

Prevalence of different gallbladder pathologies and usefulness of mucin histochemistry in early diagnosis of metaplasia and carcinoma of gallbladder



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ABSTRACT

Background: Non-neoplastic and neoplastic diseases of gallbladder are most prevalent in northern and north-eastern states of Uttar Pradesh, Bihar, Orissa, West Bengal, and Assam in India. Chronic cholecystitis, the most common pathology of gallbladder is often associated with metaplasia of epithelium, leading to increased susceptibility to malignant transformation. Along with histopathological examination, mucin histochemistry is useful in early detection of metaplasia and thereby predicting metaplasia-dysplasia-carcinoma sequence. **Aims and Objectives:** The objective of this study was to estimate the prevalence of neoplastic and non-neoplastic diseases of gallbladder and to determine the usefulness of mucin histochemistry in early diagnosis of metaplasia and carcinoma of gallbladder. **Materials and Methods:** A cross-sectional observational study was conducted in R. G. Kar Medical College, Kolkata, over a period of 18 months. Total 401 cholecystectomy specimens were studied. In every case, following sequence of examinations was performed-gross examinations, histopathological examination, and mucin histochemistry. **Results:** All the available information was meticulously documented in tables and charts, software was used to calculate the statistical significance and efficacy of mucin histochemistry as a diagnostic tool. **Conclusion:** We found that mucin histochemistry is statistically significant and has positive predictive value in early diagnosis of metaplasia and carcinoma of gallbladder.

Key words: Gallbladder metaplasia; Carcinoma; Mucin histochemistry

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INTRODUCTION

Gallbladder is one of the organs having a wide spectrum of diseases ranging from congenital anomalies, calculi and its complications, and non-inflammatory and inflammatory lesions to neoplastic lesions.¹ The non-neoplastic lesions mainly include cholelithiasis, cholecystitis, xanthogranulomatous cholecystitis,² and cholesterolosis. Neoplastic category includes adenoma, carcinoma, and mesenchymal tumors. The management of neoplastic

and non-neoplastic lesions varies considerably with gallbladder carcinoma having very poor prognosis.³ Since clinical presentation of both is very similar, it is essential to diagnose gallbladder malignancy early on histopathology. Even seemingly benign chronic cholecystitis may be associated with hyperplasia and metaplasia of gallbladder epithelium⁴ and it has been suggested that metaplastic epithelium is more susceptible to malignant transformation than the normal mucosa and the intestinal metaplasia-dysplasia carcinoma sequence exists in the gallbladder.^{5,6}

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Hence, it is essential to detect metaplastic lesions of gallbladder early and mucin histochemistry study may be useful in this regard. Ganesh et al., observed in their study that in normal gallbladder mucosa neutral mucins are predominant whereas, in metaplastic epithelium, there was significant increase in acidic mucin. However, Sood et al., and Gupta et al., suggested in their study that metaplastic lesions showed predominantly neutral mucin.^{7,8} Hence, there are contrasting findings in different studies, and hence, more studies are required to arrive at a definite conclusion. All these reasons made us undertake the study. Our study was performed to find out if mucin histochemistry can be a useful tool for early detection of metaplastic lesions of gallbladder.

Aims and objectives

This study was conducted in a tertiary care center of eastern India to estimate the prevalence of neoplastic and non-neoplastic diseases of gallbladder and to study the expression of different types of mucin in various gallbladder pathologies to determine usefulness of mucin histochemistry in the early diagnosis of metaplasia and carcinoma of gallbladder.

MATERIALS AND METHODS

It was a cross-sectional observational study conducted in the Department of Pathology in association with Department of General Surgery, R. G. Kar Medical College of Hospital (R.G.K.M.C.H), Kolkata. The study was approved by the Institutional Ethical Committee of R.G.K.M.C.H. Female patients between 35 years and 45 years of age who have undergone cholecystectomy in the Department of General Surgery, R. G. Kar Medical College and Hospital were included in the study.

Patients who have gangrenous gallbladder, gallbladder with grossly distorted morphology, who were not willing to participate in the study, have congenital abnormality of gallbladder, have malignancy other than gallbladder malignancy, and who have undergone/undergoing chemotherapy or radio therapy were excluded from this study. Total 401 patients were studied for a period of 3 years.

Gallbladder specimens received in the department of pathology were fixed in 10% formalin, thoroughly examined noting down gross findings and multiple sections of 3–4 μ thickness were taken for histopathological and histochemical examination. The sections were processed and stained with hematoxylin and eosin stain⁹ and mucin stains for acidic and neutral mucin.¹⁰

Stains used to study mucin histochemistry were Alcian blue, colloidal iron, mucicarmine, and periodic acid-Schiff stains.¹¹

Proper written informed consent from each patient or his/her next to kin was asked after explaining the study procedure in their own language before study. We started the data collection once the synopsis was approved and completed the data collection and analysis within the stipulated time.

RESULTS

Most patients were in 35–40 years age group. Out of 401 patients, 248 (61.9%) were diagnosed as chronic cholecystitis by histopathological examination. Positive stain for neutral mucin was found in 235 (94.8%) out of these 248 cases (Figure 1). Positive stain for acidic mucin was found in 41 out of 69 cases of pyloric metaplasia (59.4%) and 9 out of 15 cases of intestinal metaplasia (60%) (Figure 2).

Chi-square test for significance of difference in mucin testing*			
Chi-square tests			
	Value	Df	P
X ²	131.473	5	<0.001
X ² continuity correction			
Yates Correction	131.473	5	<0.001
n	401		

Parameter	Estimated (%)	Lower–Upper 95% Cis	Method
Sensitivity	60.2	(50.3, 69.33)	Wilson Score
Specificity	92.41	(88.87, 94.89)	Wilson Score
Positive predictive value	71.95	(61.41, 80.52)	Wilson Score
Negative predictive value	87.77	(83.72, 90.93)	Wilson Score
Diagnostic accuracy	84.54	(80.67, 87.75)	Wilson Score
Likelihood ratio of a positive test	7.931	(7.125–8.829)	
Likelihood ratio of a NEGATIVE TEST	0.4306	(0.4093–0.4531)	
Diagnostic Odds	18.42	(10.24–33.12)	
Cohen's kappa (Unweighted)	0.5569	(0.4597–0.6541)	
Entropy reduction after a positive test	-3.733		
Entropy reduction after a negative test	18.47		
Bias index	-0.0399		

Results from OpenEpi, Version 3, open source calculator – diagnostic test

DISCUSSION

Cholecystectomy specimens are one of the most frequently submitted specimens in the department of pathology.

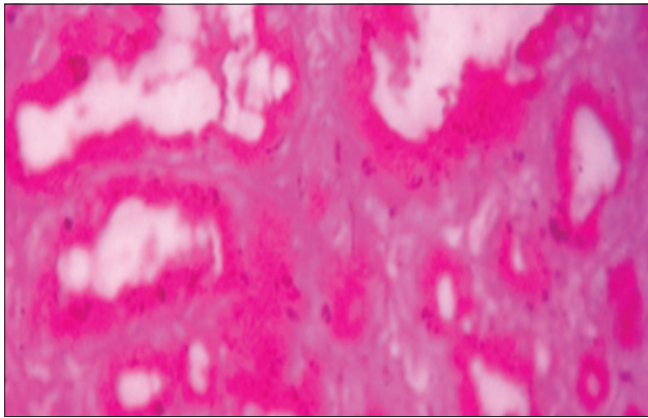


Figure 1: Periodic acid-Schiff (PAS) positive mucin in glands in pyloric metaplasia of gallbladder (PAS stain, x40)

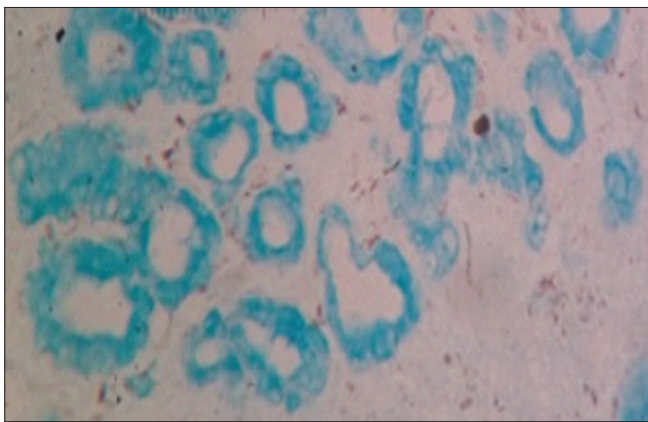


Figure 2: Alcian blue positive mucin in glands of pyloric metaplasia of gallbladder (Alcian Blue stain, x40)

Age of the patient in the present study ranged from 35 to 45 years. Maximum cases were of chronic cholecystitis 61.9% (248/401) cases, followed by acute cholecystitis 5.2% (21/401) cases and xanthogranulomatous cholecystitis 8.5% (34/401) cases. 17.2% (69/401) cases were of pyloric metaplasia and 3.7% (15/401) cases were of intestinal metaplasia.

Gall stones were present in 68.1% (273/401) cases and 31.9% (128/401) cases had no gall stones.

Length of normal gallbladder is 7–10 mm. In this study, 22.7% (91/401) cases were of normal length while 77.3% (310/401) were <7 cm.

Wall thickness of normal gallbladder is <4 mm. In this study, 69.3% (278/401) cases had normal wall thickness while 30.7% (123/401) cases had increased wall thickness (>4 mm).

Out of 401 cholecystectomy specimens, mucin histochemistry was done in all 401 cases. In metaplastic group, acid mucin was positive in 60.2% (59/98) while, in inflammatory group, neutral mucin was positive in 92.4% (280/303).

Thus, metaplastic and malignant lesions showed predominantly acidic mucin. In this study, all the cases of inflammatory lesions of gallbladder without metaplasia of the epithelium showed predominance of neutral mucin. The histochemical analysis of mucin showed definite altered expression in the diseased gallbladder epithelium in terms of amount and type of mucin expressed in cases of metaplasia. The acid mucin content increased while neutral mucin content decreased in cases of metaplasia and malignancy.

Halagowder et al., in 2007¹², studied mucin glycoarray in gastric and gallbladder epithelium and discovered mucin as a critical cytoprotective glycoprotein. Structural alteration of epithelium due to mucin has been described in different pathological conditions. Findings of this study support our observations.

A study conducted in June 2016 in the Department of Pathology, Muzaffarnagar Medical College and Hospital concluded that cholelithiasis is most common gallbladder lesion and there is a definite association between metaplasia-dysplasia-carcinoma sequences. It also revealed that mucin histochemistry in metaplastic cases can lead to early diagnosis of gallbladder carcinoma.⁷

Findings of above study are similar to findings of our study.

Mucin histochemistry is a simple, cost-effective and reliable method that can be carried out in hospital setting. If it is done routinely in cholecystectomy specimens, it will provide us added information.

Limitations of the study

This study is not without potential limitations. We have carried out this study with limited number of cases (n=401). Necrotic cells and macrophages often stain magenta in Periodic Acid Schiff stain and can be misinterpreted as mucin.

CONCLUSION

Within the constraints of small sample size, we conclude that there is a definite association between metaplasia-dysplasia-carcinoma sequences. Early detection of mucin histochemistry in metaplastic cases can lead to early diagnosis of carcinoma gallbladder as acidic mucins are more predominant than neutral mucins in cases of metaplasia and carcinoma. Hence, irrespective of the gross appearance and seemingly benign nature of the disease affecting the organ, every gallbladder removed by cholecystectomy should be subjected to histopathological examinations and mucin histochemistry.

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Authors Contributions:

JS- Concept and design of study, acquisition of data and prepared first draft of manuscript; **SC-** Acquisition of data revising it critically, interpreted the results; **US-** Reviewed the literature and revision of the manuscript. **SKB-** Statistical analysis; **RMM-** Overall coordination and interpretation.

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