

Morphological Characterization of Gallstones in Cholecystectomy Specimens: A Four Years Study

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Abstract

Background: Gallstones are the major culprits for gallbladder pathologies. More than 95% of biliary tract disease is attributable to cholelithiasis (gallstones). Most patients are unaware of the disease and remain asymptomatic for life. **Aim:** To determine morphological characterization of gallstones in cholecystectomy specimens to understand its association with etiology. **Material and Methods:** All cholecystectomy specimens having gallstones received at the Department of Pathology over a period of four years were studied for morphological characterization. **Results:** Out of 178 gallbladders showing calculi, patients of 5th and 7th decade (24.15% and 21.92%) were commonly affected and majority were females (69%) when compared to males (31%). Most common calculi were mixed type of stones (64.04%). 48.88% gallbladders were having round to oval shaped calculi, 24.15% were polyhedral, multifaceted and 26.97% were irregular in shape. **Conclusion:** The population of the present study showed mixed type of calculi as common observation which depicts its etiology of change in lifestyle with following western diets and concurrent infectious cause.

Keywords: Cholelithiasis, gallstones, multifaceted.

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INTRODUCTION

Gallstones are the major culprits for gallbladder pathologies. More than 95% of biliary tract disease is attributable to cholelithiasis. Gallstones afflict 10% to 20% of adult populations in developed countries [1]. The prevalence varies with age, sex and ethnic group. It occurs predominantly in females [2]. Most patients are unaware of the disease and remain asymptomatic for life. The current changes in lifestyles of individuals pertaining to indulgence in unhealthy fat-rich food, lack of exercise, obesity, sedentary lifestyles and various other factors have once again focused our attention to gall stones and diseases of the gall bladder [3].

CHOLELITHIASIS is a common disorder, afflicting 10% to 20% of adult population in developed countries [1]. It has been called as costly digestive disease in developed countries [4]. Its incidence has markedly increased in Asian countries owing to adoption of western type of dietary habits [5].

The estimated prevalence of Gall stone disease in India has been reported as 2% to 29%. In India, this

disease is ten times more common in the North India than in South India. Hormones, repeated pregnancies, obesity have been implicated as the major factors in females, who are more prone for gall bladder disease [6,7]. Oestrogen exposure, including oral contraceptive use and during pregnancy increases the expression of hepatic lipoprotein receptors and stimulates hepatic HMG-CoA reductase activity, enhancing both cholesterol uptake and biosynthesis respectively. The net result is excess biliary secretion of cholesterol. Common variant of sterol transporter encoded by ABCG8 gene is associated with increased risk [1].

Gallstones predominantly composed of cholesterol, bilirubin and calcium salts with lesser amounts of other constituents. These are classified into cholesterol and non-cholesterol gallstones based on the relative amount of cholesterol in the stones. Cholesterol stones are common in Western countries whereas pigment stones are common in other parts of the world including Asia [8]. Cholesterol gallstones are composed of cholesterol crystals agglomerated by a mucin glycoprotein matrix. Non-cholesterol gallstones include black or brown pigment stones. Black pigment stones are formed from calcium salts of unconjugated bilirubin

in a polymerized matrix and brown pigment stones are formed within bile ducts and contain bacterial degradation products of biliary lipids, calcium salts of fatty acids, unconjugated bilirubin and precipitated cholesterol [8].

Pathogenesis of Stone Formation

Cholesterol stones: When cholesterol-to-phospholipid ratio exceeds 1, that is exceeding solubilizing capacity of bile (supersaturation), cholesterol can no longer remain dispersed and nucleates into solid cholesterol monohydrate crystals [1]. An increase in biliary cholesterol output- either due to increased synthesis or increased uptake by hepatocytes is the most common cause of supersaturation and subsequent stone formation [9]. Cholesterol supersaturation can also arise as a consequence of bile acid hyposecretion [10, 11].

Pigment stones: As aetiology, associated clinical conditions, morphology and chemical composition are different and hence it is necessary to distinguish between black and brown pigment stones [12]. Unconjugated bilirubin being precursor for calcium bilirubinate plays a central role in the formation of both brown and black pigment stones.

Brown stones: Any condition resulting in elevated levels of unconjugated bilirubin can predispose to stone formation. Biliary infection contributing to bile stasis is a common cause of brown stones, as bacterial overgrowth generates hydrolases that can form free bile acids from conjugated bile salts. Hence the bacteria are present within the matrix of most brown stones [13].

Black stones: are not associated with bacterial infection. It originates from an increment in the secretion of bilirubin conjugates as occurs in haemolysis [14] and chronic alcoholism, followed by non-bacterial enzymatic or non-enzymatic hydrolysis. (Other conditions associated with black gallstones include malaria, pancreatitis, total parenteral nutrition and advanced age) Calcium bilirubinate sludge containing an increased amount of phospholipid forms the core of pigment gallstones. Carbohydrate rich diets stimulate enzymes which are important in the synthesis of phospholipids [15].

Gallstone formation is also favoured by bile stasis due to gallbladder dyskinesia resulting from gallbladder wall pathology and also due to exposure of gallbladder mucosa to high concentrations of mucus, calcium and lipids [16].

Cholelithiasis produces diverse histopathological changes in gallbladder mucosa namely acute and chronic cholecystitis, granulomatous inflammation, cholesterosis, glandular hyperplasia, metaplasia, dysplasia, and carcinoma [17].

MATERIAL AND METHODS

Source of Data

The present study was done on all the cholecystectomy specimens showing calculi received at the Department of Pathology of a tertiary care hospital over a period of four years June 2014 to May 2018.

Sample Size

All the cholecystectomy specimens showing calculi (178) received at the Department of Pathology, over a period of four years which satisfied inclusion and exclusion criteria were considered in the present study.

Inclusion criteria: All cholecystectomy specimens showing calculi were included in the study.

Exclusion criteria: Autolysed, gangrenous specimens and also specimens without calculi were excluded from this study.

METHOD OF COLLECTION OF DATA

The gall bladder specimens were collected in 10% formalin following scrutinizing the patient details and identity. The gallbladder specimens were cut and allowed to fix in fresh formalin for 24 hours. Detailed gross examinations were carried out and descriptions of the specimens were noted. Tissue bits were taken from representative areas of the fundus, body and neck of the gall bladder for histopathological examination. Additional tissue bits were taken from abnormal appearing areas.

This was followed by processing with routine histological techniques for paraffin embedding and sectioning at 3-5micron thickness. The tissue sections were placed on a slide warmer for deparaffinisation and further deparaffinisation using xylene, followed by Haematoxylin and Eosin stain. Special stains were used wherever required. Microscopical examination was done under different objective powers of light microscope and changes in all cases were noted.

Statistical Analysis: Statistical analysis was done using SPSS software.

RESULTS

Out of 178 gallbladders showing calculi, majority 123 (69%) were from females when compared to males (55, 31%) (Table-1).

Table-1: Sex distribution of gallbladders with calculi

Gross examination	Number of males (Percentage %)	Number of Females (Percentage %)	Total
Gallbladders with calculi	55 (31%)	123 (69%)	178 (100%)

Age distribution based on calculi in the present study

Among them, 5th decade was most commonly affected with 43 cases (24.15%), followed by 7th decade 39 (21.92%) (Figure-1).

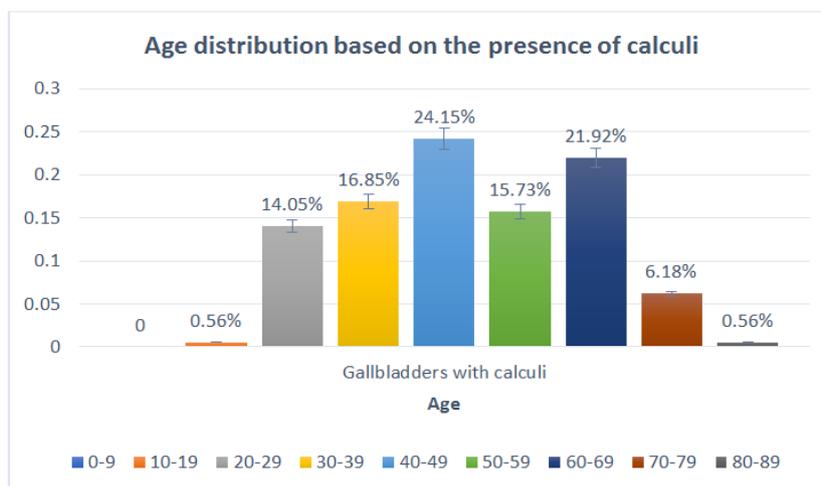


Fig-1: Age distribution based on calculi in the present study

Distribution based on type of calculi in the present study

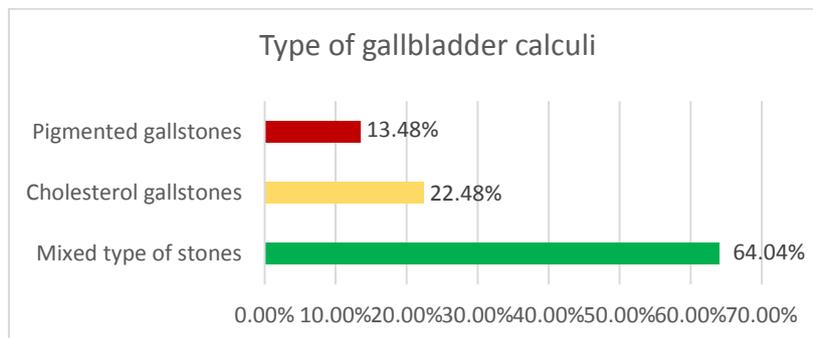


Fig-2: Distribution based on type of calculi in the present study

Most common calculi were mixed type of stones (114 gallbladders, 64.04%), followed by cholesterol gallstones (40 gallbladders, 22.48%) and

least was pigmented gallstones (24 gallbladders, 13.48%) (Figure-2).

Table-2: Distribution based on number of calculi in the present study

Type of gallbladder calculi	Single calculus	Multiple calculi	Total
Mixed type of stones	25	89	114
Cholesterol gallstones	4	36	40
Pigmented gallstones	7	17	24
Total	36 (20.22%)	142 (79.78%)	178 (100%)

Out of 178 gallbladders showing calculi, 142 (79.77%) were having multiple calculi and remaining 36 (20.22%) gallbladders had single calculus. Among the

gallbladders with multiple calculi, 89 (62.7%) were mixed stones, 17 (12%) were pigmented stones and 36 (25.3%) were cholesterol stones. (Table 2, Figure 4,6,7)

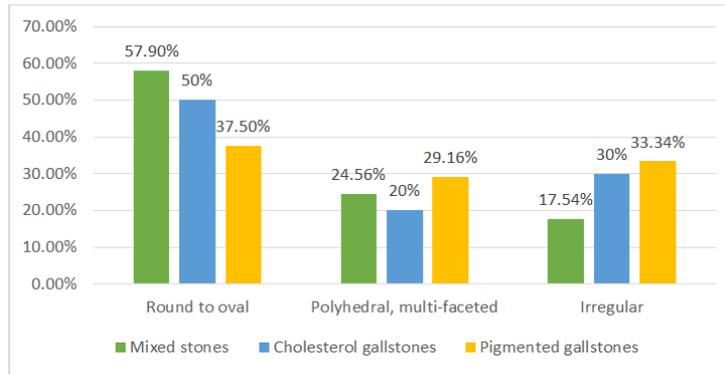


Fig-3: Distribution based on morphology of calculi in the present study

Out of 178 gallbladders with calculi, 87 (48.88%) gallbladders were having round to oval shaped calculi, 43 (24.15) were having polyhedral, multifaceted calculi and 48 (26.97%) were having calculi irregular in shape (Figure 3, 4 & 5).



Fig-4: Gross photograph of chronic cholecystitis with calculi, cut section showing multiple, smooth, yellow coloured polyhedral multi-faceted gallstones (Inset showing congested external surface of the gallbladder)

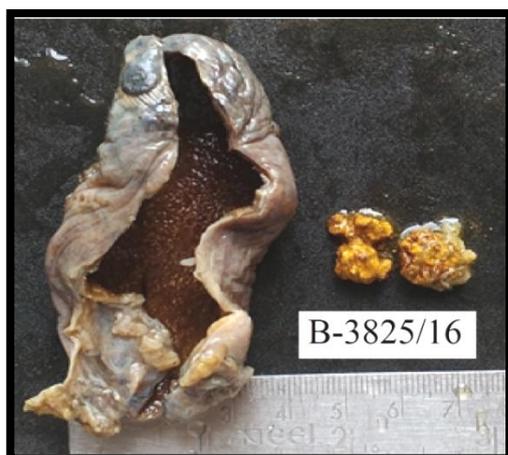


Fig-5: Gross photograph of xanthogranulomatous cholecystitis with calculi showing cut surface of the gallbladder having yellowish brown mucosa. Gallstones were irregular and yellow coloured

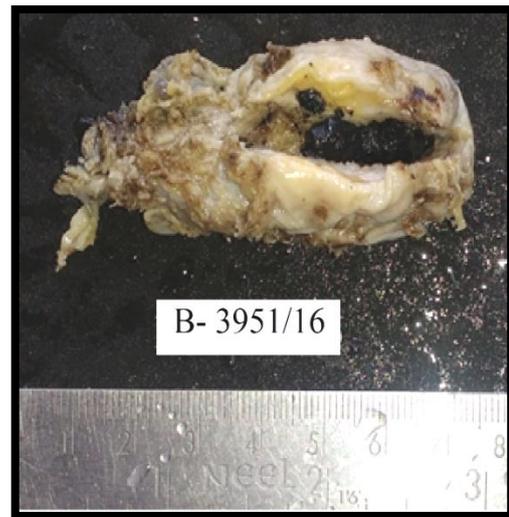


Fig-6: Gross photograph of chronic cholecystitis with calculi showing cut surface of the gallbladder with thickened wall and having irregular black pigmented gallstones



Fig-7: Gross photograph of chronic cholecystitis with calculi showing cut surface of the gallbladder having irregular, smooth and black pigmented gallstones



Fig-8: Gross photograph of mixed type of gallstones showing multiple, polyhedral, multi-faceted and brown in colour. Cut section of the stones showing central greyish black core surrounded by radial arrangement of grey brown to grey yellow area (Inset)

DISCUSSION

In the present study though 316 gallbladder specimens were received only 56.33% of the cases were associated with gallstones. Whereas studies conducted by Beena D *et al.*, [18], Karlatti SS *et al.*, [19], Awasthi N [20], H Mohan *et al.*, [21] and Goyal S *et al.*, [22]

showed higher percentage of gallbladder with calculi 65%, 72.73%, 95.2%, 95.45% and 90% respectively. As we received sectioned gallbladders most of the time, surgeons sending the gallbladder specimens without including gallstones may be the cause for the lower values.

Table 3: Comparison of presence of calculi in the present study with other studies

Study by	Total number of gallbladders studied	Total number of gallbladders with calculi	Percentage
Awasthi N [20]	732	697	95.2%
Beena D <i>et al.</i> , [18]	200	130	65%
Sharma I <i>et al.</i> , [23]	348	305	87.64%
H Mohan <i>et al.</i> , [21]	1100	1050	95.45%
Kumari G <i>et al.</i> , [24]	550	437	79.45%
Goyal S <i>et al.</i> , [22]	346	313	90%
Karlatti SS <i>et al.</i> , [19]	143	104	72.73%
Present study	316	178	56.33%

According to the present study, majority were females (69%), which is similar to the studies conducted by Murmu S *et al.*, [25], Awasthi N [20], Mondal B *et al.*, [26] and Kumar H *et al.*, [27].

Comparison of most common type of calculi in the present study with other studies

Mixed type of gallstones was the most common type of gallstones found in the present study (64.04%). This was in concordance with the studies conducted by H Mohan *et al.*, [21] (62.3%), Goyal S *et al.*, [22] (68%), Nayak G [28] (71.4%) and Karlatti *et al.*, [19] (81.12%) suggesting multifactorial involvement of change in dietary habits along with infectious origin.

Study by	Total number of gallbladders with calculi studied	Most common type of gallstone present	Total number of gallbladders showing most common type	Percentage
Beena D <i>et al.</i> , [18]	130	Pigment stones	117	58.5%
H Mohan <i>et al.</i> , [21]	1050	Mixed type of stones	686	62.3%
Kumari G <i>et al.</i> , [24]	437	Pigment stones	540	51.03%
Goyal S <i>et al.</i> , [22]	313	Mixed type of stones	213	68%
Nayak G [28]	70	Mixed type of stones	50	71.4%
Karlatti SS <i>et al.</i> , [19]	104	Mixed type of stones	84	81.12%
Present study	178	Mixed type of stones	114	64.04%

Comparison of number of calculi in the present study with other different studies

In the present study, majority (79.78%) of the gallbladders with calculi were having multiple

gallstones which was comparable to studies done by Nayak G *et al.*, [28] (84.3%) and Goyal S *et al.*, [22] (69.65%).

Study by	Total number of gallbladders with calculi	Calculi	
		Single	Multiple
Nayak G [28]	70	11 (15.7%)	59 (84.3%)
Goyal S <i>et al.</i> , [22]	313	95 (30.35%)	218 (69.65%)
Present study	178	36 (20.22%)	142 (79.78%)

CONCLUSION

Out of gallbladders associated with calculi, majority (69%) were from female patients. Most common age group having gallbladder lesions with calculi was 5th decade (24.15%) and majority of the gallstones were found to be mixed type of stones (64.04%).

These results suggest factors like female gender, lifestyle and dietary habits play a major role in the formation of different types of gallstones. And changing the habits may reduce its occurrence. Our study didn't have scope for chemical examination of the stones, which would be helpful for clear differentiation of the causes.

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