ORIGINAL RESEARCH PAPER

Anatomy

# HISTOLOGICAL STUDIES OF HUMAN GALLSTONE DISEASES IN NORTH INDIA

**KEY WORDS:** Cholecystitis, Gall Stones, Malignancy, histopathology, Confidence Interval Value

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ABSTRACT

**Background:** Gall bladder stones diseases are common in the northern part of India with symptoms of frequent abdomen pain. The removals of gall stones by operation or cholecystectomy are the most common methods are being adapted to cure this disease globally. This studies had main focus on to achieve the histopathological changes occurred during the gallstone disease and also analyze to clinical and biochemical changes observe at the time of gall stone disease.

**Methods:** This study was conducted at Rama medical college, Hospital and Research centre, Kanpur which is a tertiary care hospital from November 2015 to February 2017. Total 100 patients were chosen for this study and their diagnosis of cholelithiasis and cholecystectomy results were analyzed. The study of clinical and biochemical data along with pre and post-operative analysis data were collected.

**Results:** During our study about 60% of the cases were females with a female to male ratio of 1.5:1.0. Most of the cases 60% were operated between one to three years of the commencement of symptoms. In all the cases serum cholesterol levels was recorded higher from normal. Most of the patients 60% were suffered from multiple gall stones of mixed type and the composition of stones were cholesterol, bilirubin, calcium carbonate and calcium oxalate. The majority of patients 95% were found with histological characteristics of persistent cholecystitis.

**Conclusions:** The gall bladder diseases (Cholelithiasis) are commonly present in females who having the higher cholesterol content in their blood. The histological changes are connected in cholelithiasis is persistent cholecystitis. In our study premalignant symptoms are observed only in a case. Thus, with the use data of this study, early measures may be taken to prevent from cholecystitis and associated gall bladder diseases.

**Introduction:**

Gall bladder is an important organ of human body in which bile get store and concentrates mainly for the digestion of fat (1, 2). The common diseases in gall bladder are cholecystitis, congenital anomalies, inflammatory, non-inflammatory, neoplastic, invasive and noninvasive. The carcinoma of gall bladder is also common which affects the mortality and morbidity of human population. Cholelithiasis disease alone affects 10-20% of populations in developed countries (3). In the cholesterol gallstone disease excess amount of cholesterol in the bile get store as a solid crystals resulting the formation of gallstone. Cholesterol is only slightly soluble in aqueous media, but it is sparingly soluble in bile and mixed with micelles made by bile salts and phospholipids (4). Excessive cholesterol gets accumulated inside gall bladder and creating deficiency of bile salts (5). If these conditions persist then cholesterol crystals get aggregate, fuse and finally create pathologic gallstones. There are several genes involved in the regulation of cholesterol, bile salts and lipids. The gene responsible for cholesterol secretion are regulated by Abcg5 and Abcg8 (6) and the secretion of phospholipids is maintained by Abcb4 gene. Similarly, the cholesterol converted to bile acid by the regulation of cholesterol 7□-hydroxylase (CYP7A1) gene. Furthermore, it is supposed that bile salts are also playing the role in the formation of gallstone.

The factors concerned in the development of gall stone disease has been separated into two categorizes viz. non-modifiable and modifiable. The non-modifiable parameters contain ethnic environment, age factor, gender especially female, family backgrounds and genetically issues, while the modifiable parameters are obesity, rapid weight loss, and a passive life (7). In literature many reports have publicized a specific parallel relation between gall bladder carcinoma and cholelithiasis (8, 9).

In side gall bladder due to presence of columnar epithelial cells which are lined by a blanket of mucus gel-like secretion which separates the host mucosal cells from the external environment (10). The role of gallbladder mucus is to enhance the formation of cholelithiasis due to nucleation of stones (11). Gall bladder mucus combined with calcium and lipids resulting the formation of gallstones (12). In the gall bladder mucus there is a compound named mucin which plays a key role in gallstone formation. Though, there is very less knowledge available about the variety of mucins and the exact role of gallstone formation and its composition (13).

Another common disease of gallbladder is carcinoma which has big mortality rate in the human populations. It commonly occurs due to long duration of chronic inflammation in gall bladder may be due is considered an important etiological role in carcinogenesis. The frequency of carcinoma gall bladder coupled with gallstones is around 0.3 to 12% (14, 15). In this situation the only cure is available to perform surgical intervention so far. (16) In the available literature, it has been clearly mentioned that gallstone disease has a multidimensional reasons, contain earlier gall bladder infection, less gall bladder motility due to surgery and because of weight loss. The reason may be due to ileal diseases such as Crohn's, haemolytic diseases, genetically hypercholesterolemia and metabolic defects in hepatic bilirubin glucuronidation (17, 18). To cure it proper there is need of regular histopathological analysis for the diagnosis of early carcinomas. In most of the tertiary care hospitals including community health centre in India health workers are following poor diagnosis of gall bladder. Histopathology studies are constrained to only for the few specimens, which show coarse abnormalities. This discriminatory

method is not seems good because it increases patient's financial load.

This study was performed to evaluate the histopathological studies for the gall bladder diseases in the tertiary care unit of Rama Medical College, Hospital and Research Centre, Kanpur (India). To perform histopathology study of every specimen of gallbladder we conducted ultrasonography of abdomen of all the patients. Laparoscopic cholecystectomy was studied in all cases along with the anatomical distortion of selected cases.

**Methods and Materials:** The study was performed at general surgery, anatomy and central research laboratory departments of Rama Medical College, Hospital and Research centre, Kanpur (India). This is a newly stabilized well facilitated medical college in the country and conducting the health care of a large number of patients from the different parts of northern India. The study period was for 2 years from March 2015 to February 2017. The study was considered with all the patients registered for gall stones and cholecystectomy at this institution all through the study period.

**Inclusion criteria:** The written with duly signed consent form were collected prior to diagnosis from the patients. This study was conducted with patients bearing gall stones between the ages of 10 to 70 years.

**Exclusion criteria:** The patients recorded for gall bladder pathologies other than stones were also considered in this study. Random sampling method was adapted. A questionnaires form were filled from all the patients by asking them associated problems and history backgrounds for fulfilling the eligibility criteria to diagnose this disease. Sample size was calculated based on a similar study performed by Khanna et al., (19).

Formula applied for sample size determination was; n= (Zα) 2pq/d2.

Here n= sample size, Zα= 1.96, p= prevalence, q=100-p, d= 20% of prevalence.

By putting the values in the formula, n was calculated equal to 54. To get authentic and reliable results the sample size was considered around twice (n=100). Before starting the study institutional research committee approval was received along with the clearance from the ethics committee of the institution. All the participated patients were proper acknowledged about the study and written consent was obtained to them. All the participated patients admitted with diagnosis of cholelithiasis and surgeries were interviewed with the pre-planed, standard questionnaires to get their history record. Questionnaires form contains about the details of family history of gall stones, drug history and details of symptoms associated. Besides these parameters such as blood haemogram, liver function test, serum cholesterol level was also considered. The patients were cured and treated as the need of departmental rule. Some selected patients were selected for laparoscopic cholecystectomy study. After the operation of patients, study and analysis of stone was done. The stone were analyzed bio-chemically and their number also considered. In some patients the removal of gall bladders were analyzed in details for histopathological study. The histopathological results of cholecystectomy were analyzed in detail to documentation of changes in the tissues. The statistical analysis was done with the use of Epi Info (CDC) software. The statistical significance (p-value) was calculated up to significant level i.e. 0.05.

**Results:** The study was performed on 100 patients who suffered from gall stones and considered for cholecystectomy of gall stones. The study was mainly focused to find out different histopathological changes occurred in gall bladder and related to gall stones. During the study the patients who were subjected to cholecystectomy for cholelithiasis were also considered. The 80% studied patients age was of between the 30 to 65 years.

The majority of the studied patients (60%) were females with a female to male ratio of 1.5:1.0. The majority of patients (62%) had got the body mass index (BMI) in between 26 to 30.1. Around 70% patients have operated within one year of commencement of pain. On the other had 15% patients had recorded pain in the duration between 1 and 2 years and remaining 15% patients had acquired pain in the duration of between 2 and 5 years. The 60% studied females had 2 children in her life. Around 10 patients had claimed the history record of bone and joint pain. Other symptoms such as history of typhoid fever and estrogen administration were not recorded with any patient.

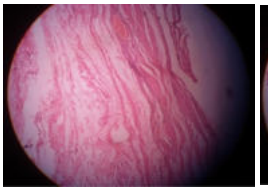

**Fig.1**

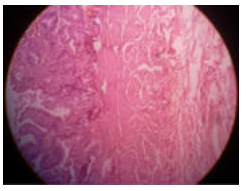

**Fig.2**

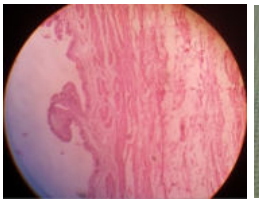

**Fig.3**

**Fig.4**

Fig, 1; A photomicrograph of a section in the cholecystitic human gallbladder showing a PAS positive staining reaction in the epithelial surface with the apical part of the columnar cells.
Fig, 2; A photomicrograph of a section in the cholecystitic human gallbladder showing a disrupted epithelium and stones at the irregular surface of its epithelium cells.
Fig, 3; A microscopy photograph showing of chronic cholecystitis in gall bladder.
Fig, 4; The photograph of a longitudinal cut dissected gallbladder just to expose the mucosal surface.

The haemoglobin (mean value) of the patients was found 12.18 grams per decilitre (gm/dl) with 95% CI value between 11.95 and 12.41gm/dl. The total leukocyte count mean value was found around 7951 with 95% CI value between 7424 and 8478. The mean erythrocyte sedimentation rate (ESR) was recorded 28 mm/h. The mean platelet count was noticed 2.73 lakh cells per cubic millimetre.

**Table: 1; Estimation of biochemical parameters of the patients**

| S.N. | Parameter | Mean value | OR (95% CI) | P-Value |
|---|---|---|---|---|
| 1. | BMI | 28.05 | 1.0 | 0.01 |
| 2. | Haemoglobin | 12.18 | 0.8 | 0.04 |
| 3. | Total leukocyte count | 7951 | 0.7 | 0.06 |
| 4. | Erythrocyte sedimentation rate | 28 mm/h | 1.3 | 0.01 |
| 5. | Total bilirubin | 1.019 mg/dL | 0.9 | 0.02 |
| 6. | Direct bilirubin | 0.36 mg/dL | 1.0 | 0.11 |
| 7. | AST/SGOT | 40.84 IU/L | 0.8 | 0.68 |
| 8. | ALT/SGPT | 50.59 IU/L | 1.0 | 0.09 |
| 9. | Alkaline phosphatase | 78 IU/L | 0.8 | 0.76 |
| 10. | Total protein content | 5.98 g/dL | 0.7 | 0.01 |
| 11. | Albumin content | 3.99 g/dL | 1.0 | 0.79 |
| 12. | Serum cholesterol | 222.90 mg/dL | 1.1 | 0.02 |

The total bilirubin mean value of was found 1.019 mg/dL with 95% CI value between 1. 002 to 1.037mg/dL. The direct bilirubin mean value of was recorded 0.36 mg/dL with 95% CI value

between 0.26 to 0.49 mg/dL. The aspartate transaminase (AST/SGOT) mean value was observed 40.84 IU/L with 95% CI value and its lies between 34.97 to 46.71 IU/L. The alanine transaminase (ALT/SGPT) mean value was observed 50.59 IU/L with 95% CI value and lies between 40.91 to 60.28 IU/L. The alkaline phosphatase mean value was observed 78 IU/L. The total protein mean value was noticed 5.98 g/dL. The protein albumin mean value was recorded 3.99 g/dL. The serum cholesterol mean value was found 222.90 mg/dL with 95% CI and its value lay between 210.30 and 235.40.

Out of 100 patients studied, 60% of the patients were suffered from multiple gall stones. 40% patients were got single or two gall stones inside. The total 100 patients of gall stones were studied and found 60% stones were made up of cholesterol, bilirubin, calcium carbonate and calcium oxalate. Remaining 40% patient's gallbladder stones were made up of cholesterol, calcium carbonate and calcium oxalate. Out of the 100 examined gall bladder patients 95 patients demonstrated the characteristics of chronic cholecystitis. One patient was found of chronic cholecystitis with ulceration while three patients were observed of chronic cholecystitis with gangrenous modifications. While, one patient was found to be suffered from premalignant lesion as a pyloric metaplasia. Remaining one patient was observed to have features of xanthogranulomatous cholecystitis like symptoms.

**Discussion:**

In this study the main victims of gall bladder stones were females with a female to male ratio of 1.5:1.0 and this finding are strongly correlated with the finding of Giri *et al*., 2013 (20) where female: male ratio had been recorded 1.5:1.11. This report has its novelty and may be first report of gall stone disease related to histological aspects in the patients of North India. In this region gall stones are the common linked threat with carcinoma and its correlation is around 60 to 90% in younger generations. The symptoms duration was noticed between one to three years in 90% of studied patients. In another study conducted by Tyagi et al., 1992 the mean duration of this disease was recorded 2.8 years (21). The histopathological modifications are totally depending on the time of gall stones (22, 23). Most of the females around 60% were having 2 children in their life. Serum cholesterol levels were recorded prominent in most of patients with its mean value of 222.90 mg/dL although other blood factors were not presented any considerable divergence from normal values. The presence of high concentration of cholesterol (bile acids) in gallstones has been the main reason of the non-surgical cure of gall bladder stones. Approximately, 60% of the examined patients had noticed with multiple gall stones. Similarly, study performed by Mathur *et al.*, 2012 (23) it has been noted that the number of stones recorded was varied form from a single and double stone in 40% patients while multiple stones were observed in the residual majority 60% of the patients (23). In the majority of cases (60%) the composition of gall stones was found mixed type and its content was cholesterol, bilirubin, calcium carbonate and calcium oxalate. It has been mentioned by Tyagi et al., 1992 (21) the gall stones of mixed diversity were high in the 78.2% of the cases. Similarly, In the finding of Mathur *et al*., 2012 (23) it has been mentioned that the mixed gall stones were recorded in the majority of cases that is 60.4%. Chronic cholecystitis was observed in 95% of the cases while one patient was found suffered with premalignant stage in the form of pyloric metaplasia. Although, similar co-workers have been found the premalignant and malignant changes proportion of high rate. Mathur *et al*., 2012 has been mentioned cholesterolosis in 6% cases while cholecystitis with hyperplasia in 8% cases along with cholecystitis with metaplasia in 18% patients ((23). Although, in the study conducted by Giri *et al*., 2013 epithelial hyperplasia has been recorded in 46.20%, intestinal metaplasia in 25.54%, cholesterolosis in 14.61% (20). Similarly, the study conducted by Abassi *et al.*, 2012, two patients from the 100 had been found positive with carcinoma (24). During the study of Tyagi *et al.*, 2012 chronic cholecystitis has been recorded the principle histological diagnosis (50.8%). Other lesions had found of adenomyomatosis (8.2%), adenomatous hyperplasia (10.1%), cholecystitis (4.1%), cholesterolosis (2.7%) and acute cholecystitis (4.1%) (21) in the gall bladder patients.

The main reason of the factors touching the premalignant lesion was not explored due to only one specimen has observed of premalignant change and there was no any significant malignancy observed. The reason of low occurrence of premalignant and malignant change may be found due to the small sample size taken for this study. Although, we have explore out sufficient amount of data related to the histological study of gall bladder stone in our study.

**Conclusion:**

In the conclusion we have to say that the most of the patients suffering from cholecystitis were females and they have disease symptoms in between the one to three year. Majority of studied patients have got higher serum cholesterol level which may be a main cause of gall bladder stone. In this study we have found that most of the patients were got multiple gall stones of mixed type containing of cholesterol, bilirubin, calcium carbonate and calcium oxalate. Using this study the major focus was attained on histopathological changes which is the main cause of chronic cholecystitis. At last, we hope that our study would fulfill the requirements and make the strength of data generated during this study for the proper cure of cholecystectomy in gall bladder patients mainly on northern India population.

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**References:**

1. Guyton and Hall textbook of medical physiology: 588-595
2. K. Sembulingum essential of medical physiology 6th edition: 590-600.
3. Bladder G. Extrahepatic biliary tree and ampulla. In: Mills SE, editor. Sternberg s Diagnostic Surgical Pathology. 5 th ed., Vol. II. Wolters Kluwer, 2010. p. 1600-51.
4. Mittal R, Jesudason MR, Nayak S. Selective histopathology in cholecystectomy for gallstone disease. Indian J Gastroenterol 2010; 29: 211.
5. Jayasundara JA, de Silva WM. Histological assessment of cholecystectomy specimens performed for symptomatic cholelithiasis: Routine or selective? Ann R Coll Surg Engl 2013; 95:317-22.
6. Yu L, Gupta S, Xu F, et al. Expression of ABCG5 and ABCG8 is required for regulation of biliary cholesterol secretion. J Biol Chem 2005; 280: 8742-8747.
7. Lyons MA, Wittenburg H. Cholesterol gallstone susceptibility loci:a mouse map, candidate gene evaluation, and guide to human LITH genes. Gastroenterology 2006; 131: 1943-1970.
8. Portincasa P, Di Ciaula A, Wang HH, et al. Medicinal treatments of cholesterol gallstones: old, current and new perspectives. Curr Med Chem 2009; 16: 1531-1542.
9. Tyagi SP, Tyagi N, Maheshwari V, Ashraf SM, Sahoo P. Morphological changes in diseased gall bladder: a study of 415 cholecystectomies at Aligarh. J Indian Med Assoc. 1992; 90 (7):178-81.
10. Kuver R, Klinkspoor JH, Osborne WR, Lee SP. Mucous granule exocytosis and CFTR expression in gallbladder epithelium. Glycobiology 2000; 10:149-157.
11. Afdhal NH. Choleterol crystal nucleation: A decade- long search for the missing link in gallbladder pathogenesis. Hepatology 1990, 11:669-702.
12. Jacyna MR: Interactions between gallbladder bile and mucosa; Relevance to gallstone formation. Gut 1990, 31:568-570.
13. Kim HJ, Kim JS, Kim KO,Park KH, Yim HJ, Kim JY, et al. [Expression of MUC3, MUC5AC, MUC6 and epidermal growth factor receptor in gallbladder epithelium according to gallstone composition] Korean J Gastroentero. 2003; 42:330-336.
14. Ganesh IM, Subramani D, Halagowder D. Mucin glycoarray in gastric and gallbladder epithelia. Journal of Carcinogenesis 2007, 6:10 doi: 10. 1186/1477-3163-6-10.
15. Satoh H, Koga A. Fine structure of cholesterolosis in the human gallbladder and the mechanism of lipid accumulation. Microsc Res Tech 1997; 39: 14-21.
16. Dawson PA, Lan T, Rao A. Bile acid transporters. J Lipid Res 2009; 50: 2340-2357.
17. Bassi N, Aggio L, Ghiro S, Meggiato T, Di Mario F, Del Favero G, Scalon P, Molin M, D'Amico D, Naccarato R. X-ray diffraction study of biliary calculi. Morphological and composition correlations of calculi] G Clin Med. 1990; 71: 331-335. (PubMed)
18. Hatsushika S, Tazuma S, Kajiyama G. Nucleation time and fatty acid composition of lecithin in human gallbladder bile. Scand J Gastroenterol. 1993; 28:131-136. (PubMed).
19. Khanna R, Chansuria R, Kumar M, Shukla HS. Histological changes in gallbladder due to stone disease. Indian J Surg. 2006; 68: 201-4.
20. Giri S. Histopathological changes in gallbladder mucosa associated with cholelithiasis. IJCRR. 2013; 5(4):126-9.
21. Tyagi SP, Tyagi N, Maheshwari V, Ashraf SM, Sahoo P. Morphological changes in diseased gall bladder: a study of 415 cholecystectomies at Aligarh. J Indian Med Assoc. 1992; 90(7):178-81.
22. Goyal S, Singla S, Duhan A. Correlation between gallstones characteristics and gallbladder mucosal changes: A retrospective study of 313 patients. Clin Cancer Invest J. 2014; 3: 157-61.
23. Mathur SK, Duhan A, Singh S, Aggarwal M, Aggarwal G, Sen R, et al. Correlation of gallstone characteristics with mucosal changes in gall bladder. Trop Gastroenterol. 2012; 33(1): 39-44.
24. Abassi A, Qasmi SA, Ghafoor A, Kiani F, Abassi H. Frequency of carcinoma gallbladder in cases of cholelithiasis undergoing cholecystectomy at tertiary care hospital. RMJ. 2012; 37(4): 406-8.