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Mucin Histochemistry in Chronic Calculous Cholecystitis and Its Correlation with Histomorphology

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Abstract

Objective: 1. To study the mucin spectrum in cholelithiasis, 2. To study the histomorphology of calculous cholecystitis, 3. To correlate mucin histochemistry with the histopathological findings. *Materials and Methods:* Histopathologically diagnosed cases of chronic calculous cholecystitis were taken. The study included 30 cases. Special stains like PAS (for neutral mucin), alcian blue-ph2.5(for sialomucin) and alcian blue pH 1(sulfomucin) were done on all 30 cases. Slides were assessed as per proforma. *Results:* The gall stone disease showed female preponderance. There was increase in sialomucin and neutral mucin in contrast to normal gall bladder(where sulfomucin predominates). Pigment stones seen more common than cholesterol stones. Gastric metaplasia is more common than intestinal. *Conclusion:* In chronic calculous cholecystitis, intraepithelial sulfated mucin reduces and sialomucin and neutral mucin increases. Cases with severe inflammation(grade III) showed the maximum decrease in sulfomucin and increase in sialomucin scores.The sialomucin showed increasing scores with degree of fibrosis. Gastric metaplasia had highest incidence(53.8%) in severe inflammation and was associated with increase in sialomucin.

Keywords: Alcian Blue; Cholecystitis; Metaplasia; Mucins; Periodic Acid Schiff.

Introduction

The gallbladder is among the most commonly surgically resected organs, and the numbers of cholecystectomies has increased more than 50% in the past decade [1,2,3]. As much as 1L of bile is secreted by the liver per day. Between meals,bile is stored in the gall bladder, where it is concentrated. More than 95% of biliary tract disease is attributable to cholelithiasis(gallstones)[4]. It occurs predominantly in females and the incidence increases with age, possibly resulting from a progressive increase in the secretion of biliary cholesterol. [5].

Cholesterol stone formation requires the supersaturation of bile with cholesterol, which results from increased biliary cholesterol output, decreased bile acid synthesis, or both [6,7,8]. Gallbladder hypomotility and mucin hypersecretion promote the precipitation and agglomeration of cholesterol monohydrate crystals into stones [9].

Pigment gallstones are complex mixtures of abnormal insoluble calcium salts of unconjugated bilirubin along with inorganic calcium salts [4].

Cholelithiasis produces diverse histopathological changes in gallbladder mucosa namely acute inflammation, glandular hyperplasia, granulomatous inflammation, cholesterosis, dysplasia and carcinoma [10].

Histochemically, the mucin produced by both the lining cells and the neck mucous glands is mainly of sulfated acid type (in contrast to that of metaplastic glands) the cells of metaplastic glands contain nonsulfated acid mucin and neutral mucin but little sulfated acid mucin[11].

The gallbladder mucus play a regulatory role in cholelithiasis as it promotes the nucleation of stones.

Mucin, a high molecular weight glycoprotein secreted by the gallbladder and biliary duct epithelium, is a pronucleating agent in experimental and human

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gallstone disease [12]

However, there is little on record a systematic study of mucin carbohydrate changes in gallstone formation. So we have attempted to investigate mucin changes in cholelithiasis using mucin histochemistry.

Materials and Methods

A total number of 30 cases, which were histopathologically diagnosed as chronic calculous cholecystitis were taken. The study was conducted in pathology department of Bangalore medical college and research institute. This was a prospective study undertaken during a period of 6 months from may 2015 to October 2015. Three sections were cut from each case for special stains to demonstrate mucin. The stains used were per-iodic acid Schiff (PAS), alcian blue of pH 2.5 and alcian blue of ph 1 to demonstrate neutral, sialomucin and sulfated mucins stains respectively. Section from normal kidney was taken as control for periodic acid Schiff stain,small intestine for alcian blue pH 2.5 and large intestine for alcian blue pH 1.

First H&E sections were screened and various morphological features seen were noted. A proforma was prepared for assessment of the slide. Intensity of inflammation and degree of fibrosis, were graded as mild, moderate and severe (I, II, III). Rokitansky Aschoff sinuses, Gastric metaplasia and intestinal metaplasia were also noted and indicated as present or absent. Type of stones namely, cholesterol and pigmented were noted as per their gross appearance. A scoring system for mucin stain was devised, based on the percentage positivity of cells in each field under low power examination (10X), as:

75% -100 %= 5+, 50% - 75% =4+, 25% - 50% =3+, 5% - 25% =2+, 0% - 5% = 1+ .(Figure 3a, 3b, 3c).

Results

- Out of 30 cases, 20 were female (66.7%) and 10 were male (33.3%), proving female preponderance (a ratio of 2:1-F:M).
- Histomorphology of gall bladder showed-
- Inflammation: 5(16.7%) cases had Grade I inflammation in the form of mild lymphocytic infiltrates. Out of 30 cases 12 (40%) showed grade II inflammation having moderate degree of lymphocytic infiltrate and other 13 (43.3%) cases showed large lymphoid aggregates which were regarded as grade III inflammation(Figure 1a).
- Fibrosis: Mild degree of fibrosis seen in 10(33%) cases, moderate degree in 11(36%) cases, Severe degree in 09 (30%) cases.
- Rokitansky sinus seen in 20(66.7%) cases. (Figure 1b)
- Gastric metaplasia (Figure 2a & 2b) was evident in 12 cases (40%), intestinal metaplasia (2c & 2d) in 6(20%) cases, remaining 12 did not show any metaplasia (40%).
- Pigment stones were seen in 19 cases(63.4%) and cholesterol stones in 11 cases (36.6%).
- Mucosal hyperplasia-15(50%) cases.(Figure 1c & 1d).

Comparison of mucin histochemistry with inflammation and fibrosis showed the following results-

1. With increasing grades of inflammation, neutral and sialomucin showed increasing scores. Grade III inflammation showing highest score of 4 (for PAS) and 4.3 (for Alcian blue 2.5). Sulfated mucin showed decreasing scores with increasing grades of inflammation (Table 1).
2. Fibrosis: PAS score(neutral mucin) was highest in grade III fibrosis, where as least in grade II. Sialomucin scores showed increasing values with increasing degree of fibrosis. Sulfated mucin did not show much of variation(a difference of 0.1 decimel among the three grades) (Table 2).

Comparison of grades of inflammation with type of metaplasia-Highest number of gastric metaplasia (7 cases) were seen in grade III inflammation. Four cases and one case of gastric metaplasia were seen in grade II and grade I inflammation respectively. Intestinal metaplasia did not correlate with grades of inflammation. One, three and two cases of intestinal metaplasia were seen in grade I, grade II and grade III inflammation respectively (Table 3).

Comparison of inflammation with types of stones- 8 cases of pigmented stones and 5 cases of cholesterol stones elicited grade III inflammation. 7 cases of pigmented stones and 5 cases of cholesterol stones showed grade II inflammation. Lastly 4 cases of pigmented stones and 1 case of cholesterol stones showed grade I inflammation (Table 4)

Comparison of metaplasia with types of stones- Out of 19 cases of pigmented stones 8 cases showed gastric metaplasia and 5 cases showed intestinal metaplasia, rest 6 cases did not show any metaplasia. Out of 11 cases of cholesterol stones 4 cases exhibited gastric metaplasia and 1 case intestinal metaplasia and

Table 1: Comparison of mucin histochemistry with inflammation

Inflammation grade	Periodic acid Schiff (average score)	Alcian blue ph 2.5 (average score)	Alcian blue ph 1 (average score)
Grade I	2.2	2.2	2.5
Grade II	3.2	3.3	1.4
Grade III	4.0	4.3	0.9

Table 2: Comparison of fibrosis and mucin histochemistry

Fibrosis	Periodic acid Schiff (average score)	Alcian blue ph 2.5 (average score)	Alcian blue ph 1 (average score)
Grade I	3.3	2.5	1.1
Grade II	2.8	3.5	1.2
Grade III	3.5	4.1	1.3

Table 3: Comparison of inflammation with metaplasia

Inflammation Grade	Gastric Metaplasia	Intestinal Metaplasia
Grade I	1 (20%)	1 (20%)
Grade II	4 (33.4%)	3 (25%)
Grade III	7 (53.8%)	2 (15%)

Table 4: Comparison of inflammation with gall stones

Inflammation grade	Pigment stones (19)	Cholesterol stones (11)
Grade I	4	1
Grade II	7	5
Grade III	8	5

Table 5: Comparison of metaplasia with types of gall stones

Types of Metaplasia	Pigment Stones	Cholesterol Stone
Gastric(12)	8	4
Intestinal(6)	5	1

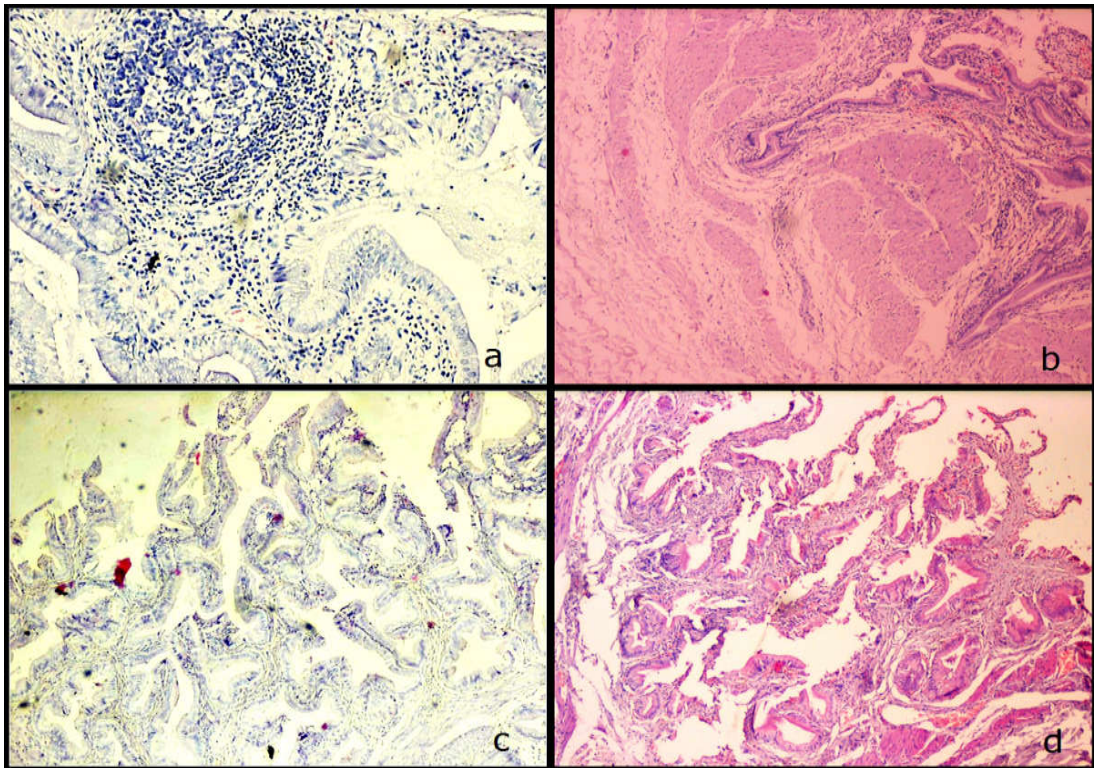

Fig. 1(a): Grade III inflammation showing lymphoid aggregate.(H&E, 5x) **Fig. 1(b):** Rokitansky aschoff sinus (H&E,5x) **Fig. 1(c)&(d):** Mucosal hyperplasia (H&E,5x)

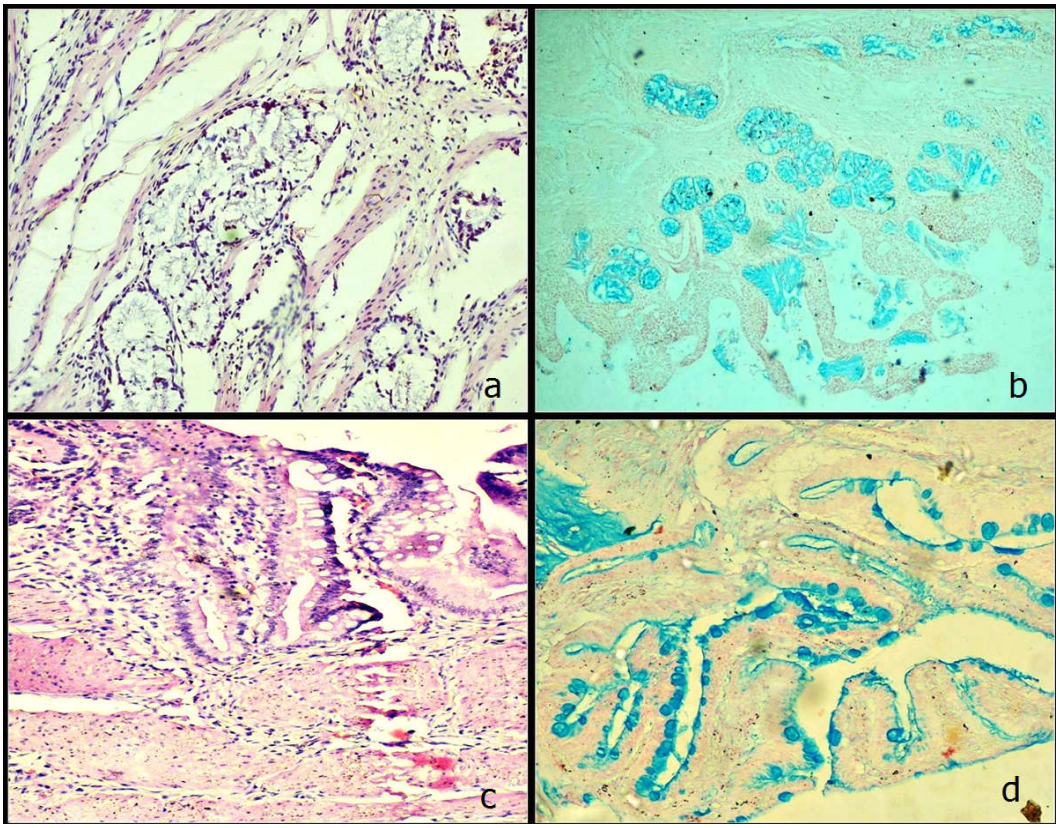

Fig. 2(a): Gastric metaplasia (H&E, 5x). **Fig. 2(b):** Metaplastic glands positive for sialomucin (Alcian blue ph 2.5, 5x). **Fig. 2(c):** Intestinal metaplasia (H&E, 5x). **Fig. 2(d):** Goblet cells highlighted by sialomucin (Alcian blueph 2.5,5x)

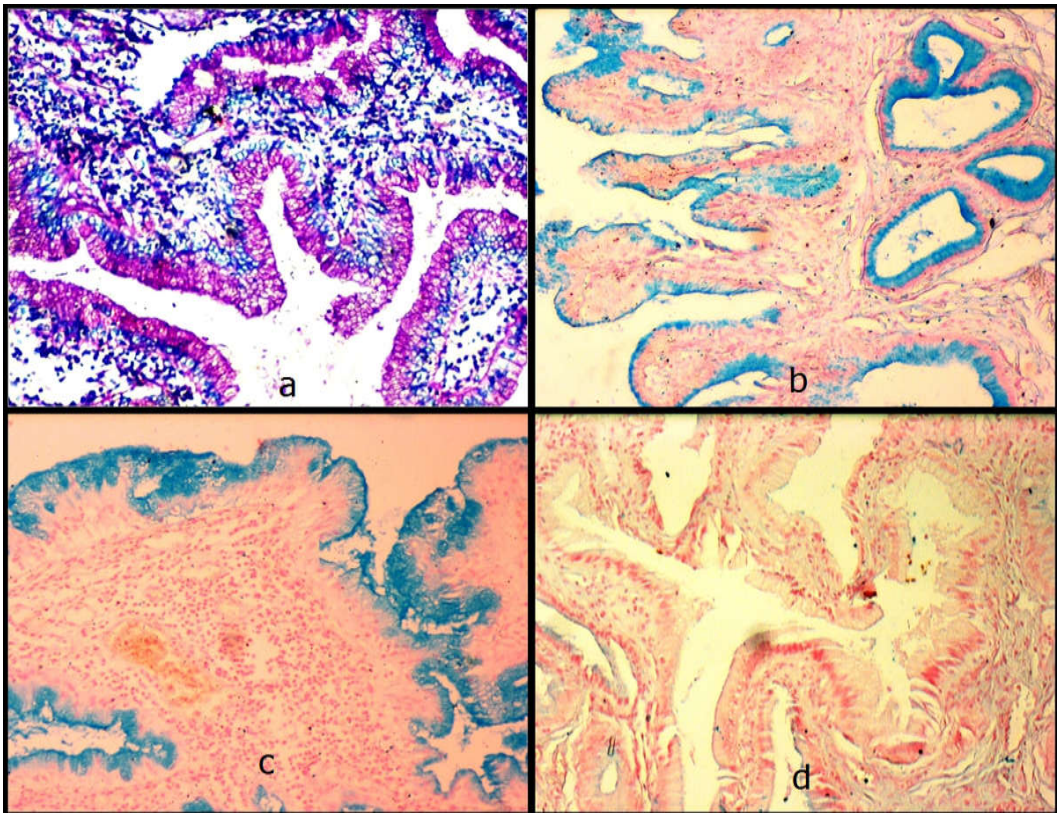

Fig. 3(a): Pas Score 5+(75%-100%)(10x) **Fig. 3(b)&(c):** Alcian blue 2.5(sialomucin)-Score 5+(10x) **Fig. 3(d):** Alcian blue 1(sulfomucin): Score 1(0-25%)(10x)

remaining 6 did not show metaplasia (Table 5).

Discussion

The pathogenesis of gall stone formation involves four factors, namely- 1)supersaturation of bile with cholesterol, 2) hypomotility of the gall bladder, 3) accelerated cholesterol crystal nucleation, 4) hypersecretion of mucus in the gall bladder.This mucus traps the nucleated crystals, leading to accretion of more cholesterol and the appearance of macroscopic stones[4]. So it is well known that mucus hypersecretion plays one of the important role in pathogenesis. Our study aimed to evaluate the changes in mucin spectrum in cases of cholelithiasis and to see if there is any correlation with histomorphological findings and also to study histomorphology of chronic calculous cholecystitis.

The characteristic microscopic features observed were namely, Inflammation ranging from mild to severe grade, fibrosis ranging from mild to severe degree, presence of rokitansky aschoff's sinus, mucosal hyperplasia,gastric and intestinal metaplasia.

Normal gall bladder mucosa is known to conatin predominantly sulfomucin. Traces of sialomucins and neutral mucins also may present [13]. We attempted to demonstrate neutral,sialo and sulfomucin using PAS, alcian blue of pH 2.5 and alcian blue of pH 1 respectively.

- In our present study there is significant decrease in the intraepithelial sulfomucin and increase in sialomucin and neutral mucin. This decrease in intraepithelial sulfated mucin is likely to be a reflection of increased secretion of sulfomucin into bile which is known to occur in calculous disease.
- The cases with severe inflammation (grade III) showed the maximum decrease in sulfomucin and increase in sialomucin scores.
- The sialomucin showed increasing scores with degree of fibrosis. Sulfomucin did not show much variation and neutral mucin was least in grade II fibrosis.
- Gastric metaplasia had highest incidence(53.8%) in severe inflammation and was associated with increase in sialomucin(Figure 2b). Intestinal metaplasia was least in grade III inflammation.

The mucin profile, changes with progressive transformation to neoplasia, from normal with sulfomucin predominating through metaplastic and dysplastic showing increase in amounts of sialomucin, to full-fledged neoplastic with sialomucins predominating.

Our findings correlated with that of study conducted by Anupama et al, where it is concluded that the sulfomucin content decreases in chronic calculous cholecystitis and with severe inflammation, neutral mucin increases, and there is a higher incidence of gastric metaplasia and pigment stones[14].

In the study by Santosh Upadhyaya Kafle et al, the gastric metaplasia was present in 33.0% of cases and intestinal metaplasia in 8.0%. Maximum positivity among the three mucins (neutral, sulfated and sailo) was of neutral mucins (35.0%), followed by sulfated mucins (21.0%) and sailomucins (1.0%) [10].

Similar type of study done by Ganesh I M et al suggested that the sulfated mucins have a greater role in gallstone formation than the neutral mucins. Also, the sialomucins and sulfomucins play an important role in cancer progression and metastasis [15].

In the study by Gupta SC et al, 150 surgically resected gallbladder specimens were included to evaluate the relationship between the prevalence of gall stones and histochemical alteration in sequential changes of metaplasia, dysplasia and neoplasia in gallbladder epithelium. Results revealed increase in sailomucin with a corresponding decrease in sulfated mucin was observed from metaplasia to malignancy. Neutral mucin was increased in metaplastic cells but reduced in neoplastic cells [16].

Main limitations of our study are less sample size and lack of biochemical confirmation of gall stones. Gall stones were categorized based on gross findings and no biochemical tests were done for confirmation. There is a need to conduct study on larger sample size to know mucin changes in metaplasia-dysplasia-carcinoma sequence and also to clearly identify role of mucin in cholelithiasis which will further aid in treatment of gall stone disease.

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