patients [18%] had no cholesterol. Calcium oxalate was seen in 38% of patients .8% of patients showed 60% of bilirubin, 56% of patients had no bilirubin.10% patients had magnesium. Biliverdin, hematin and fibrin were seen in most cases, in variable amounts.

Histopathological changes in the gallbladder mucosa, showed features of chronic cholecystitis such as Rokitansky Aschoff sinuses 33 (66%), 1 patient had features of benign papillary hyperplasia. One patient with B-thalassemia showed Xantho granulo matous changes, two patients with mixed stones showed features suggestive of malignancy, one patient had well differentiated adenocarcinoma with focal areas of Rokitansky-aschoff sinuses, one patient had well differentiated adenocarcinoma (papillary variant), the most common histopathological change observed was features of Rokitansky-aschoff sinuses with hyperplasia, it was seen in 94.7% of the cholesterol stones, 90.9% of the mixed stones, 77.8% of the pigment stones. 5.3% (one) of the patients with cholesterol stones showed Cholesterosis. 9.1% (2 subjects) of the mixed stones showed features of well-differentiated adenocarcinoma. 11.1% (1 subject) of the pigmented stone showed features of Xanthogranulomatous cholecystitis, P value = 0.09 not significant. There was No significant difference found between type of stone and mucosal changes. This was in correlation with a study conducted by S K Mathur et al³⁰, 2014 in which cholecystitis, hyperplasia, metaplasia and carcinoma all mucosal changes were more commonly seen with mixed stones. In carcinoma, 88% (7 out of 8 cases) had mixed stones. In cases with metaplasia, besides the 34 (58%) cases with mixed stones, 19 (32%) had combined stones. While 15 (66%)

of the 24 cases of hyperplasia had mixed stones, in rest 9 cases cholesterol, combined and pigment stones appeared in equal numbers (3 each). The association of mucosal response with type of stone was not found to be statistically significant ($p=0.326$). 25 patients (89.3%) of the multiple stone presented with chronic cholecystitis and one patient (3.6%) of multiple calculi was present with benign papillary hyperplasia, one patient (3.6%) of multiple calculi was present with well-differentiated adenocarcinoma, one patient (3.6%) of multiple calculi was present with xantho granulomatous. 20 patients (90.9%) of the single stone was present with chronic cholecystitis and one patient (4.5%) of single calculi was present with well-differentiated adenocarcinoma, one patient (4.5%) of single calculi was present with Cholesterosis significant difference found between number of stone and mucosal changes. 2 patients with malignant changes one each with solitary and multiple stones showed average size of gallstones was found to be 0.762cm in cases with inflammation and benign conditions, whereas in cases with carcinoma was greater (0.902 cm). In our study, the average weight of the stone was 0.688 gm in cases of chronic inflammatory changes, whereas in carcinoma average weight was found to be 0.889gm.

Discussion

Diagnosis of gallstone is by history and physical examination and correlating with ultra sonography, CT scans. Gall stones are categorized as cholesterol, mixed, black pigment, or brown pigment stones. Cholesterol and Two thirds of gallstones patients are asymptomatic and have a benign natural course; the progression of symptomatic disease, ranges from 10 to 25% such as acute or chronic cholecystitis, biliary colic, pancreatitis or obstructive jaundice. Common presenting symptom is intermittent pain below the right ribcage radiating to the

back and to the shoulder blades. Nausea or vomiting and dyspepsia might be present. On Clinical examination tenderness may be elicited on deep palpation of the right upper quadrant of the abdomen (Murphy's sign). Fever and tachycardia, enlarged gallbladder may be palpated in mucocele or empyema of the gall bladder. Hyperaesthesia between the 9th and 11th ribs posteriorly on the right side is seen in acute cholecystitis called as Boas's sign. Complete or partial obstruction of the common bile duct manifests as jaundice. Mixed gallstones are formed from biliary sludge, stays for a long time in the gallbladder lumen. Pigment stones are calcium salts of unconjugated bilirubin, with varying amounts of cholesterol and protein.¹¹ The following microscopic diagnostic criteria are used:¹² **Hyperplasia:** Pseudo stratification of epithelium, nuclear crowding, tall columnar cells, occasional mitotic figures. **Dysplasia**

Pseudo stratification of epithelium, nuclear crowding, some loss of architecture, disorganization of epithelium, nuclear atypia. **Intestinal metaplasia:** Presence of goblet cells. **Antral metaplasia:** Branched tortuous glands in lamina propria. **Carcinoma in situ:** Large vesicula on hyperchromatic nuclei having one or more prominent nucleoli, eosinophilic cytoplasm, clear or mucous secreting, occasional giant cells, loss of cell polarity, atypical mitotic figures, absence of stromal invasion.

The management of cholelithiasis are non-surgical or surgical. Non-surgical by dissolution of gallstones either oral or invasive contact with organic solvents, extracorporeal shock wave lithotripsy. The surgical management is open or laparoscopic cholecystectomy.⁵

Cholelithiasis produces diverse histopathological changes in gallbladder mucosa namely, acute inflammation, chronic inflammation, granulomatous inflammation, hyperplasia, Cholesterosis, dysphasia and carcinoma. Frequently, chronic cholecystitis presents a large range of

associated lesions such as cholesterolosis, muscle hypertrophy, parietal fibrosis, polypoid and adenomatous proliferation of mucous glands, metaplasia, hyperplasia and dysplasia. Occasionally, it may be associated with diffuse calcifications known as porcelain gallbladder. The metaplasia can be of goblet cell (intestinal) or pyloric (antral) type, the former being accompanied by appearance of Paneth cells and endocrine cells.

Gallbladder may show fibrosis, muscle hypertrophy, encrusted stones or nodular collections of foamy macrophages. Irregularly shaped tubular structures are present within the wall which are usually lined by columnar or cuboidal epithelium and may contain bile or gall stones. These tubular structures are Rokitansky-Aschoff sinuses, and are thought to represent herniations or diverticulations resulting from increased intraluminal pressure.

Follicular cholecystitis, Diffuse lymphoplasmacytic cholecystitis, Xantho granulomatous cholecystitis are some pathological changes. The initial inflammation is chemically induced and not by bacterial origin. Gallstone impaction leads to mucosal damage, which in turn leads to release of phospholipase. Phospholipase acts on lecithin converts into toxic lysolecithin, which further damages mucosa. Inflammation resolves in some 80% of cases with conservative treatment. Tension within the gallbladder lifts the stone impacted in the Hartmann's pouch leading to decompression and resolution of the inflammation. Jaundice may present in 20-25% of the patients with acute cholecystitis but common bile duct stones were detected only in 10-12% of these patients. A plain radiograph may reveal stones in 10-20% as calculi are radiopaque. The WBC count is usually high and liver functions are mildly altered. USG and radionuclide scans are confirmatory. Chronic cholecystitis of the gallbladder is most commonly due to stones (> 90%)

Carcinoma gallbladder is the fifth cause of carcinoma in the gastrointestinal tract. Majority of the cases are associated with gall stones, and the malignant change is found in approximately 0.9%. Untreated, chronic symptomatic gallstones are the major risk factor. Carcinoma of the gallbladder and extrahepatic bile ducts are diseases of older age groups. Most patients are in the 6th or 7th decades of life. Gallbladder carcinomas have a strong female preponderance. Although gallstones are considered a risk factor, the overall incidence of carcinoma of the gall bladder in patients with cholelithiasis is less than 0.2%; this percentage varies with race, sex, and length of exposure to the stones. Some authors have reported a correlation between gallstone size and the risk of cancer. Diffuse calcification of the gallbladder wall (porcelain gallbladder) is associated with carcinoma in 10-25% of cases. The signs and symptoms are not specific, often resembling those of chronic cholecystitis. Carcinoma of the gallbladder appears as an infiltrating grey white mass. Some carcinomas may cause diffuse thickening and induration of the entire gallbladder wall. The gallbladder may be distended by the tumour, or collapsed due to obstruction of the neck or cystic duct. It can also assume an hourglass deformity when the tumour arises in the body and constricts the lateral walls.^{8,9} Papillary carcinomas are usually sessile and exhibit a polypoid or cauliflower-like appearance. Mucinous and signet ring cell carcinomas have a mucoid or gelatinous cut surface. There are two histological variants of mucinous adenocarcinomas of the gall bladder and extrahepatic bile ducts: one variant is characterised by neoplastic glands distended with mucin and lined by columnar cells with mild to moderate nuclear atypia, and the second variant is characterized by small groups or clusters of cells surrounded by abundant mucin. The chemical composition of gallstones is essential to study

etiopathogenesis of gallstone disease. The major elements which are involved in the formation of gallstones are cholesterol, bile pigments, calcium, hepatic bile composition, biliary glyco protein, infection, age, sex, genetics, oestrogen, dietary factors, geographical prevalence, and cirrhosis of the liver.¹⁷ Chandran et al (2007) during chemical analysis of gallstone found total cholesterol as the major component and total bilirubin, phospholipids, bile acids, fatty acid, soluble proteins, calcium, magnesium, sodium, potassium, inorganic phosphates, oxalates and chlorides as minor components have been reported. The pigment stones were reported to be rich in total bilirubin, bile acids, calcium, oxalates, magnesium, sodium, potassium, chloride and soluble proteins compared to cholesterol and mixed stones. Verma et al (2002) found calcium and trace elements in chronic cholelithiasis. Gallstone formation is relatively increased with consumption of dietary fats rich in saturated fatty acids. Patients with hemolytic anemia generally develop gallstone composed of bilirubin, a bile pigment. Mostly gallstones are a mixture of cholesterol, acids, lipids and soluble protein. This study describes an extensive quantitative biochemical and inorganic constituent of gallstones, including cholesterol, bilirubin, bile acids, phospholipids, calcium, magnesium, inorganic phosphate and oxalate and their association to induce neoplastic changes. In Northern India, gallstones are seven times more common with overall incidence of about 2.29%.⁹ In a study done by Swati Jain et al in 2015, it was found that the incidence of gallstones were found to be high in Northern population than the Southern part of India which correlates with our study in which, majority of the subjects were from North India, 38 (76%) and South India (12) 24%. [Table 3]. Multiple stones have been found in (60.4%) than solitary stones (39.6%) as same was in our study as well as previous reports. This

indicates that cases with multiple number of stones are more symptomatic than those with solitary stones. There was No significant difference found between type of stone and mucosal changes. This was in correlation with a study conducted by S K Mathur et al, 2014 in which cholecystitis, hyperplasia, metaplasia and carcinoma i.e., all mucosal changes were more commonly seen with mixed stones. Our study showed 2 patients with malignant changes one each with solitary and multiple stones. Mathur SK et al observed that benign, Prema lig nant and malignant lesions were more frequently associated with multiple gallstones. A strong correlation has been reported between the size of the gallstones and the incidence of gallbladder carcinoma. In a study of more than 1600 patients with gallbladder disease, vittetta et al reported that 40% of the patients with gallbladder carcinoma had stones that were >3 cm in size. But case control studies by Roa et al found no relationship between stone size and gallbladder cancer. In our study, the average weight of the stone was 0.688 gm in cases of chronic inflammatory changes, whereas in carcinoma average weight was found to be 0.889gm.

Conclusion

Management of asymptomatic gallstones is still a controversy we noted that, asymptomatic gallstones also had a series of epithelial pathological changes in the gallbladder mucosa which could be precursor lesions for gallbladder carcinoma hence cholecystectomy could be offered to all asymptomatic gallstone patients.

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