

An Evaluation of Histopathological Changes of Gallbladder Mucosa in Cholelithiasis

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Received: 03-06-2022 / Revised: 08-07-2022 / Accepted: 02-08-2022

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Conflict of interest: Nil

Abstract

Introduction: Bile deposits that have hardened into gallstones can form in the gallbladder. They come in sizes and shapes ranging from a golf ball's size to a sand particle. Gallstones develop when one or more of the bile's chemical components precipitates out as a result of an imbalance in the composition of bile. Although cholelithiasis is a medical disorder that can affect everyone, there is a substantial geographic variation in the prevalence rate, with African populations having the lowest incidence. There have been several attempts to diagnose gallstones early and to associate the condition of cholelithiasis with that of abnormal histology of gallbladder wall.

Aims and Objectives: To find out the association between several parameters pertaining to the gallstone properties with that of gall bladder pathological conditions.

Methods: This prospective study considered patients who underwent cholecystectomy and had cholelithiasis. Biochemical histopathological examinations were conducted to assess the several properties of gallstones and histological status of the gallbladder.

Results: The study has found that there is significant association between the size of the gall stones with that of histological diagnosis ($p < 0.05$). The study has found no association between any characteristic and the histopathological condition of the gallbladder ($p > 0.05$).

Conclusion: The study has highlighted that there is a significant association between the size of the gallstones and mucosal response leading to abnormal histology of the gall bladder.

Keywords: cholelithiasis, gall bladder, gall stones, Cholecystitis

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Introduction

Gallstones are bile deposits that have solidified and can develop in the gallbladder. They range in shape and size from the size of a golf ball to a sand particle. When there is a discrepancy in the chemical components of bile, one or more of these components precipitate out, resulting in gallstones. Gallstones are hard aggregates

in the gallbladder that resemble pebbles. Gallstones often develop as a result of the bile becoming overloaded either with cholesterol or bilirubin, followed by supersaturation, nucleation, and precipitation of crystals of cholesterol monohydrate, which expand to form a stone-size aggregation. Gallstones mostly

form due to changes in the pathology of the gallbladder mucosa. It has been said that cholelithiasis is a disease of civilization. Cholelithiasis is a medical condition that affects people all around the world, however, the prevalence rate shows significant geographic diversity, with African populations having the lowest rates. Most of the time, they do not result in symptoms, and only 13% and 22% will finally start to exhibit symptoms within 6 years and 20 years of the diagnosis. The gallbladder mucosa undergoes various histological alterations due to cholelithiasis, including hyperplasia, cholesterolosis, dysplasia, chronic inflammation, acute inflammation, granulomatous inflammation, and cancer. Therapeutic action is required if a cholelithiasis patient develops symptoms. The gallbladder is a flask-shaped, hollow viscus with a blind end. With a 35–55 ml capacity, it primarily functions as a bile reservoir. Cholelithiasis is the most frequent biliary disease, making cholecystectomy the most common abdominal operation worldwide [1,2,3,4].

Cholelithiasis is controlled by the mucus in the gallbladder, which promotes the formation of gallstones. The interaction of lipids, calcium, and mucus results in the production of gallstones. The large amounts of hazardous chemicals, both exogenous and endogenous, that are discharged into the primary bile are not shielded from the gallbladder and biliary tract epithelium. Gallbladder disorders including cholelithiasis and cholecystitis are extremely common in fatty, fertile, and females in the age range of 45-50 although males and children also experience the same symptoms. In India and the western world, the incidence of cholecystitis and cholelithiasis appears to be rising during the past couple of years due to the growing adoption of diets heavy in fat and calories. Total lipid content did not change, but the biliary cholesterol saturation index, glycoprotein, prostaglandin E₂, and arachidonate levels all increased

noticeably. The nucleation time also shrank. Crystals of cholesterol first appeared before nucleation times shortened. Prostaglandin E₂ levels significantly increased, followed by arachidonate levels significantly increasing and glycoprotein levels significantly increasing. These findings support the following hypotheses: (a) declines in nucleation period are required prior to actually cholesterol crystals shape in the gallbladder in obese patients destined to cholelithiasis by very-low-calorie diets; (b) the synthesis of bile and glycoprotein secretion are formed by the conversion of biliary arachidonate to prostaglandins; (c) biliary glycoprotein helps in the promotion of nucleation; (d) the cholesterol nucleation does not necessarily require an increased concentration of bile in the gallbladder [5,6].

Early gallbladder cancer is infamously difficult to diagnose without histology since it exhibits no clinical symptoms or indications and offers no hints during an ultrasound examination. A considerable proportion of early malignant lesions of the gallbladder are missed by cholecystectomy conducted with a preliminary diagnosis of benign diseases based upon computed tomographic scanning, clinical, and ultrasonological. Therefore, every specimen of cholecystectomy must be checked regularly histologically in order to prevent such errors with negative outcomes. Large or multiple cholesterol gallstones that initially may interrupt with the mechanical operation of the gall bladder are intimately linked to gall bladder carcinoma. Gallstones' size and number in the gall bladder may both have a significant role in the development of a gallstone filling deficiency that could result in long-term mechanical harm to the gall bladder mucosa. Gallbladder carcinoma has one of the highest cancer fatality rates. In patients having surgical treatment for chronic cholecystitis with cholelithiasis, it is a common underlying condition; long-term chronic inflammation brought on by

gallstones is thought to play a significant etiological role in carcinogenesis. Between 0.5 and 13% of cases of gallstone-related gallbladder cancer occur [7-10].

Materials and Methods

Research Design

This is a prospective study which was conducted during the period of eleven months, involving 120 patients who underwent cholecystectomy for symptomatic cholecystitis caused by cholelithiasis. The patients who underwent the mentioned surgery in our hospital were considered. A gross examination is done on the gallbladder to reveal the following characteristics: size, mucosa, and thickness of the wall. Biochemical testing was also done on the gallstones (whether they were received in a container or a sample). The tissue was appropriately collected and prepared using standard histological methods for paraffin embed and sectioning at a thickness of 4 microns. On the basis of the sections' hematoxylin and eosin staining, a histopathological diagnosis was made. Two from the body of the gallbladder and one each from the neck and fundus of the gallbladder were used to create the four parts that made up the whole wall. If more were needed, pieces of the aberrant mucosa were removed. Regarding the quantity, size, and morphological type of the stone, the pattern of responses in the mucosa of the gallbladder was investigated (s). Following that, the different morphological responses were divided into four major groups: cholecystitis, metaplasia, hyperplasia, and cancer. The following factors were used to describe the stones' physical attributes. The type of stones based on morphology, number of stones (single, double, or multiple), the size of the stones (average of 2 significant diameters). The greatest and smallest stones' diameters were noted when there were several gallstones. Using Oser's approach, crushed stone crystals were biochemically analyzed for the qualitative presence of mixed components, bile pigments, and

cholesterol. Several properties of gallstones were found out and statistical analysis was conducted to evaluate any possible association between the characteristics of gallstones and the histopathological conditions of the gallstones.

Inclusion and Exclusion Criteria

The patients who underwent cholecystectomy in our hospital were only included. The included patient had to give consent for the whole study process. The patient who underwent all the required tests were only included. The patients who had underlying chronic conditions including liver diseases, were excluded. The patients who could not finish or undergo the required examinations were also excluded. Applying inclusion and exclusion criteria, the study finally considered 120 patients.

Ethical Approval

The study has been approved by the Ethical Committee of the hospital. The study process has been explained to each patient clearly and required consent was obtained from all the patients.

Statistical Analysis

The study used SPSS 25 and excel software for effective statistical analysis. The descriptive measurements were expressed as mean±standard deviation (sd). The study employed ANOVA for analysis of the parameters. The level of significance considered to be $\alpha = 0.05$.

Results

The study summarizes the baseline characteristics of the patients in this study. The study shows the percentages of patients are more with cholecystitis (81.6%) and least with carcinoma (1.6%) and the percentage of females affected are more than the male patients. It was found that 82.6% of the patients with cholecystitis are females while 87.5% of the patients with hyperplasia were females. Table 1 shows the detailed findings of baseline characteristics.

Table 1: The baseline characteristics of the patients in this study

Parameters		Cholecystitis N =98	Hyperplasia N =8	Cholecystitis with metaplasia N =12	Carcinoma N =2
% Of patients of total		81.6%	6.6%	10%	1.6%
Mean age (years), mean±SD		45.23±15.2	41.13±16.33	43.35±15.43	47.11±14.55
Gender	Male (n, %)	17,17.3%	1, 12.5%	2, 16.6%	Gender
	Female (n, %)	81, 82.6%	7, 87.5%	10, 83.3%	
BMI (kg/m ²)		24.52±1.2	25.32±1.7	25.33±1.3	23.13±1.5

Table 2 shows that the number of stones are more in the cholecystitis than the other 3 groups. Of the 120 cases, the morphological type of stone distribution is mixed (55%), combined (27.5%), pigmented (10%), and cholesterol (7.5%). The size of the gallbladder is normal in 58.3%, fibrotic in

29.1%, and enlarged in 12.5%. 56.6% of the cases have <3mm of the wall thickness of the gallbladder and 43.3% have >3mm of the wall thickness of the gallbladder. Again, the study has found no association between any characteristic and the histopathological condition of the gallbladder ($p>0.05$).

Table 2: The assessment of the gall bladder stones (cholelithiasis)

Gall stone characteristic		Cholecystitis N =98	Hyperplasia N =8	Cholecystitis with metaplasia N =12	Carcinoma N =2	p- value
Number of stones	One or two	54	7	5	1	0.958
	Multiple	44	1	7	1	
Stone Type	Cholesterol	9	0	0	0	0.784
	Pigment	10	1	1	0	
	Mixed	54	4	7	1	
	Combined	25	3	4	1	
Size of the gallbladder	Normal	57	5	7	1	0.699
	Fibrotic	29	2	3	1	
	Enlarged	12	1	2	0	
Thickness of gallbladder wall	< 3 mm	56	3	9	0	0.157
	> 3 mm	42	5	3	2	

The study has found that there is significant association between the size of the gall stones with that of histological diagnosis ($p<0.05$). The size of the stone increases from cholecystitis, hyperplasia, cholecystitis with metaplasia and finally patients with gall bladder carcinoma had

shown the largest size of gall stones. Hence, the size gall stones significantly associates with the histological diagnosis which can have importance in clinical setting. In table 3 the size of the stone is large in carcinoma cases and least in case of cholecystitis.

Table 3: The size of gall stones in association with the mucosal response

Histological diagnosis	Mean size of stone \pm SD (cm)	p-value
Cholecystitis	0.73 \pm 0.35	0.0124*
Hyperplasia	1.57 \pm 1.5	
Cholecystitis with metaplasia	1 \pm 0.43	
Carcinoma	4.5 \pm 0.23	

Discussion

Various changes in the histopathological features of the gallbladder are seen in patients with cholelithiasis. By assessing various parameters like size, number, weight, volume, and morphology of the gallstones by taking sections from the neck, fundus, and body of the gallbladder states that as the size, volume, and weight of the gallstone increases, mucosal changes in the gallbladder is seen from cholecystitis, hyperplasia, and metaplasia and then to carcinoma. The number and the type of gallstone are not significant in producing precancerous lesions. Different chemicals make up gallstones. Cholesterol, pigment or mixed stones are possible. Ultrasound was used to determine the diagnosis. Following surgery, gallstones were submitted for laboratory analysis to check for cholesterol and calcium bilirubin. In contrast to gallbladders holding mixed and cholesterol stones, which were seen in our study, gallbladders carrying pigment stones did not exhibit adenomatous hyperplasia or Rokitansky-Aschoff sinuses. The production of cholesterol-rich stones may be caused by glandular hyperplasia, which may be more potently stimulated by cholesterol. Du Bois et al. first employed laparoscopic cholecystectomy in 1988 after it was first introduced by Mouret in 1987. Despite cholecystectomy remaining the standard of care, a major problem is the therapeutic management of gallstone formation in vulnerable patients. [4,11,12,13].

A key contributor to the development of gallbladder cancer is gallstones. We discovered that silent gallstones, as well as cholelithiasis, developed a number of epithelial pathological alterations in the

gallbladder mucosa while asymptomatic, which may have been a precursor lesion to gallbladder malignancy. Premalignant alterations in the gallbladder epithelium, including atypical hyperplasia, metaplasia, dysplasia, and carcinoma, are known to be linked to gallstones. It may be possible to identify patients who should receive prophylactic cholecystectomy to lower their risk of developing cancer by recognizing the factors linked to these alterations in patients with gallstones. Premalignant epithelial alteration in the gall bladder mucosa is four times more likely to occur in people with gall bladder walls thicker than 3 mm than it is in those with walls thinner than 3 mm, and this difference is statistically significant. For this group of patients, preventive cholecystectomy should be taken into consideration. The discovery of premalignant changes in the morphologic backdrop of chronic cholecystitis supports current recommendations for cholecystectomy and supports the metaplasia-dysplasia-neoplasia sequence. Without the apparent connection to lipid profiles, the number, size, and cholesterol of gallstones increase the likelihood of gall bladder cancer [14,15,16,17].

A prospective study was conducted to investigate the histological characteristics of the gallbladder mucosa in 100 control participants and 85 patients with symptomatic gallstones divided by age and sex. The latter group's stone count and size were also examined. 35% of the controls had aberrant histological findings, mostly chronic cholecystitis, which was more common in older women. Acute inflammatory alterations were present in 6% of individuals with asymptomatic

gallstones, while chronic cholecystitis and/or cholesterolosis were present in all patients. A solitary stone was discovered in 54% of them. These findings imply that the development of microscopic gallstones in the gallbladder can precede the occurrence of persistent inflammatory alterations in the gallbladder mucosa [18].

The defunctionalisation of the gallbladder in individuals who have undergone nonsurgical excision of gallstones but who are not able to undertake surgical/laparoscopic cholecystectomy has been attempted using chemical gallbladder sclerosis. Without harming the common bile duct, cystic duct laser thermocoagulation enables fast gallbladder sclerotherapy. Gallbladder mucosa is denuded as a result of ethanol and sodium tetradecylsulfate-induced sclerosis. However, due to reepithelialisation, a single treatment session with prompt removal of the cholecystostomy catheter was insufficient for gallbladder ablation in this type [19].

35 patients with cholelithiasis had their gallbladder bile tested for carcinoembryonic antigen (CEA), some of whom had incredibly high levels. The relevance of high CEA levels in the bile was assessed by examining the immunohistochemistry reactivity of the gallbladder tissue. In 10/35 patients, gallbladder tissue showed a favorable reaction to anti-CEA serum absorption with nonspecific cross-reacting antigen (NCA). The presence of elevated levels of CEA in the bile was linked to this immunoreactivity. In these nine cases, studies employing anti-CEA serum taken with either biliary glycoprotein-I or regular human bile allowed for further investigation of the reactivity (BGP-I). Five of the nine cases involving immunoreactivity to anti-CEA serum absorbed with regular human bile had negative results, whereas none of the tests using anti-CEA serum absorbed with BGP-I. According to the observations, the

gallbladder epithelium may manufacture CEA and/or some CEA-related antigens, with the exception of NCA and BGP-I, and the inflammatory phase of cholelithiasis may cause an increase in the CEA level in bile [20, 21].

Conclusion

The study has concluded that there is no significant association between the histological types of gallstones with that of number of stones, types of stones, size of the gallbladder and thickness of gallbladder wall. However, there is significant association between the size of the gallstones and mucosal response leading to abnormal histology of the gall bladder. The size of the stones increases with the abnormal histology sequentially from Cholecystitis, Hyperplasia, Cholecystitis with metaplasia and Carcinoma. The study found this finding to be one of the most contributing conclusion in terms of importance in early diagnostics and treatment.

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