

## Effects of Chemical Composition of Cholesterol and Pigment Stones on the Gallbladder Mucosa

Sanum Ali, Shahid Rasul, Surrendar Dawani, Sarah Zahid, Sehrish Hussain, Ovais Sarwar, Tanweer Fatima, Zainab Tariq

### ABSTRACT:

**Objective:** To compare the effect of chemical composition of cholesterol and pigment stones on gallbladder mucosa.

**Study design & setting:** This comparative cross-sectional study was conducted in the Department of Anatomy, BMSI, JPMC, Karachi, Pakistan, from April to September 2021.

**Methodology:** Total n= 120 gallbladder specimens were collected after cholecystectomies in 10% formalin. Gross morphological features of gallbladder and stones were observed. Specimens were processed and 5µm sections from fundus, body & neck of gallbladder were stained and examined for microscopic changes. Chemical analysis of stones was carried out to differentiate between cholesterol & pigment stones.

**Results:** We evaluated different histomorphological alterations in gallbladder with reference to both type of gallstones. Muscular wall thickness in pigment stone group was noted to be (143.89±78.66) and in cholesterol group (158.37±83.95). Both pigment and cholesterol stone showed increased tendency of muscular hypertrophy. Mucosal ulceration, chronic inflammatory cell infiltration, capillary congestion and hyperplasia (p-value: 0.04) were observed in both type of stones. Statistical analysis showed that large cholesterol stones produce significant histopathological changes (p-value: 0.004); greater the size of stone, more profound was the mucosal hyperplasia. Rokitsansky-Ashcoff sinuses were predominant in gallbladders with cholesterol stone.

**Conclusion:** Gallstones cause series of histopathological changes ranging from chronic cholecystitis to pre-malignant and malignant changes. Hyperplasia was observed to be more frequent with long standing, large cholesterol stones.

**Key words:** Cholelithiasis, Gallstones, Gallstone disease, Hyperplasia.

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### INTRODUCTION:

Gallbladder is a flask-shaped viscus, firmly attached to the visceral surface of the right lobe of liver. It stores and concentrates bile secreted by the liver. It is lined by tall columnar epithelial cells, covered by duvet of mucous, which separates the epithelium from luminal contents<sup>1</sup>.

Gallstone disease (GSD) is a worldwide common health problem. According to the literature the prevalence of gallstones varies by region and ethnicity. Approximately 10-20% of adults in the United States are said to be affected by GSD, followed by European population with an estimated

prevalence of 15%<sup>2</sup>, while Hispanics and Amerindians from the US (25-30%) are said to be the most susceptible ethnic groups<sup>3</sup>. Developing countries such as Pakistan has also been facing the rapidly increasing burden of GSD, which is around 10.2%, this increase in the frequency could be associated with the consumption of carbohydrate rich diet and physical inactivity. It is also reported that prevalence rates vary with age, sex and ethnicity, with females being the most predominant gender especially during the fourth decade of their lives<sup>4</sup>.

Literature suggests that gallstone formation is favoured by factors including; biliary supersaturation of cholesterol or bilirubin, nucleation of mucous with bile salts and bile stasis due to gallbladder dyskinesia<sup>5</sup>. Gallstones can be classified into three main types on the basis of their chemical composition; cholesterol stones comprising of crystalline cholesterol monohydrate, pigment stones consist of bilirubin calcium salts and mixed stones which are the mixture of both types of stones. These stones can also be distinguished by their morphology e.g., cholesterol stones are large, oval and yellowish in colour, whereas, pigment stones usually appear as small, multiple and black coloured. Mixed stones are multiple, multifaceted and can be of variable size<sup>6</sup>.

Cholelithiasis manifest diverse microscopic changes, ranging from acute and chronic cholecystitis, xanthogranulomatous cholecystitis and cholesterosis in majority of specimens then proceeding to advanced pre-cancerous varieties in some cases, like hyperplasia, metaplasia & dysplasia, eventually leading to carcinoma<sup>7</sup>. As most of the gallstones exhibit irregular surfaces, their irritation is said to be responsible for the initiation of above-mentioned changes that patients harbouring gallstones for a longer duration are seen with a higher risk of developing gallbladder carcinoma, which although found rare but is the most common neoplasm of the biliary tract and ranks fifth among gastrointestinal cancers<sup>8</sup>. Due to lack of gallbladder serosal layer adjacent to liver, hepatic invasion and metastasis is common. As the survival rate is quite low i.e., around 5%, early diagnosis could increase life expectancy<sup>9</sup>. This study is our contribution to observe the effects of mechanical irritation as well as chemical composition of gallstones on gallbladder mucosa of patients with cholelithiasis. Proper examination of cholecystectomy specimens with respect to gallstone type and size could increase the chances of early diagnosis of Gallbladder cancer which may reduce the morbidity.

We evaluated various microscopic changes in gallbladder and investigated their association with age, gender, ethnicity, stone size & stone type.

#### **METHODOLOGY:**

This comparative cross-sectional study was conducted from April to September 2021, at the Department of Anatomy, Basic Medical Sciences Institute in collaboration with Surgical Units of Jinnah Postgraduate Medical Centre

(JPMC), Karachi, after getting ethical approval from Institutional Review Board of JPMC (NO.F.2-81?2021-GENL/57122/JPMC). Total 120 patients diagnosed with cholelithiasis of ages, genders and ethnicities were recruited in the study. A sample size of 120 achieves 80.055% power to detect the difference (P1-P0) of 0.0850 using a two-sided exact test with a significance level (alpha) of 0.05. These results assume that the population under the null hypothesis (P0) is 0.1000. Sample size was based on a similar study that studied the histopathological changes in the gallbladders of Indian patients with gall stones (prevalence rate (10%)<sup>10</sup>. Patients were divided into groups on the basis of gender, ethnicity and stone type. Autolyzed specimens and specimens from patients suffering from diseases other than cholelithiasis i.e., Hepatitis A & B, Primary sclerosing cholangitis and Gallbladder carcinoma were excluded from the study<sup>11</sup>.

Specimens were collected post cholecystectomy and fixed in 10% formalin for 24 hours. Sections were taken from fundus, body and neck of gallbladder after taking measurements (length\*width\*breadth of gallbladders & diameter of stones) and observing the gross morphological features of all specimens & stones. 5 µm thick sections were cut & stained with H&E. Microscopic analysis was performed under light microscope (Nikon Eclipse 50i; Japan) connected to video link digitalizing board system (DS Camera control unit- DS-L2). Two slides per specimen and three areas (fundus, neck & body) per slide were observed, (histology slides of normal human gallbladder from department of Anatomy was used as control). Histomorphological features examined were; mucosal ulceration, Rokitansky-Ashcoff sinuses, chronic inflammatory cell infiltration, capillary congestion & hyperplasia. They were scored on an arbitrary scale denoted by numbers as; nil (0), mild (1), moderate (2) & severe (3). Muscular hypertrophy was measured through Image J Fiji software and mean values were noted. Gallstones were sent for chemical analysis to JSMU lab and results were obtained to differentiate between cholesterol & pigment stones on the basis of their chemical composition.

Statistical analysis of data was done by using SPSS version 22. The comparison of quantitative variables such as; age, stone size and volume of GB was done by using Student t-test to compare their mean difference. The categorical data such as ethnicity, groups of stone type, stone size and stone number were compared for their association with microscopic findings by Chi-Square test of dependence. The results were considered significant at P-value <0.05.

#### **RESULTS:**

In the period of six months, total no: of specimens studied were 120, among which 109 were from females (90.83%) and 11 were from male patients (9.16%) (Table-1).

Out of 120, 90 patients had cholesterol stones (75%), having mean age of  $37.02 \pm 10.66$  and 30 patients had pigment stones (25%), having mean age of  $41.07 \pm 13.14$ .

Frequency of GSD was found to be more frequent in Urdu speaking people (43.33%), followed by Pathans (23.33%) and then Sindhis (15.83%). Cholesterol stones were the predominant type among all ethnicities.

Out of 90 specimens containing cholesterol stones, 24 were solitary, 8 had two, 5 specimens had three stones while 53 carried multiple stones with mean size of  $0.79 \pm 0.57$ .

In 30 specimens containing pigment stones, number of stones varied from solitary stone in 6 specimens while 3 specimens had two and multiple in the remaining 19 specimens with mean size of  $0.3477 \pm 0.098$  (Table-1).

This study depicted many microscopic alterations within the gallbladder. The details are presented in (Table: 2).

Out of 30 specimens containing pigment stones, 16.7% had mild, 63.3% had moderate & 20% were having severe mucosal ulceration.

Out of 90 specimens containing cholesterol stones, 31.1% had mild, 53.3% had moderate & 15.6% were having severe mucosal ulceration (p-value: 0.305) (Table. 2).

Out of 30 specimens harbouring pigment stones, 20% had mild chronic inflammatory cell infiltration, 66.7% had moderate & 13.3% had severe infiltration.

Out of 90 specimens harbouring cholesterol stones, 45.6% had mild, 50% had moderate and 4.4% had severe infiltration (p-value: 0.023) (Table. 2).

Mean size of smooth muscle within the gallbladder specimens was found to be of  $154.75 \pm 82.58$ .

Out of 30 specimens carrying pigment stones, 10% exhibited mild, 6.7% exhibited moderate and none showed severe hyperplasia.

Out of 90 specimens carrying cholesterol stones, 32.2% exhibited mild, 11.1% had moderate and 2.2% showed severe hyperplasia (p-value: 0.04) (Table. 2).

Rokitansky-Ashcoff Sinuses were more pronounced and deep with cholesterol stones (58.9%) than pigment stones (33.3%).

## DISCUSSION:

Cholelithiasis is regarded as the commonest cause of chronic cholecystitis and is responsible for severe mechanical and chemical irritation of gallbladder mucosa. This in conjunction with bile stasis and chronic inflammation may lead to premalignant changes within the gallbladder, which could culminate to carcinoma<sup>12</sup>. In this research we have tried to assess the histological changes in gallbladder in association with the chemical composition of gallstones, age, gender and ethnicity.

Table 1: Physical Parameters

Variables	Cholesterol (n=90)	Pigment (n= 30)	t-value	p-value
Age (years)	37.02±10.66	41.07±13.14	1.69	0.09
Stone size (cm)	0.7953±0.576	0.3477±0.098	4.22	0.001
Volume of Gallbladder (cm <sup>3</sup> )	28.23±18.43	29.09±23.023	0.207	0.836
<b>Gender</b>				
Male (n=11) (9.16%)	7	4	Chi - square = 0.834	0.361
Female (n=109) (90.83%)	83	26		
<b>Ethnicity</b>				
Urdu speaking (n=52) (43.33%)	36	16	Chi - square =8.38	0.45
Pathan (n=28) (23.33%)	24	4		
Sindhi (n=19) (15.83)	13	6		
Punjabi (n=9) (7.5%)	8	1		
Baloch (n=7) (5.833%)	5	2		
Balti (n=1) (0.833%)	1	0		
Hazara (n=1) (0.833%)	1	0		
Hindko (n=1) (0.833%)	0	1		
Kashmiri (n=1) (0.833%)	1	0		
Kathiawari memon (n=1) (0.833%)	1	0		
<b>Number of Stones</b>				
One (n=30)	24	6	Chi - square =0.834	0.361
Two (n=11)	8	3		
Three (n=7)	5	2		
Multiple (n=72)	53	19		

Table 2: Microscopic Findings  
Applied Chi- Square test

Variables			Mucosal Ulceration			Chronic Inflammatory Cell Infiltration			Hyperplasia			
			Mild	Moderate	Severe	Mild	Moderate	Severe	Nil	Mild	Moderate	Severe
Stone Type	Pigment (n=30)	Count	5	19	6	6	20	4	25	3	2	0
		%	16.7	63.3	20.0	20.0	66.7	13.3	83.3	10.0	6.7	0.0
	Cholesterol (n=90)	Count	28	48	14	41	45	4	49	29	10	2
		%	31.1	53.3	15.6	45.6	50.0	4.4	54.4	32.2	11.1	2.2
p-value		<b>0.305</b>			<b>0.023</b>			<b>0.04</b>				
Number of Stones (n=120 specimens)	One (n=30)	Count	10	12	8	13	15	2	20	4	6	0
		%	33.3	40.0	26.7	43.3	50.0	6.7	66.7	13.3	20.0	0.0
	Two (n=11)	Count	5	6	0	5	6	0	8	3	0	0
		%	45.5	54.5	0.0	45.5	54.5	0.0	72.7	27.3	0.0	0.0
	Three (n=7)	Count	2	5	0	4	3	0	4	3	0	0
		%	28.6	71.4	0.0	57.1	42.9	0.0	57.1	42.9	0.0	0.0
	Multiple (n=72)	Count	16	44	12	25	41	6	42	22	6	2
%		22.2	61.1	16.7	34.7	56.9	8.3	58.3	30.6	8.3	2.8	
p-value		<b>0.162</b>			<b>0.803</b>			<b>0.357</b>				
Stone Size (in cm)	0.20 to 0.70	Count	19	44	12	28	40	7	51	20	2	2
		%	25.3	58.7	16.0	37.3	53.3	9.3	68.0	26.7	2.7	2.7
	> 0.70	Count	14	23	8	19	25	1	23	12	10	0
		%	31.1	51.1	17.8	42.2	55.6	2.2	51.1	26.7	22.2	0.0
p-value		<b>0.711</b>			<b>0.312</b>			<b>0.004</b>				

Our results showed a trend that made it apparent that the prevalence of cholelithiasis is greater in females in comparison with males, this finding is consistent with a previous Pakistani study that reported, that among the population of 454 GSD patients, 73.6% were females of reproductive age, this may be due to elevated estrogen levels during the child bearing age, which increases cholesterol secretion and saturation of bile<sup>13</sup>. Moreover, a previous gender-based study is also in accordance with our findings that the number of females is higher than the males, however, the number of emergency admissions in hospital was seen more common in males. Although, there is no single article proving that this may be related to anatomical variants associated with gender<sup>14</sup>.

In terms of ethnic connection to GSD, we observed that majority of the cases diagnosed with cholelithiasis, were from Urdu speaking females (43.33%), migrated from Indo-Pak subcontinent region, and in this population cholesterol stones were predominant (table-1). Our results coincide with a study proposing significant association of ethnic group migrated to Karachi from northern Indian region which is said to be the stone belt of India. Oily and spicy food, sedentary habits and multiparity in women were suggested to be few associated reasons<sup>15</sup>, however, other ethnic groups e.g., Pathan and Sindhi speaking patients also showed an increased trend of developing GSD in our study (23.33%) and (15.83%) respectively.

Age has always been linked with an increased risk of developing gallstones. Our findings demonstrated that the most frequent age group was of 28-48 which is consistent with another local study where the peak incidence of GSD was observed in the age group of 26-35<sup>16</sup>. Moreover, these findings are also supported by an Indian study that reports greater incidence of GSD in the fourth decade of life<sup>17</sup>.

Regarding the microscopic findings, our observations revealed that moderate to severe mucosal ulceration was associated with both variety of stones, however we did not find any significant difference between cholesterol and pigment stone groups, when compared with each other (table-2). Literature suggests that this surface irregularity could be due to constant mechanical and chemical irritation of gallbladder mucosa by stones. We also observed that the size and number of stones were responsible to cause greater erosion and ulceration of the GB mucosa as suggested by other studies<sup>18</sup>.

An interesting observation in our study was regarding Rokitansky-Ashcoff sinuses. Their clinical relevance is important because adenomas could extend along these sinuses and stimulate the development of adenocarcinomas, likewise other studies also propose that the synthesis of cholesterol stone cause inward proliferation of the mucosa due to increase in the intraluminal pressure and weakening of the wall by distension, leading to the formation of Rokitansky-Ashcoff sinuses<sup>19</sup>.

In our study chronic inflammatory cell infiltration and capillary congestion were commonly seen with both type of stones but infiltration was extensively scattered in specimens containing cholesterol stones, some specimens also had multiple lymphatic nodules within the lamina propria to be more associated with cholesterol stones (table.2). Similarly, literature indicates that presence of stones triggers inflammatory response<sup>20</sup>.

According to current studies, chronic irritation by gallstones and their size increases the proliferation and mitotic activity of gallbladder epithelium resulting in precancerous changes like hyperplasia, which may progress to carcinoma<sup>21</sup>, our study displayed hyperplastic changes in 74 out of 120 specimens (61.6%) and it was profound in large size, multiple cholesterol stones (p-value < 0.05) (table-2).

The limitation of our study is that we cannot generalize our results regarding ethnicity because this is a single centre study and the sample size is not large enough.

### CONCLUSION:

This study revealed that gallstones cause series of histopathological changes ranging from chronic cholecystitis to pre-malignant and malignant changes. These changes are observed more with the long standing, large cholesterol stones. Urdu speaking females among age group of 28-48 were found to be more susceptible to GSD in our study.

Authors Contribution:	
<b>Sanum Ali:</b>	Corresponding author, research plan, experiment conduction, interpretation of data & analysis
<b>Shahid Rasul:</b>	Surgical relevance in write up & critical review
<b>Surrender Dawani:</b>	Surgical expertise
<b>Sarah Zahid:</b>	Experimental work, manuscript drafting and data analysis
<b>Sehrish Hussain:</b>	Literature review, data collection and experimental work
<b>Ovais Sarwar:</b>	Literature review, data collection and experimental work
<b>Tanweer Fatima:</b>	Literature review, data collection and experimental work
<b>Zainab Tariq:</b>	Statistical expertise and data analysis

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