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Study of Histopathological Examination of Gallbladder After Cholecystectomy

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Abstract
To study the histological changes in the gallbladder for which cholecystectomy was required. Permission to conduct research using human subjects was obtained from the Research and Ethical Committee of the Index Medical College, Hospital and Research Centre, Malwanchal University. Histological assessment was performed on all 60 cases, resulting in the categorization of tissue samples based on their mucosal response patterns. Cases with multiple mucosal responses per specimen were assigned a predominant pattern. The majority of these cases displayed chronic inflammation, with chronic cholecystitis being the most prevalent, affecting 47 patients. Five cases showed chronic cholecystitis with metaplasia, while two cases were diagnosed with carcinoma. Interestingly, all patients with carcinoma were female. After analyzing the relationship between mucosal response and gallstone type, the chi-square test yielded a value of x2=3.824 and a P-value of 0.867, indicating no significant association. However, when examining the association between mucosal response and gallstone size, the test statistics revealed a value of 12.923 and a P<0.05, suggesting a significant relationship. Chronic cholecystitis can exhibit various histo-morphological patterns, such as acute on chronic cholecystitis and xantho-granulomatous cholecystitis. This condition can occur with cholelithiasis at any age, as observed in this study group, where chronic calculous cholecystitis was the most common finding. Additionally, pigment stones were the most frequently identified type of stone, as opposed to mixed and cholesterol stones.

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Key Words
Gallbladder, histological, gallstones, calcification, morphological, bile ducts

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INTRODUCTION

The gallbladder, situated below the liver, is a vital organ in the digestive system that stores and concentrates bile produced by the liver[1]. A comprehensive understanding of the structural, cellular and imaging alterations in the gallbladder is crucial for the accurate diagnosis and effective treatment of different clinical diseases[2,3]. This versatile technique enables thorough evaluation, facilitating the prompt identification and management of gallbladder diseases[5].

First, research on morphological changes entails an examination of the overall anatomical characteristics of the gallbladder[6]. Morphological changes such as the presence of gallstones, polyps, or structural abnormalities can have a substantial impact on organ function and make individuals more susceptible to several issues. Gallstones, such as those found in gallbladder diseases, are frequently observed morphological features[4,5]. These calcified accumulations of bile constituents can block the bile ducts, resulting in inflammation, infection, or, perhaps, causing the gallbladder to burst[8]. Through the utilization of imaging tools such as ultrasound or computed tomography (CT), healthcare practitioners can detect and analyze morphological alterations, enabling the development of suitable strategies for managing these anomalies[6,7].

Histological examination provides a more in-depth analysis of cellular and tissue makeup of the gallbladder[9]. It offers vital information on the pathogenic mechanisms that contribute to the observed changes in morphology in imaging studies[10]. Histological investigation enables the detection of inflammatory disorders, such as cholecystitis, which can vary in severity from acute to chronic and may be accompanied by consequences such as gangrene or perforation[11]. Moreover, histological assessment is essential for the diagnosis of gallbladder cancer[12]. Gallbladder cancer is frequently diagnosed during advanced stages because of its nonspecific symptoms, underscoring the importance of histological examination for early detection and treatment strategies[13]. Therefore, the aim of the present study was to study the histological changes in the gallbladder for which cholecystectomy was required.

MATERIALS AND METHODS

Permission to conduct research using human subjects was obtained from the Research and Ethical Committee of the Index Medical College, Hospital and Research Centre, Malwanchal University. A detailed history was obtained from patients directly and from medical records, as necessary. After cholecystectomy, the gall-bladder specimens were collected in 10% buffered formaldehyde as follows: Histological procedure: Tissue pieces (5 mm×5 mm) were taken from the fundus, body and neck of the gallbladder for processing (from fixation to wax impregnation). The samples were stored in 10% formaldehyde solution for 8 h. The tissue samples were then processed as follows.

- 70% alcohol: 1 hour
- 90% alcohol: 1 hour
- Absolute alcohol I: 1 hour
- Absolute alcohol II: 1 hour
- Absolute alcohol III: 1 hour
- Absolute alcohol IV: 1 hour
- Xylene I: 30 mins
- Xylene II: 30 mins
- Paraffin (low melting) I: 1 hour
- Paraffin II: 1 hour
- Paraffin III: 1 hour

The total processing duration was 18 h:

Block Making: Paraffin blocks will made by using Leukhart's L pieces. The metal molds were lightly smeared with glycerin to prevent wax from sticking during solidification. Sectioning:15-20 µm sections were obtained using a rotatory microtome. Floating and mounting of sections: Individual sections were cut from the ribbon and lowered to the surface of water in a water bath (-5-10°C below the melting point of paraffin wax). Staining with hematoxylin and eosin: The sections were stained with xylene I for 3 min, followed by xylene II for 1 min to dissolve paraffin wax. The sections were dried using blotting paper and treated with descending grades of alcohol (90%, 70%, and 50%), followed by holding under slow running tap water for 1 min. The sections were stained with Harris's hematoxylin for 5 min and placed in slow-running tap water for 1 min. Overexposure of the stain was controlled, if necessary, by dipping the section in an acid-alcohol solution. The sections were then placed under running tap water for 2 min. The sections were then stained with eosin for 3 min, followed by washing under slow-running tap water for 30 s. Random samples were observed directly under a microscope to ascertain the depth and degree of staining. The sections were then dehydrated with ascending grades of alcohol (50%, 70%, 90% and absolute alcohol). The sections were blotted until dry and treated with xylene for 5 min, followed by mounting with a coverslip using D.P.X. The slides were ready for study and photographs were obtained using a digital photographic microscope.

The Following Microscopic Diagnostic Criteria were Used to Study Stained Gallbladder Sections:

- **Hyperplasia:** Pseudo-stratification of gall bladder epithelium, nuclear crowding, tall columnar cells, and occasional mitotic figures.

- **Dysplasia:** Pseudo-stratification of the gall bladder epithelium, nuclear crowding, some loss of architecture, disorganization of epithelium and nuclear atypia.
- **Metaplasia:** Presence of goblet cells.
- **Antral metaplasia:** branched tortuous glands in the lamina propria.
- **In situ carcinoma:** hyperchromatic nuclei with one or more prominent nucleoli, eosinophilic cytoplasm, clear or mucous secreting, occasional giant cells, loss of polarity, atypical mitotic figures, and absence of stromal invasion.

Statistical Analysis: The units of expression for all data are presented as mean and standard deviation (±). Data analysis was conducted using SPSS 20.0. Pie charts and bar diagrams were used to statistically represent results. To ascertain whether a significant association exists between at least two categorical variables, Fisher's Exact Test was used. It is commonly employed in lieu of the Chi-Square Test of Independence when one or more sample sizes are approximately five. Fisher's exact test is technically applicable to samples of any size. Given the limited sample size of 60 participants in this investigation, the correlation test remains valid. These observations were subsequently compared with contemporary standard texts and literary works produced in the same field.

RESULTS AND DISCUSSIONS

Histological evaluation was performed for all 60 cases and the tissue samples were categorized based on their mucosal responses. The predominant pattern was used for categorization of cases with multiple mucosal responses per specimen (one condition per case). Among these cases, the majority exhibited chronic inflammation, with chronic cholecystitis being the most common, affecting 47 patients. Next, chronic cholecystitis with metaplasia was observed in 5 cases. In two cases, carcinoma was diagnosed and intestinal metaplasia was identified in one case. Notably, all patients with carcinoma were female.

Upon analyzing the relationship between mucosal response and gallstone type, the chi-square test yielded a value of x2= 3.824 and a P-value of 0.867, indicating no significant association. However, when examining the association between mucosal response and gallstone size, the test statistics revealed a value of 12.923 and a $P<0.05$, suggesting a significant relationship.

Cholelithiasis is responsible for almost 95% of biliary tract illnesses. As chronic cholecystitis is linked to cholelithiasis in over 90% of cases, individuals at risk for cholelithiasis are also at risk for chronic cholecystitis. The presence of excessive bile increases the likelihood of developing chronic inflammation and, in most cases, stone formation. Bacteria such as E. coli and enterococci can be grown in the laboratory from bile in approximately 33% of cases. Biliary symptoms often arise because of the prolonged presence of gallstones and chronic low-level inflammation[17].

Chronic cholecystitis is a rare occurrence without the presence of gallstones., however, pure cholesterol and calcium bilirubinate stones can exist without inflammation. A thickened gallbladder wall is consistently present, sometimes to a significant extent, and can be accompanied by widespread calcification, leading to a medical condition called porcelain gallbladder.

The stones in the gallbladder are usually composite in nature. When examined under a microscope, the mucosa of the gallbladder shows varying degrees of mononuclear cell infiltration and fibrosis due to chronic inflammation. Epithelium may exhibit normal, atrophic, hyperplastic, or metaplastic changes. Metaplasia can manifest as either goblet cell metaplasia (intestinal type) or pyloric metaplasia (antral type), characterized by the presence of Paneth and endocrine cells in the former.

Unlike the typical glands found in the neck of the gallbladder, metaplastic gland cells contain non-sulfated acid mucin and neutral mucin, but only a small amount of sulfated acid mucin. The prevalence of metaplastic alterations increases with increasing age. The gallbladder may exhibit fibrosis, muscle hypertrophy, encrusted stones, and nodular collections containing foamy macrophages[17].

Tubular structures with irregular shapes were found within the wall in more than 50% of the cases. Their inner surface is covered by a layer of columnar or cuboidal epithelial cells and may contain bile or stones. The term "Rokitansky-Aschoff sinuses" refers to herniations or diverticula resulting from elevated pressure within the lumen. The subserosal layer on the hepatic side contains tiny tubular forms called Lushka ducts.

Adenomyoma refers to instances of gallbladder diverticulosis characterized by localized or segmental muscle hypertrophy, which is more pronounced than usual. In gallbladders affected by chronic cholecystitis with pyloric gland metaplasia and segmental adenomyomatous hyperplasia, intra-and perineural invasion can be observed. Chronic cholecystitis manifests as several morphological forms.

In a groundbreaking study conducted by[17] individuals with cholelithiasis who had not undergone surgery were closely monitored. The results revealed that a considerable proportion (one-third to one-half) of these patients later developed serious symptoms or complications related to the condition[10-17].

Based on these findings, prophylactic removal of the gallbladder, including stones, is recommended for all candidates suitable for surgery. The National Cooperative Gallstone Research has reached similar

Table 1: Gallbladder size of the study population specimen

Gall bladder size	Number
Normal	35
Enlarged	16
Fibrotic	9

Table 2: Gallbladder thickness of the study population specimen

Gallbladder thickness	Number of patients
< 3mm	34 (56%)
≥ 3 mm	26 (54%)

Table 3: Subject distribution in accordance with diverse mucosal responses

Mucosal response on histological examination	Number
Chronic cholecystitis with cholelithiasis	47
Chronic cholecystitis with metaplasia and cholelithiasis	5
Adenomatous hyperplasia with cholelithiasis	2
Adenomyomatous hyperplasia with cholelithiasis	2
Acute on chronic cholecystitis with cholelithiasis	1
Xanthogranulomatous cholecystitis with cholelithiasis	1
Papillary carcinoma	1
Intestinal metaplasia and cholelithiasis	1

conclusions. Inflammatory polyps have been consistently associated with chronic cholecystitis. Adenomyomatous hyperplasia and adenomyomatous hyperplasia are reactive changes in the mucosa that occur because of inflammation and/or the presence of gallstones. The lining cells and neck mucous glands primarily consist of sulfated acid mucin, with minimal presence of non-sulfated acid mucin[1-8].

Jha[12] and other researchers examined the morphological alterations of gallbladder specimens obtained from cholecystectomy procedures. Female individuals were disproportionately affected, with a male-to-female ratio of 1:6.5. The average age was 43.6 years, with the majority of cases occurring in individuals aged between 30 and 49 years. Cholelithiasis was identified in 85.3% of the patients. The primary histological diagnosis was chronic cholecystitis (CC). Other abnormalities observed included adenomyomatosis, adenomatous hyperplasia, granulomatous cholecystitis, cholesterosis, acute cholecystitis, acute chronic infection, subacute cholecystitis and gallbladder malignancy.

Mori[18] reported a significantly higher prevalence of gallstones in young women taking oral contraceptives. However, the opposite was observed in older age groups. Additionally, a study by Sood[19] reported that gastric metaplasia was observed in 33% of cases, whereas intestinal metaplasia was found in 8% of cases. Neutral mucin exhibited the highest level of positivity among the three mucins, followed by sulfated and sialomucin. The presence of sulfated mucin in both gastric and intestinal metaplasia showed a statistically significant result with a low p-value, while the presence of neutral mucin showed a statistically significant correlation with stomach metaplasia.

A study conducted by Hwang[20] revealed that xanthogranulomatous cholecystitis is more commonly observed in females between the sixth and seventh decades of life. In another study[21], researchers found a notable increase in the occurrence of gallstones in gallbladders that had undergone metaplastic, dysplastic and neoplastic changes in the mucosal lining. The transition from metaplasia to malignancy was accompanied by an elevation in sialomucin levels and a reduction in sulfated mucin levels. Furthermore, the presence of neutral mucin was elevated in metaplastic cells, but was significantly diminished in neoplastic cells. According to a study by[34], sulfated mucins play a larger role in the production of gallstones than neutral mucins. Additionally, sialomucins and sulfomucins significantly contribute to the progression and spread of cancer[34].

In this study, females showed a heightened susceptibility to chronic cholecystitis, with a prevalence of 91%. Cholelithiasis and chronic cholecystitis were observed. In some cases, gastric and intestinal metaplasias occur. Well-established risk factors for gallstones include female sex and advancing age, while modifiable risk factors include obesity, fast weight loss, and gallbladder stasis. The primary dietary risk factor for gallstones is excessive caloric consumption, whereas protective factors include a substantial amount of dietary fiber, vegetable protein, nuts and regular physical activity. Chen[21] found that obese patients exhibited a higher prevalence of benign gallbladder disease.

In the current investigation, there was a higher number of females than males, with a male-to-female ratio of 1:11. The majority of incidents occurred in individuals in their forties. The histological findings included chronic cholecystitis with stones, chronic cholecystitis without stones, acute cholecystitis and xanthogranulomatous cholecystitis.

In this study, only one male patient, aged 44 years, was diagnosed with xanthogranulomatous cholecystitis. This patient had pigment stones and was found to have no gallbladder cancer. Additionally, only one male patient aged 37 years was observed to have acute chronic cholecystitis, specifically characterized by the presence of pigment stones.

CONCLUSION

Chronic cholecystitis has a wide range of histo-morphological patterns, including acute on chronic cholecystitis, chronic and xantho-granulomatous cholecystitis, which can be associated with cholelithiasis at all ages. The predominant histo-morphological pattern observed in this study group was chronic calculous cholecystitis, and the most common type of stone found was pigment stone, compared to mixed and cholesterol stones. The involvement of younger age groups is another noteworthy finding of the present study.

Conflict of Interest: There is no conflict of interest among the present study authors.

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