Histopathological and Histochemical Study of Human Cholecystitis

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Author’s contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

In our study the following inferences were made. Chronic calculous cholecystitis was seen in the age group of 41 to 50 years. A female preponderance was noted with male to female ratio of 2:3. The most common signs and symptoms were upper abdominal tenderness and right hypochondria pain. The mucosa showed ulceration in 63%, hyperplastic mucosa in 34% and metaplasia in 5%. 22% of cases showed mild (Grade-I) inflammation while 41% of cases and 27% of cases showed moderate (Grade-2) and severe (Grade-3) inflammation respectively. 44% of cases showed mild (Grade-I) fibrosis while 22% of cases and 15% of cases showed moderate (Grade-2) and severe (Grade-3) fibrosis. With increase in severity of inflammation and fibrosis, the total acid mucin content decreased while the neutral mucin content increased. Similar changes were observed in cases of metaplasia too.

Keywords: Chronic calculous cholecystitis; metaplasia; Inflammation.

1. INTRODUCTION

Biliary tract disorders affect a large percentage of the world’s population. Cholelithiasis is responsible for more than 95% of biliary tract illness. In affluent areas, gall stones affect 10 to 20% of the adult population. It is a very common practice to receive cholecystectomy specimens in Histopathology laboratory following cholelithiasis, acute cholecystitis, chronic or acute superimposed on chronic cholecystitis [1].
When cholecystectomy specimens are subjected to histopathology examination, it created very important role for pathologists to diagnose the lesion that led to cholecystectomy. Histochromically the mucins produced by both the lining cells and the neck mucous glands is mainly of sulphated acid type in physiological condition [1-3].

In contrast the cells of metaplastic glands contain nonsulfated acid mucin (sialomucin) and neutral mucin but little sulphated mucin. Histochemical stains for mucins carnes play an important role in the evaluation of the disease with clinical knowledge, the observations by histopathologic examination of gallbladder under light microscope and histochemical study of mucin present on the glands enable us to arrive at useful conclusions [4]. Several studies have suggested progression from metaplasia, through dysplasia, to adenocarcinoma of gallbladder. The existence of such a pathway has not been definitely proven [5].

2. MATERIALS AND METHODS

This is a prospective study carried out in the Department of Pathology at Sree Balaji Medical College and Hospital, Chennai. The specimens of cholecystectomy received from Surgery Department.

Surgically removed 59 gallbladder (cholecystectomy) specimens were received from Department of Surgery Sree Balaji Medical College and Hospital and the same were studied in the Department of Pathology, 59 specimens of chronic calculous cholecystitis.

2.1 Methods of Collection

The specimen was collected in 10% neutral buffered formalin along with clinical details scrutinization of the patient. The specimens of cholecystectomy were fixed in fresh formalin for 24 hours. Gross descriptions of all the specimens were done. Portions of the fundus, body, and neck of the gall bladder were obtained, as well as sections of abnormally looking mucosa.

This was followed by paraffin embedding and sectioning at a thickness of 4 microns using standard histological procedures. The tissue sections were deparaffinized on a slide warmer, then further deparaffinized with xylene, followed by Hematoxylin and Eosin, PAS, and Alcian blue staining, as listed below. Morphological changes in all cases were studied with special reference to degree of inflammatory reaction characterized by plasma cells, macrophages and lymphocytic infiltration in the mucosa and subserosal tissue, presence of Rokitansky-Aschoff sinuses and focal accumulation of cholesterol laden macrophages in the lamina propria and metaplastic glands containing non sulphated acid mucin and neutral mucin but little sulphated acid mucin.

3. OBSERVATIONS AND RESULTS

In this study conducted on 59 patients who underwent Cholecystectomy in Sree Balaji Medical College & Hospital, Chennai the following findings were observed.

**Age wise distribution of cases:** The age of the patient varied from 10 years to 71 years. The highest incidence was seen in the 41-50 years age group. The mean age of the patients was 41.8 years.

**Genderwise distribution of cases:** Out of the 59 cases studied, it was observed that 23 males (39%) and 36 females (61%) were operated. Therefore a female preponderance was noted with male to female ratio of 2:3.

Our findings were based on average ages of 42.5, 43.6, and 45.3 years. Females made up 61 percent of the patients we looked at [Table 1].

**Clinical symptoms:** Though the patients presented with various symptoms, the most common symptom was right hypochondria Pain noted in 54 cases (91.5%) followed by flatulent dyspepsia noted in 30 cases (50.8%).

| Gender   | No of case | Percentage (%) |
|----------|------------|----------------|
| Male     | 23         | 38.98          |
| Female   | 36         | 61.02          |
| Total    | 59         | 100            |
Physical signs: The most common physical finding noted on examination by the clinician was upper abdominal tenderness seen in 21 patients (36.2%). About 33 cases had no signs at the time of presentation [Table-2].

Mucosal characteristics: The mucosal features included ulceration, hyperplasia and metaplastic changes. Ulceration was observed in 37 cases, hyperplasia in 20, and metaplasia in three. One patient had intestinal metaplasia, while the other two had gastric metaplasia.

Inflammation: On examination of the stained sections the inflammatory cells were counted under 400x magnification. The grading was done [Table-3].

Fibrosis: The distribution of fibrosis in the muscle mucosae was assessed. The grading 1st based on collagen distribution and presence of lamellar fibroplasias. [Table-4].

Correlation of Mucin Score with Grades of Inflammation and fibrosis: Alcian Blue and PAS were used to stain the sections. The percentage of positive cells was calculated and linked with inflammation and fibrosis severity. The percentage positive of cells in each field under low power inspection (100X) was used to create a score system, as shown below:

Correlation of Alcian Blue stain with inflammation: The mean scores for Alcian Blue positive mucins in the three groups of inflammation were tabulated [Table-5].

In the three grades of inflammation, the mean scores for Alcian Blue positive (acid) mucins in the superficial and deep mucosa are displayed. With increasing degrees of inflammation, the mean scores for Alcian Blue positive (acid) mucins in the superficial and deep mucosal epithelium decline.

Correlation of PAS with inflammation: The mean scores for PAS positive mucins in the three groups were tabulated [Table-6].

The scores are higher in Grade III inflammation, than in Grade 1 inflammation. Grade II inflammation however shows random scores, not conforming to any pattern.

Table 2. Signs of chronic calculous cholecystitis

| Signs                        | Number of Cases | Percentage (%) |
|------------------------------|-----------------|----------------|
| Upper Abdomen Tenderness     | 21              | 36.2%          |
| Icterus                      | 5               | 8.47%          |
| No signs                     | 33              | 55.9%          |

Table 3. Grading of inflammation

| 0 | No inflammatory cells |
|---|-----------------------|
| 1 | Diffuse,<10 inflammatory cells per HPF in any layer |
| 2 | Diffuse, between11 to 30 cells per HPF |
| 3 | Diffuse, more than 31 cells per HPF or follicle |

Table 4. Grading of fibrosis

| 0 | No Fibrosis |
|---|-------------|
| 1 | Uneven collagen deposition in <20% of material |
| 2 | Uneven collagen deposition in 21% to 70% material |
| 3 | Uneven collagen or lamellar fibroplasias in >71% of material |

Table 5. AB (Acid mucin) mean score with grades of inflammation

| Inflammation grade | Alcian blue [acid mucin] means core |
|--------------------|-------------------------------------|
|                    | Superficial | Deep |
| I (13 cases)       | 3.23        | 3.54 |
| II (24 cases)      | 2.12        | 3.03 |
| III (16 cases)     | 1.8         | 2.52 |
Table 6. PAS (Neutral Mucin) mean score with grades of inflammation

| Inflammation grade | Pas [neutralmucin] Mean score |
|--------------------|--------------------------------|
|                    | Superficial | Deep |
| I (13 cases)       | 0.27        | 0.11 |
| II (24 cases)      | 0.15        | 0.29 |
| III (16 cases)     | 0.33        | 0.46 |

Table 7. AB (Acid Mucin) mean score with grades of fibrosis

| Fibrosis grade | Alcianblue [acidmucin] Mean score |
|----------------|----------------------------------|
|                | Superficial | Deep |
| I (26 cases)   | 2.44        | 2.81 |
| II (13 cases)  | 2.12        | 3.22 |
| III (9 cases)  | 1.88        | 2.18 |

Correlation of Alcian Blue Stain with Fibrosis the Mean Scores For the 48 Cases with Fibrosis are Tabulated: Alcian Blue scores (acid mucins) are slightly lower in Grade III fibrosis, compared with Grade I [Table 7].

5. CONCLUSION

Chronic calculous cholecystitis was more common in the age group of 41 to 50 years, with a female majority, and more cases with moderate inflammation and mild fibrosis were identified in our study. In order to improve therapeutic and prognostic relevance, additional cases of severe inflammation, gastric metaplasia, and intestinal metaplasia with decreased acid mucin (sialomucin) in high-risk groups should be researched.

The altered expression of mucin plays an important role in the diagnosis of chronic cholecystitis and gall stone disease. In severe inflammation, gastric metaplasia and intestinal metaplasia were associated with increase in sialomucin. The risk of neoplasia is greater, making these patients high risk. Normal human gall bladder mucosa contains predominantly sulphated acid mucin. This sulphated mucin content is found increased in gall stone disease. On the other hand the metaplastic and neoplastic gall bladder epithelium shows an increase in sialomucins and decrease in sulphomucin.

CONSENT

As per international standard or university standard, patients’ written consent has been collected and preserved by the author(s).

ETHICAL APPROVALS

We conducted our research after obtaining proper IEC approval.
COMPETING INTERESTS

Author has declared that no competing interests exist.

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