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A Histopathological Study Of Neoplastic And Non-Neoplastic Diseases Of Gall Bladder With Mucin Histochemistry In A Tertiary Health Care Centre In Bihar(North India) – An Initiative To Rise Up To The Modern Era Of Diagnostics.

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I. Introduction

Gall bladder diseases are becoming a common health problem throughout the world. Diseases of the gallbladder commonly manifest as gallstones and gallbladder cancer.[1-2] Cholelithiasis is a common disorder afflicting 10 –20% of adult populations in the developed countries.[3-4] A remarkable shift in the trend of gall-stone disease to young asthenic females in their twentieth decade has been witnessed, which was earlier prevalent in middle aged, fair, fertile and fat females, often in their forties.[5] Chronic cholecystitis is the most common disease of gallbladder with a diverse histomorphological spectrum. Acute on chronic cholecystitis, chronic cholecystitis with cholesterolosis, xanthogranulomatous cholecystitis, follicular cholecystitis are to name a few of its varied forms. The gallbladder epithelium can undergo various sorts of metaplastic changes, ranging from pyloric gland metaplasia, antral gland metaplasia, intestinal metaplasia to squamous cell metaplasia, each of which can progress to dysplasia sometimes.[6]

II. Aims And Objective

In the yesteryears, attempts have been made to correlate gallbladder histomorphology, mucin histochemistry, and composition of calculi in gallstone disease.[13,34-35] Many previous studies aimed at combining the two of any of the three above mentioned aspects, as in gallbladder histomorphology with mucin histochemistry, or either composition with calculi with mucin histochemistry, or with gallbladder histomorphology with composition of calculi. Correlating all the three aspects together have been very meagrely studied, especially in the Kosi region, where it has not been studied at all. The purpose of the study is to discuss the histomorphological spectrum of gallbladder diseases and to evaluate the role of mucin in those diseases and carcinogenesis, thus correlating them together, along with the physical or gross morphological categorization of the types of stones present. Correlating each one of them could lead to early identification of high-risk cases and groups and implementation of possible therapeutic interventions.

III. Materials And Methods

Study Design : It was a hospital based, prospective study on the cholecystectomy specimens received in the Department of Pathology from the Department of Surgery, Katihar Medical College and Hospital, Katihar for histopathological evaluation. The study was conducted with the approval of the institutional ethics review committee, on the specimens recovered either through open cholecystectomy or laparoscopic cholecystectomy and it spanned for a period of 2 years from 2019-2021.

Hematoxylin & Eosin staining was done on each of the paraffin sections. Later, special staining for mucin, i.e. PAS & Alcian blue (pH 2.5 and 1.0) were done on representative sections from cases of metaplasia,

dysplasia and carcinoma. The slides were assessed according to the prepared proforma.

Inclusion Criteria
All cholecystectomy specimens – Both, open and laparoscopic

Exclusion Criteria
- Specimens which were too tiny or inadequate to evaluate
- Autolysed specimens

Sample Size Estimation

Sample size was calculated based on 3 years retrospective analysis in Katihar Medical College and Hospital, Katihar from July 2016 to June 2019.

A total of 243 cholecystectomy specimens had been received in Department of Pathology, Katihar Medical College and Hospital, Katihar, thus, making an average of 81 cases per year. Based on this data, the **sample size for the study was taken as 150,** for all the cholecystectomyspecimens received from July 2019 to June 2021(2 years) but, autolysed and inadequate specimens were excluded from the study.

Method

The received cholecystectomy specimens were collected in 10% formalin, following scrutiny of the patient details and identity. They were allowed to get fixed in fresh formalin for 24 hours. Gross morphological details of all the specimens were noted. Gallstones if present, their physical characteristics based on gross morphology were noted and clinical reports reviewed. Yellow and whitish stones as cholesterol stones, black and dark brown as pigment stones and brownish yellow or green as mixed stones. Small, hard, amorphous, grayish-white stones as calcium carbonate stones. Large, often barrel-shaped, dark green to greenish yellow stones as combined stones. Three sections were taken including entire wall, one each from the fundus, body and neck of the gallbladder. Additional sections were taken from any abnormal appearing area. These steps were followed by processing with routine histopathological techniques. Therepresentative sections were stained with Hematoxylin and Eosin stain. The slides were observed under the microscope. The special stain for mucin, i.e. Periodic Acid Schiff (PAS) &Alcian blue stain (pH 2.5 & 1.0) were used in cases of metaplasia, dysplasia and carcinoma. Four additional sections were cut of each of the selected blocks for special stains. The special stains for mucin were done with a view to assess the quantity and quality of mucins in variousgallbladder lesions.

Interpretation of PAS stain :-Mucin --- red/purple Background--- blue

Interpretation of Alcian blue (pH 2.5) :-
Acid mucins (sulfomucins and sialomucins)----------blue
Proteoglycans and hyaluronic acid ---------- blue
Nuclei---------- pink to red
Cytoplasm---------- pale pink

Interpretation of Alcian blue (pH 1.0) :-
Strongly acidic sulphomucins and
sulfate containing proteoglycans ---------- stained blue (lighter than pH 2.5)

Semiquantitative grading for mucin histochemistry:[7,8]
ABS : No staining
Weak : Weak throughout the section or intense staining in less than one third of themucosa
STR : Intense (Strong) throughout or in more than one third of the mucosa

Statistical Data Analysis

Data were summarized in terms of range, frequencies (number of cases) and relative frequencies (percentages), mean ± standard deviation (± SD), as appropriate. For comparing categorical data, Chi squared ($\chi 2$) test was performed and exact test was used when the expected frequency was less than 5.

A probability value (*p* - value) less than 0.05 was considered statistically significant. For statistical analysis, data were entered into a Microsoft excel spreadsheet and then analysed bySPSS (Statistical Package for the Social Science) SPSS Inc., Version 27.0,Chicago, IL, USA and GraphPad Prism version 5.

IV. Results

The selected patients were tabulated for the present study from 11 to 80 years age with a classinterval of 10 years in each group and a mean age of 45.8 years. The youngest patient was of 17 years and the oldest was 77 years old. The maximum patients were in the 3rd decade, 38 patients (25.30%) patients, out of 150.

There was a significant female preponderance with 119 cases (i.e. 79.3%), while male cases being 31,(i.e. 20.7%). Female to male ratio was 3.8:1.

Maximum number of female patients were encountered in 3rd decade (26.9%) and maximum number of male patients were seen in 5th decade (25.8%).

Table 1 - Distribution Of Gallbladder Lesions With Or Without Calculi

Calculi	No. of cases	Percentage
Present	136	90.66%
Absent	14	9.33%
Total	150	100.0%

Table 1: illustrates the distribution of gallbladder lesions with or without calculi.
Calculi were present in 136 (90.66%) cases, out of 150 and the rest 14 (9.33%) cases, didnot show any calculi.

Table 2 - Distribution Of Cholelithiasis According To VariousTypes Of Calculi

Types of stones	No. of cases	Percentage
Mixed (A)	109	80.1%
Cholesterol (B)	10	7.4%
Pigment (C)	7	5.1%
Calcium carbonate (D)	4	2.9%
Combined (E)	4	2.9%
Both mixed and combined (A E)	2	1.5%
Total	136	100.0%

Table 2 : Through morphological analysis of stones, it was found that maximum no. of cases, 109/136 cases (i.e. 80%) had mixed type of gallstones, followed in decreasing order by cholesterol stones 10 cases (7.4%), pigment stones 7 cases (5%), calcium carbonate stones and combined type, each were present in 4 cases (i.e. 3% in either of them) and 2 cases (i.e. 1.5%) had both mixed and combined stones in them.

Table 3 - Distribution Of Gallbladder Lesions Of Total Cases

Lesions	No. of cases	Percentage
Neoplastic	13	8.7%
Non neoplastic	137	91.3%
Total	150	100.0%

Table 3 : illustrates gallbladder lesions. Maximum cases were non neoplastic, i.e. 137/150(91.3%) and the remaining were neoplastic,13 cases (8.7%).

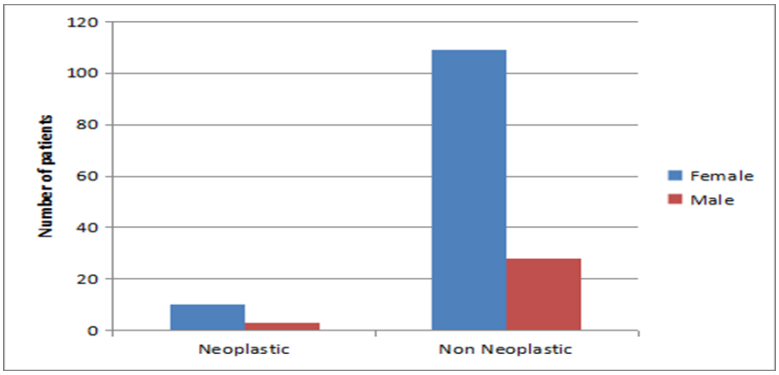
Table 4 - Distribution Of Gall Bladder Lesions According ToAge Group

Age in group	Neoplastic	Non neoplastic	TOTAL
11-20 years	0 (0.0%)	3 (2.2%)	3
21-30 years	0 (0.0%)	26 (19.0%)	26
31-40 years	0 (0.0%)	38 (27.7%)	38
41-50 years	0 (0.0%)	24(17.5%)	24
51-60 years	5 (38.5%)	23 (16.8%)	28
61-70 years	7 (53.8%)	16 (11.7%)	23
71-80 years	1 (7.7%)	7 (5.1%)	8

TOTAL	13 (8.7%)	137 (91.3%)	150

p – value - 0.0003

Table 4 : illustrates that in present study, out of 150 cases, non neoplastic cases were 91.3% (137/150) and neoplastic cases were 8.7% (13/150). Non neoplastic lesions were mostcommon in 3rd decade i.e. 38/137 cases (27.7%) and neoplastic lesions were common in 6th decade of life i.e. 7/13 cases (i.e. 53.8) % of total neoplastic cases. The p value is highly significant, i.e. < 0.005 (0.0003).



Out of 137 non neoplastic lesions, 109 cases (79.6%) were females and 28 cases(20.4%) were males. In neoplastic lesions, 10 cases (76.9%) were females and 3cases (23.1%) were males. Both either type of lesion, be it neoplastic or non neoplastic, the number of female patients is more than male patients.

Table 5 : Histopathological Spectrum Of GallbladderLesions

Provisional diagnosis on H/E	No. of cases	Percentage
AC	3	2.0%
AC with CL	2	1.3%
FC	2	1.3%
CC	11	7.3%
CC with CL	57	38.0%
CC with CS	22	14.7%
CC with IM	25	16.7%
CC with PM	12	8.0%
DYS	4	2.7%
ADENO	8	5.3%
SRCC	1	0.7%
XC	3	2.0%
Total	150	100.0%

Table 5 : shows histopathological spectrum of gallbladder lesions. Amongst thenon-neoplastic lesions, maximum patients had chronic cholecystitis with cholelithiasis (CC with CL), 57/150 (38%), which was followed by chronic cholecystitis with intestinal metaplasia (CC with IM), 25/150 (16.7%). In neoplastic ones, the most common lesion was Adenocarcinoma, with 8 cases (i.e.5.3%) and one case was of Signet Ring Cell Carcinoma (SRCC) - (0.7%)

Table 6: Histopathological Spectrum Of GallbladderLesions According To Age Group

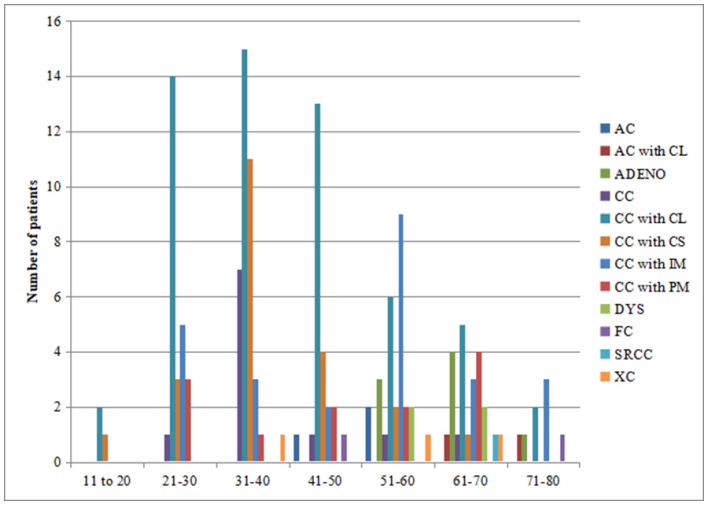


Table 6 : depicts the histopathological spectrum of gall bladder lesions. Maximum cases were reported to be chronic cholecystitis with cholelithiasis (CCwith CL), 57/150 (38%), and maximum patients were in the 3rd decade, 38/150 (25.3%).The *p* - value was significant, i.e.<0.05(0.0061).

Table 7: Sex Wise Distribution Of HistopathologicalDiagnosis

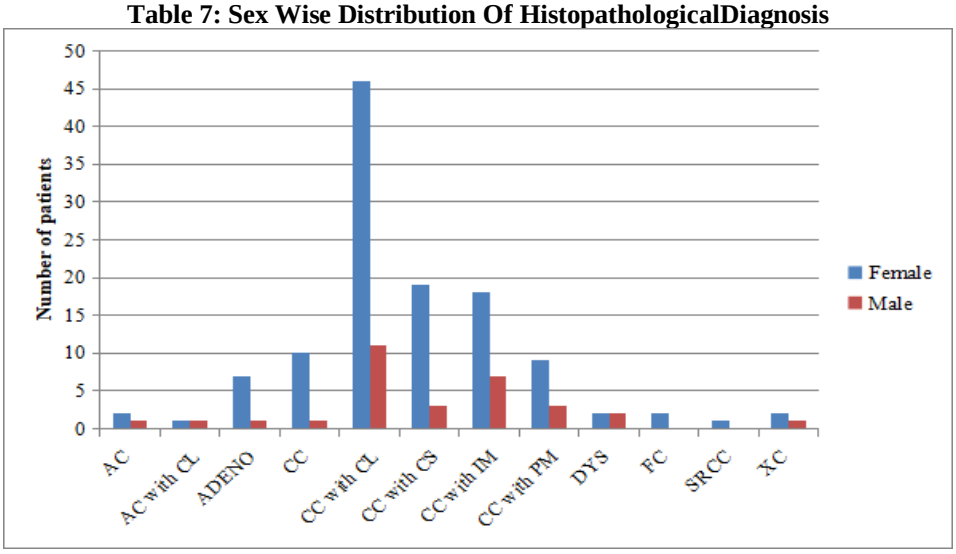


Table 7 : illustrates sex wise distribution of histopathological diagnosis of gallbladder lesions. It was observed that of the maximum number of cases of chronic cholecystitis with cholelithiasis (CC with CL) i.e. 57 cases, 46 cases (38.71%) were females and only 11 cases (35.5%) were males. Similar pattern was seen in chronic cholecystitis with intestinal metaplasia (CC with IM), out of25 cases, 18 cases (15.1%) were females and 7 cases (22.6%) were males. Malignancy, in the form of adenocarcinoma and SRCC, was found to be more infemales and only one case of adenocarcinoma was reported in male.

Table 8 - Pas Staining In Gallbladder Lesions

PAS staining	No. of cases	Percentage
STR	34	68.0%
Weak	16	32.0%
Total	50	100.0%

Table 8 : depicts the results of PAS staining on gallbladder lesions. Mucin histochemistry was carried on 50 cases showing metaplasia(37),dysplasia(4) andmalignancy(9), out of 150 . All 50 cases showed PAS positivity, of which 34 cases (68%) showed strong staining intensity and 16 cases (32%) showed weak staining intensity.

Table 9: Alcian Blue (Ab) Staining At Ph 2.5In Gallbladder Lesions

AB (pH – 2.5)	No. of cases	Percentage
ABS	1	2.0%
STR	44	88.0%
Weak	5	10.0%
Total	50	100.0%

Table 9 : shows results of Alcian blue staining at pH – 2.5. Mucin histochemistrywas carried out on 50 cases and among them 49 cases showed positivity for alcianblue (AB) at pH 2.5, which were interpreted according to their staining intensity.

44 cases (88%) showed strong intensity and 5 cases (10%) showed weak intensity.1 case (2%) was negative for AB at pH 2.5.

Table 10: Alcian Blue (Ab) Staining At Ph 1In Gallbladder Lesions

AB (pH – 1)	No. of cases	Percent
ABS	8	16.0%
STR	4	8.0%
Weak	38	76.0%
Total	50	100.0%

Table 10 : shows results of Alcian blue staining at pH – 1. Mucin histochemistrywas carried out on 50 cases and among them 42 cases were positive for alcian blue (AB) at pH 1, which were interpreted according to their staining intensity.38 cases (76%) depicted weak intensity positivity and 4 cases (8%) were strongly positive. 8 cases (16%) were negative for AB at pH 1.

The metaplastic cases i.e. 37 cases (74%), showed predominantly sialomucin in34 cases (91.9%) and predominantly sulphomucin in 3 cases (8.1%). Neoplastic cases (dysplasia and carcinoma) i.e. 13 cases (26%), showed predominantly sialomucin in 12 cases (92.3%) and 1 case (7.7%) showed predominantly sulphomucin positivity. So, Sialomucin is most commonly seen in gallbladder lesions i.e. in 46 cases (92%) and sulphomucin was seen in only 4 cases (8%) oftotal 50 cases.

Out of 50 cases, sialomucin was seen predominantly in 16 cases in the 5^{th} decade(34.8%) and sulphomucin was particularly common in 4 cases in the 6^{th} decade(100%). The *p* – value was <0.05 and was statistically significantly(0.0479).

Table 11: Type Of Mucin In Histomorphological Spectrum Of Gallbladder Lesions

	Provisional Diagnosis On H/E					
Type of mucin	**ADENO**	**CC withIM**	**CC withPM**	**DYS**	**SRCC**	**TOTAL**
SIALO	8 (17.4%)	25 (54.3%)	9 (19.6%)	4 (8.7%)	0 (0.0%)	46 (100.0%) (92.0%)
SULPHO	0 (0.0%)	0 (0.0%)	3 (75.0%)	0 (0.0%)	1 (25.0%)	4 100.0 (8.0%)
TOTAL	8 (16.0%)	25 (50.0%)	12 (24.0%)	4 (8.0%)	1 (2.0%)	50 (100.0%)

p - value – 0.0006

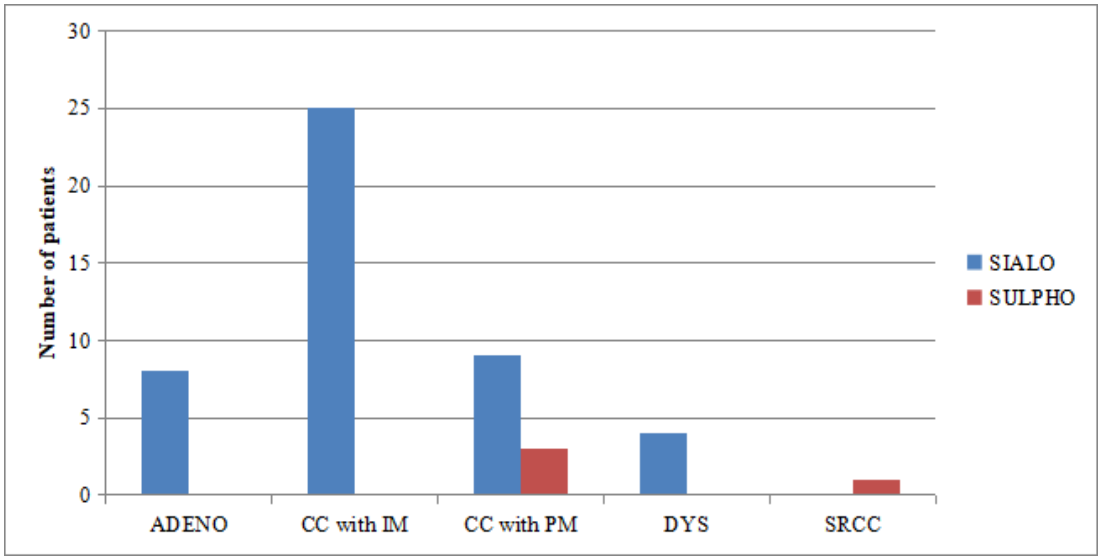


Table 11: show that out of 50 cases of gallbladder lesions studied for mucin histochemistry, all the 25 (54.3%) cases of intestinal metaplasia showed predominantly sialomucin in them, but out of 12 (24%) cases of pyloric metaplasia, 9(19.6%) cases were positive for sialomucin and the other 3 cases(75%) were positive for sulphomucin. All 4 cases of dysplasia were positive for sialomucin. All the 8(17.4%) cases of adenocarcinoma were positive for sialomucin and 1(25%) case of signet ring cell carcinoma (SRCC) was positive for sulphomucin. The *p* - value was <0.005 and was highly significant(0.0006).

V. Discussion

Cholecystitis is defined as the inflammation of the gallbladder that most commonly occurs following obstruction of the cystic duct, due to cholelithiasis. Lithiasis can be either chronic or acute inflammatory process which is usually associated with calculi (stones)[9].Cholelithiasis inflicts diverse histopathological changes in gall bladder mucosa- namely, acute inflammation, chronic inflammation, granulomatous inflammation, cholesterolosis, hyperplasia, metaplasia, dysplasia and carcinoma. Gallstones injure the mucosal epithelium and lead to metaplasia, dysplasia and neoplasia.[10-12]

It has been years since gallbladder mucus has been recognized as an important factor in gallstone development.[34-35] The implicated role of mucin in gallstone formation has been greatly studied. In support of the view, hypersecretion of mucus is seen to occur in humans and experimental animals. The normal human gallbladder predominantly contains sulfated acid mucin. In gallstone disease, the sulfated acid mucin content is increased. But, metaplastic and neoplastic gallbladder epithelium show a decrease in sulfomucins and an increase in sialomucins.[12-13]

In our study, mucin histochemical stains were used - Periodic acid Schiff (PAS),Alcian Blue at pH 2.5 and pH 1 to know about the quantity and quality of the contained mucins in different gallbladder lesions and to differentiate various types of mucins. So, the aim was to study the histomorphological spectrum of gallbladder diseases and to assess the results of mucin histochemistry in neoplastic and non neoplastic diseases. In this present study, age of patients ranged from 11-80 years. Maximum number of patients belonged to the 3rd decade, 38 patients (25.30%). The mean age in this study was 45.89 years, while in studies by **Sood et al**[7], **Banerjee et al**[14](2015), it was observed to be 43.56years and 39.3 years respectively.

Table 12 : Comparison of mean age

Present study	**45.89**
Sood et al	43.56
Banerjee et al	39.3
Damor andOzgur et al	43.2
Tyagi SP et al	43.6

In our study, neoplastic lesions were most common in older age group i.e. 6th decade of life. The results of our study were in concordance with the studies by **Baig SJ et al**[15]**, Mathur SK et al**[16] **Mohan et al**[17] **Tyagi SP et al.**[18] Maximum no. of patients were females i.e. 119 cases out of 150 cases (79.3%) with female : male ratio being 3.8:1.Similar results of female preponderance were alsoreported by **Sood et al**[7] **, Shiffman ML et al,**[19,20] **Madrid JF et al**[21]**, SwobodnikW et al**[22] **and Maki T et al**[23] with the F:M ratio ranging from 3.2:1 to 6.5:1.

Table 13 : Comparison of F:M ratio in various studies

Present study	**3.8:1**
Sood et al	4.5:1
Banerjee et al	3.3:1
Mohan et al	6.4:1
Tyagi SP et al	6.5:1
Kaur et al	3:1
Selvi et al	3.2:1
Pradhan SB et al	3.2:1

In this study, gallstones were present in 136/150 (90.66%) cases, which was consistent with the study of **Tyagi SP et al**[18] and **Sood et al**[7],which showed 85.3% and 91.55% (1300/1420) cases with cholelithiasis,

respectively. Thestones were mainly of mixed type, 80% (109/136) cases.

High levels of female hormones, estrogen as well as progesterone, contribute to gallstone disease by inhibiting gall bladder contraction, by promoting bile stasis and hypomotility, which may be responsible for the preponderance of gallstone diseases in females[24]. Pregnancy, use of birth control pills and hormone therapy,lead to increased cholesterol levels in bile and decreased gallbladder movement,resulting in gallstone formation.[25-27]

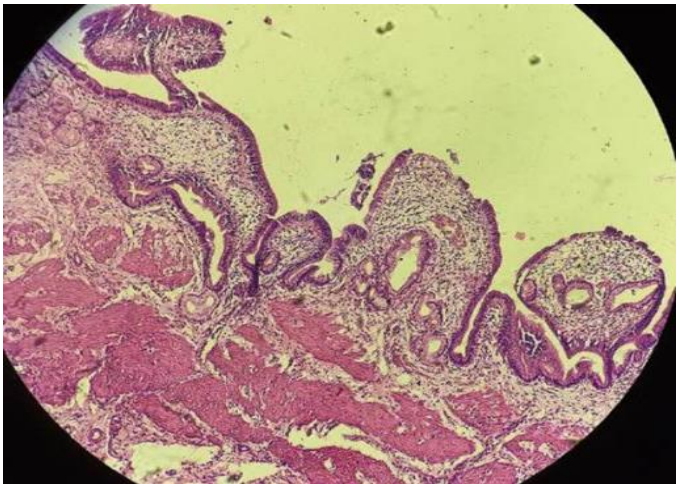


Fig :1 - CHRONIC CHOLECYSTITIS, H & E, 10x

In this study, chronic cholecystitis with cholelithiasis with 57 cases out of 150 cases (38%) was the most common pathological finding reported on histopathological evaluation. – **fig.1** shows microscopy. Results similar to this study were reported by **Tyagi SP et al**[18]**, Mathur SK et al**[16] and **Mehta et al**[31].Various other studies also observed chronic cholecystitis with cholelithiasis as the commonest lesion, ranging between 64% to 90%. The cause may be different dietary habits, geographical regions, customs, religions, ethics etc.

Table 14 : Comparison of results of chronic cholecystitis with cholelithiasisin various studies

Study	No. of cases	Chronic Cholecystitis with Cholelithiasis (Commonest lesion)
Present study	**150**	**38%**
Tyagi SP et al	415	50.8%
Mathur SK et al	330	45%
Selvi TR et al	78	85.8%
Pradhan SB et al	80	65.53%
Kaur A et al	384	75%
Vahini G et al	110	72.7%
Costa et al	1689	64.59%
Sood et al	1440	90.28%

Acute cholecystitis accounted for 2.0% (3/150 cases) which was in concordancewith study by **Sood et al**[7] and **Kaur et al**[28](2012) which reported 3.26% and 2.60% cases respectively.
Acute cholecystitis with cholelithiasis was seen in 2/150 cases (1.3%).

In the present study, chronic cholecystitis with cholesterolosis accounted for 14.7% cases (22/150 cases) – **fig.2** shows its microscopy, which was in concordance with the study by **Kaur et al**[28](2012) that showed 12.25% cases of cholesterolosis. Study by **Sood et al**[7] and **Vahini et al**[29] was in discordance withthe present study, with 1.67% and 1.8% cases of chronic cholecystitis with cholesterolosis. The reason could be the varied dietary habits, culture and different religions.

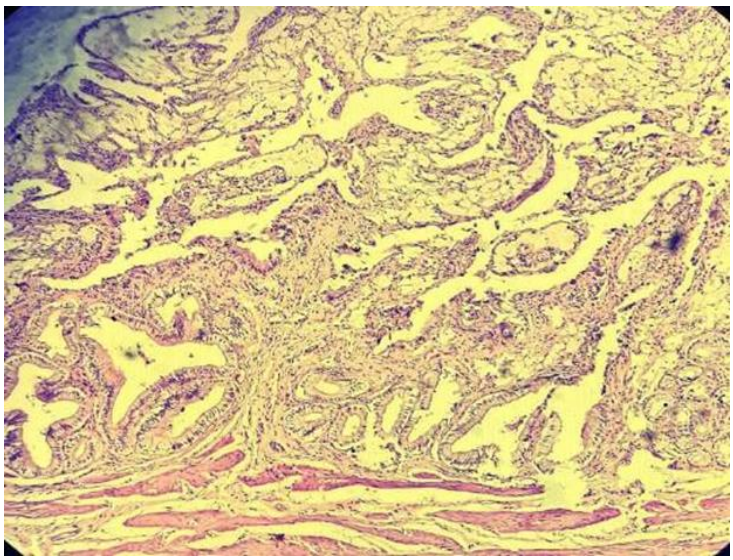


Fig : 2 - CHRONIC CHOLECYSTITIS WITH CHOLESTEROLOSIS, H & E, 10X - Section shows abundantfoamy macrophages in the mucosa.

Follicular cholecystitis – **fig.3** – shows microscopy, accounted for 1.3% cases (2/150 cases) in this study. Similar results were reported by **Sood et al**[7] and **Vahini G et al**[29],showing 0.90% and 1.81% cases of follicular cholecystitis respectively.

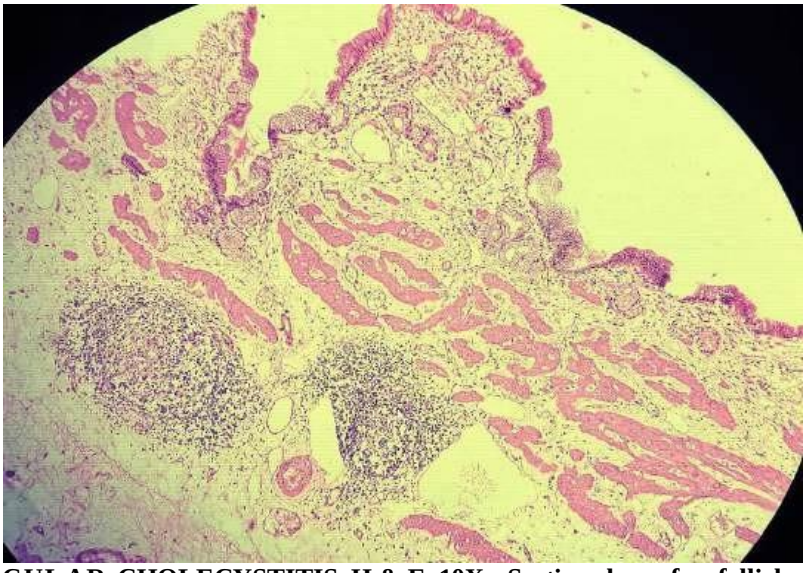


Fig : 3 - FOLLICULAR CHOLECYSTITIS, H & E, 10X - Section shows few follicles with germinal centres.

Table 15 : Comparison of results of follicular cholecystitis

Study	**No. of cases**	**Percentage**
Present study	**150**	**1.3%**
Sood et al	1440	0.90%
Vahini et al	110	1.81%

In the present study, (3/150), i.e. 2% cases were reported to be xanthogranulomatous cholecystitis. – **fig. 4 & 5 –** gross and microscopy. The study conducted by **Mathur SK et al**[16] and **Vahini et al**[29] showed similar results, with 3% and 1.81% cases of xanthogranulomatous cholecystitis, respectively. Other studies also showed xanthogranulomatous cholecystitis to be ranging from 1% to 3%.This difference in percentage could have been due to changed dietery habits and different geographical regions.

Fig : 4 - GROSS APPEARANCE OF XANTHOGRANULOMATOUS CHOLECYSTITIS

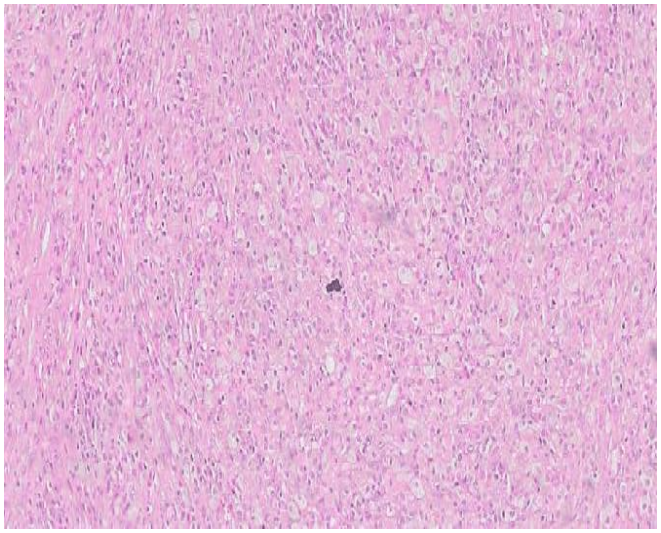


Fig : 5 - XANTHOGRANULOMATOUS CHOLECYSTITIS, H & E,40X

-Section shows sheets of foamy macrophages, plasma cells, lymphocytes and giant cells.

Table 16 : Comparison of results of xanthogranulomatous cholecystitis

Study	No. of cases	Percentage
Present study	**150**	**2%**
Mathur SK et al	330	3%
Vahini et al	110	1.81%
Kaur et al	384	1.04%

The present study showed 24.6% cases (37/150 cases) of chronic cholecystitis with metaplasia (pyloric and intestinal) – **fig.6 & 7 –** shows microscopy. The study by **Sood et al**[7] showed 12% cases of the same. In this study, 2.7% (4/150 cases) cases showed dysplasia – **fig.8 –** shows microscopy. **Costa et al**[30] and **Sood et al**[7] reported 0.2% and 0.35% cases of metaplasia, respectively in their studies.

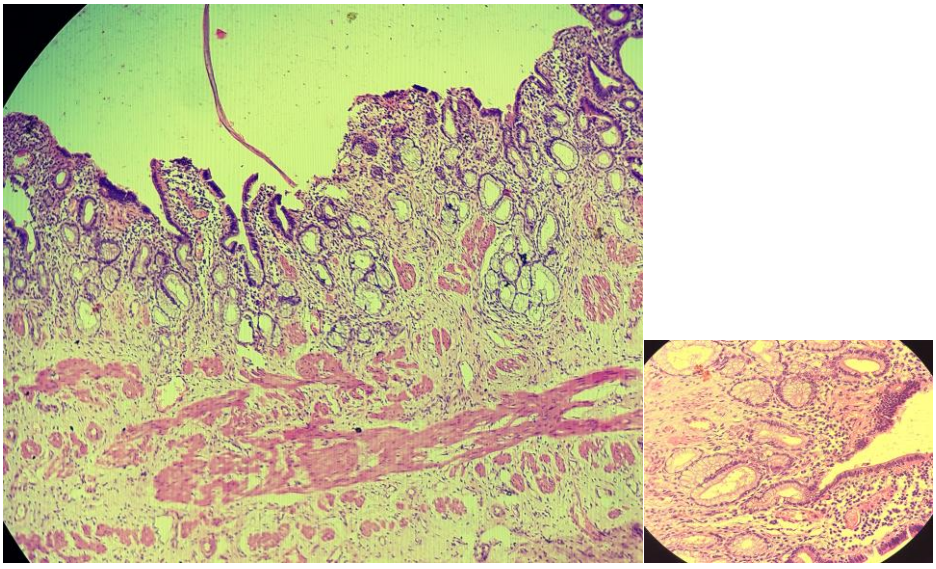


Fig : 6 - CHRONIC CHOLECYSTITIS WITH PYLORIC METAPLASIA, H & E,10X AND INSET AT 40X

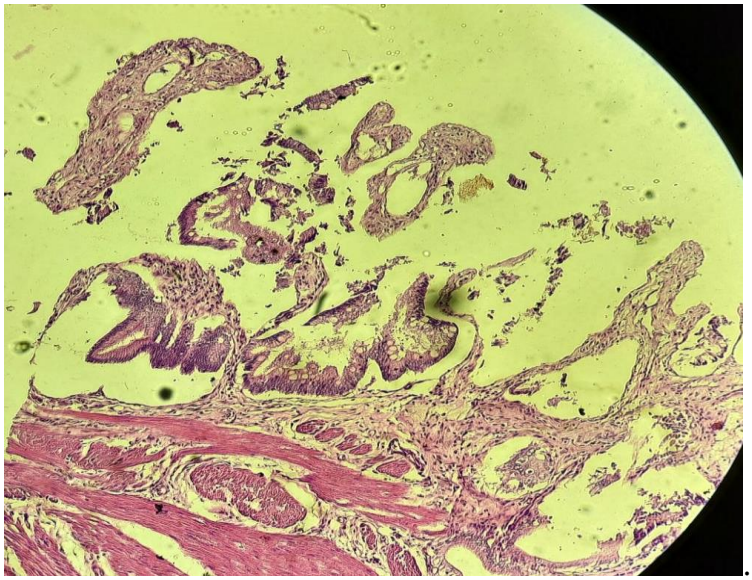


Fig : 7 - CHRONIC CHOLECYSTITIS WITH INTESTINAL METAPLASIA, H & E, 10X

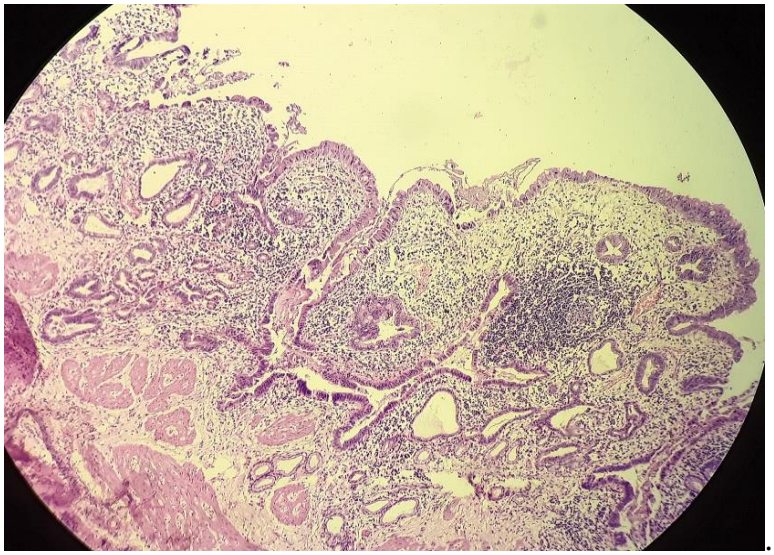


Fig : 8 - DYSPLASIA, H & E, 10X

Gall bladder carcinoma – **fig.9 & 10 -** shows microscopy, was reported in 6% (9/150 cases) in this study, which was consistent with the studies by **Mehta et al**[31],**Tyagi SP et al**[18] and **Vahini G et al**[29],showing 6%, 6.8% and 4.5% cases of carcinomas respectively.

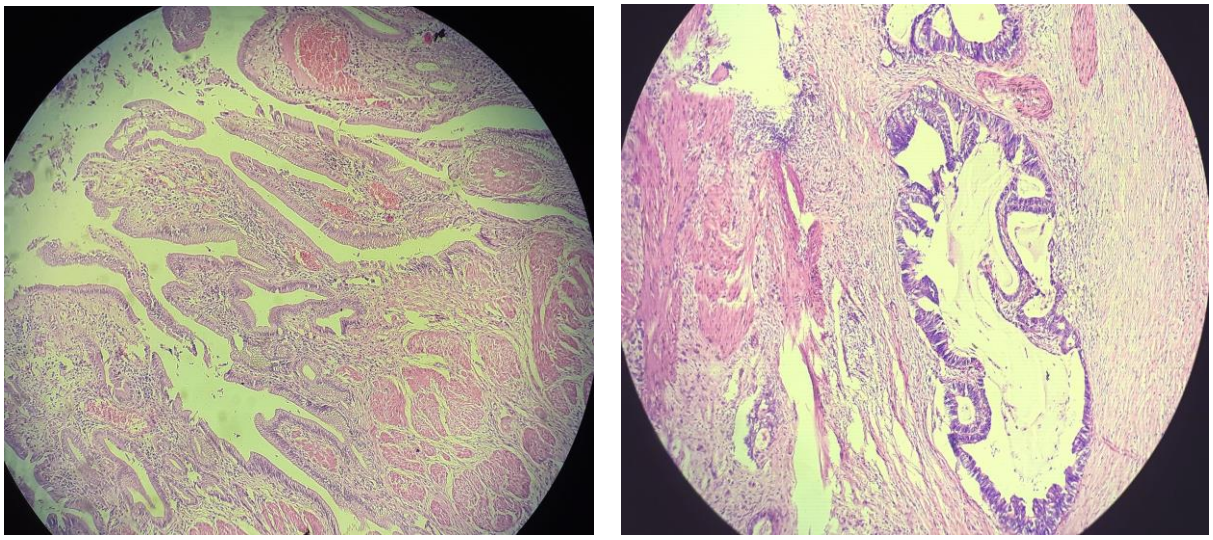


Fig: 9 & 10 - MODERATELY DIFFERENTIATED ADENOCARCINOMA, H & E, 10X - Section shows neoplastic glands infiltrating the muscular layer and their lumina with extracellular mucin.

Mucin Histochemistry

In the present study, mucin histochemistry was carried out, showing PAS positivity in all 50 cases (metaplasia, dysplasia and carcinoma). – **fig.11 –** shows PAS positivity in case of adenocarcinoma. Out of 37 cases of metaplasia, 100% cases (37/37) showed strong positivity for AB at pH 2.5 and also 100% cases (37/37) showed positivity for AB at pH 1, but with weak positivity in 91.89% (34/37) cases and only 8.1%(3/37) cases showing strong positivity for AB at pH 1, indicating that the type of mucin was predominantly sialoform in majority of the cases. **Sood et al**[7] through their study, reported that in metaplastic group, 97.46% cases depicted positivity for AB at pH 2.5 and 80.20% cases showed positivity for AB at pH 1.

In the present study, malignant lesions (consisting of dysplasia and carcinoma) depicted positivity in 98% (12/13) cases for AB at pH 2.5.- **fig.12 & 13 –** shows AB 2.5 positivity in case of adenocarcinoma. Out of 13 cases, 8 cases showed no positivity for AB at pH 1 and 4 cases showed weak positivity, which

indicated the change of mucin from sulphomucin to sialomucin.

The study of **Sood et al**[7] showed 100% positivity in malignant lesions for AB at pH 2.5.

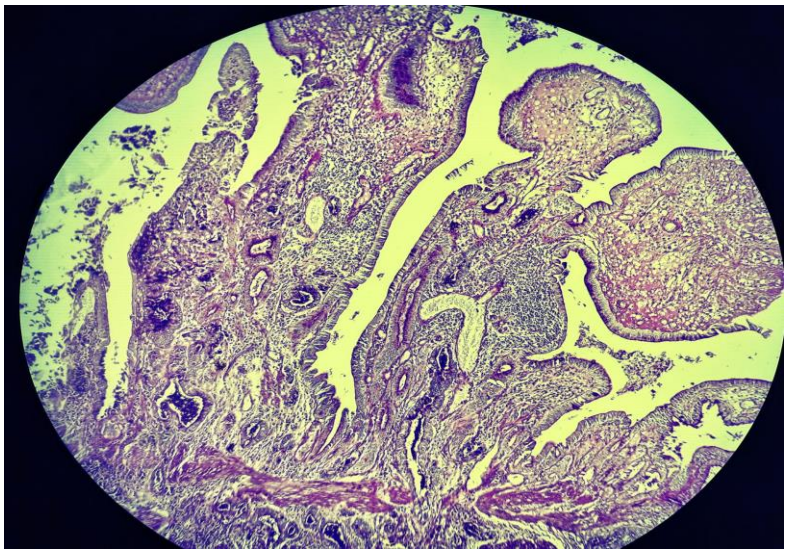


Fig : 11 - PAS STAIN POSITIVE ADENOCARCINOMA, 10X

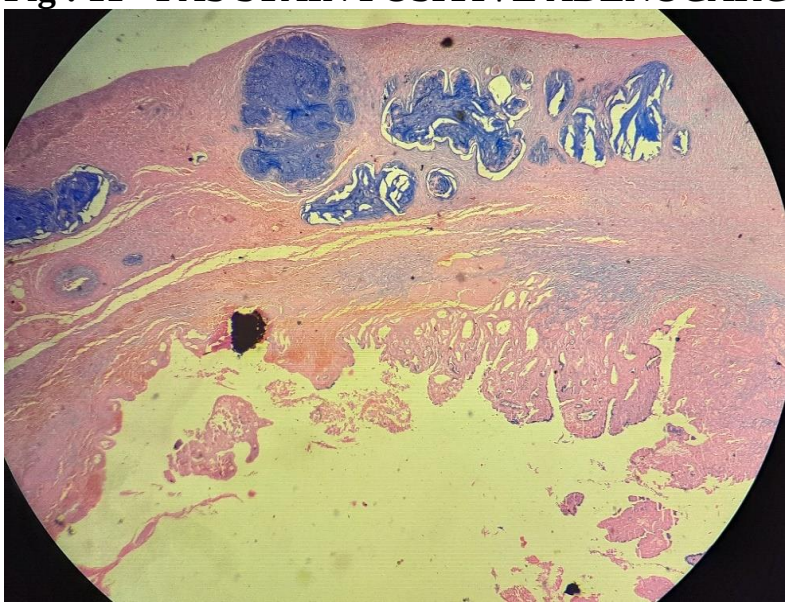


Fig : 12 - ALCIAN BLUE (pH 2.5)-SIALOMUCIN POSITIVE ADENOCARCINOMA,10X

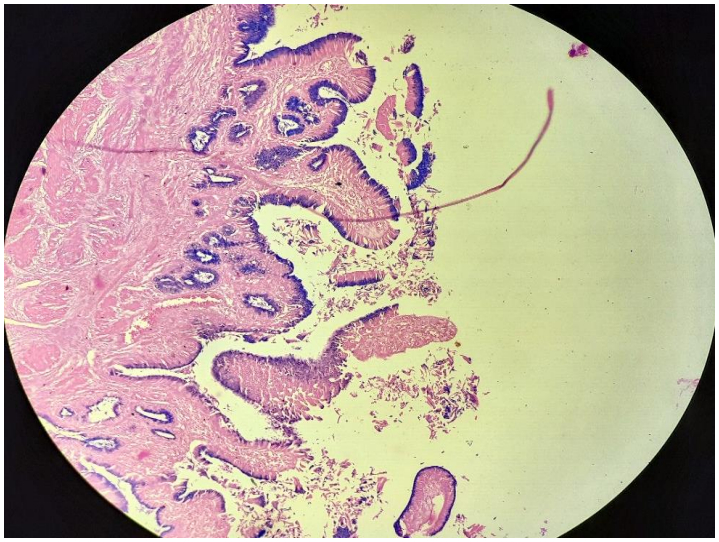


Fig : 13 - ALCIAN BLUE (pH 2.5) WEAK POSITIVE - SIALOMUCIN ADENOCARCINOMA,10X

Sialomucins are known to occur only in traces in normal gall bladder mucosa and in considerable quantities in metaplastic gallbladder mucosa[12,13].In our study, one case of SRCC shows sulphomucin, which is an exception because it is stated in literature, that SRCC is a variety of adenocarcinoma in which sialomucin is normally present. – **fig.14 –** shows gross, **fig.15&16** - shows microscopy, **fig.17**- shows sulphomucin positivity at AB pH 1.Literature regarding this is quite less as this entity is not so common. Similar results for SRCC with sulphomucin positivity were recorded by **Mehta et al**[31].Since there is only one such case in our study, so it cannot be commented upon in detail. More cases would have been required to confirm this exception and derive a definite conclusion

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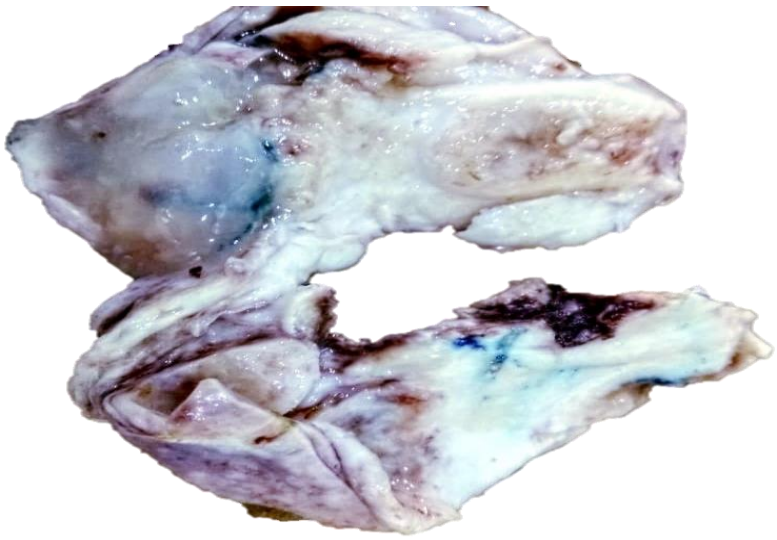
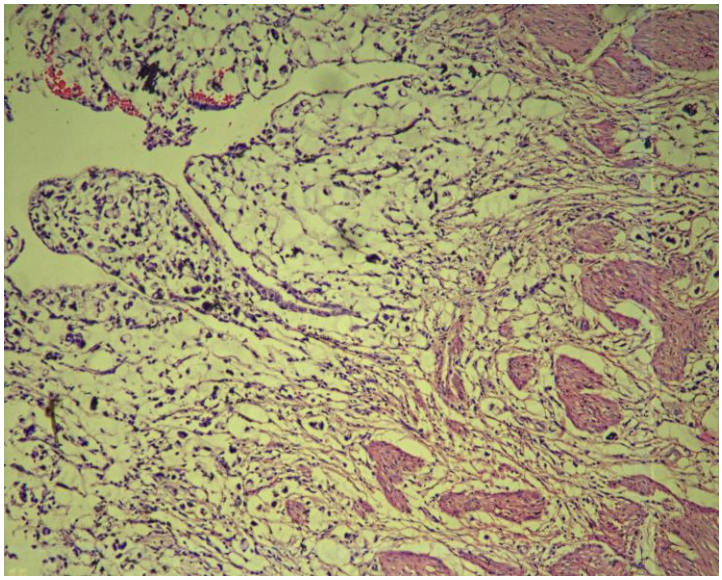


Fig. 14 - GROSS APPEARANCE OF SIGNET RING CELL CARCINOMA

Growth replacing the gall bladder with diffusely thickened wall



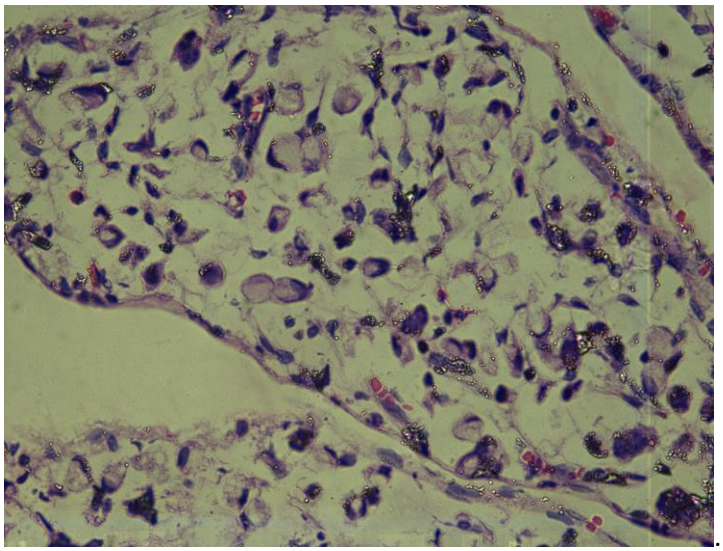


Fig.

15 & 16 - SIGNET RING CELL CARCINOMA, H & E, 10X & 40X

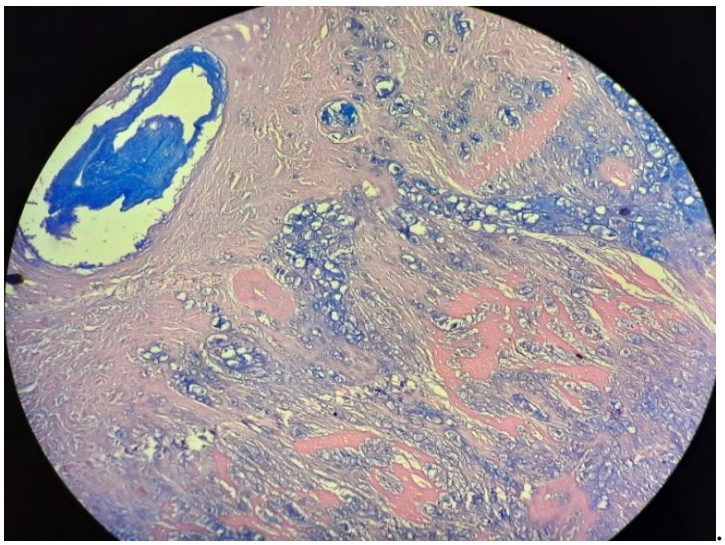


Fig. 17 - ALCIAN BLUE (pH 1) - SULPHOMUCIN POSITIVE SIGNET RING CELL CARCINOMA, 10X

Several studies are suggestive that preneoplastic lesions play an important role and there is progression in sequence from metaplasia to carcinoma through dysplasia[12,32].In normal gall bladder epithelium, sulphomucin predominates, but the amount of sialomucin increases with progressive transformation through metaplasia and dysplasia, and finally sialomucin predominates in neoplastic lesions, suggesting that sialomucin is predominantly increased in malignancy[12,13]. Similar results have been depicted by **Mehta et al**[31]**, Inada A et al**[33], **Laitio M**[34] **and Gupta SC et al**[32] in their studies.

VI. Summary And Conclusion

Summary:

The present study was conducted in the Department of Pathology, Katihar Medical College and Hospital, Katihar, in a total of 150 cases, with the aim to study histomorphological spectrum of gall bladder diseases along with mucin histochemistry. Mucin histochemistry (PAS, AB at pH 2.5 and 1staining) was carried out only on cases showing metaplasia, dysplasia and carcinoma.

- Study involved patients of the age group ranging from 11-80 years and mean age was 45.89 years.
- The maximum patients having gall bladder diseases belonged to the 3rd decade (25.30%).
- There was a female preponderance in the present study with 79.3%, whilemale being 31%. Female to male ratio was 3.8:1.
- Out of 150 cases, categorisation was done forming two groups: non neoplastic 91.3% (137/150) and neoplastic 8.7% (13/150).

- Maximum female patients were encountered in the 3rd decade and maximum male patients belonged to 5th decade.
- Non neoplastic lesions were most common in 3rd decade and neoplastic lesions were common in 6th decade of life.
- Gallstones were present in 90.66% (136/150) and absent in 9.33% (14/150)cases. The mixed type were the most common type of stones, found in 80.1% (109/136) cases.
- The maximum no. of patients were reported as chronic cholecystitis with cholelithiasis (CC with CL), 38% (57/150) followed by chronic cholecystitis with intestinal metaplasia (CC with IM), 16.7% (25/150).
- Out of 150 cases, mucin histochemistry was carried out on 50 cases showing metaplasia, dysplasia and malignancy i.e. 37, 4 and 9 cases respectively.
- Mucin histochemistry showed PAS positivity in all 50 cases (metaplasia, dysplasia and carcinoma). Out of 37 cases of metaplasia, 100% cases (37/37) showed strong positivity for AB at pH 2.5 and also 100% cases (37/37) showed positivity for AB at pH 1, but with weak positivity in 91.89% (34/37) cases and only 8.1% (3/37) cases showing strong positivity for AB at pH 1, indicating that the type of mucin was predominantly sialoform in majority of cases.
- Malignant lesions (consisting of dysplasia and carcinoma) depicted positivity in 98% (12/13) cases for AB at pH 2.5. Out of 13 cases, 8 cases showed no positivity for AB at pH 1 and 4 cases showed weak positivity, which indicated the change of mucin from sulphomucin to sialomucin. In our study, one case of SRCC shows sulphomucin, which is an exception because it is stated in literature, that SRCC is a variety of adenocarcinomain which sialomucin is normally present. Literature regarding this is quite less as this entity is not so common. Since there is only one such case in our study, so it cannot be commented upon in detail. More cases would have been required to confirm this exception and derive a definite conclusion.

VII. Conclusion:

- Sulphomucins and sialomucins play an important role in cancer progression and metastasis. There is a definitive and well established association between metaplasia - dysplasia - carcinoma sequences.
- As there is progressive transformation from normal epithelium through metaplasia to carcinoma, the nature of mucin gets altered fromsulphomucin to sialomucin and the content of sialomucin also increases.
- Drugs and compounds regulating sialylation and sulfation, might be an effective way to curb progression, invasion, metastasis and gallstoneformation, thus, opening up novel therapeutic approaches.
- Further studies on large scale may enable us to draw definite conclusions and aid in early identification of high - risk cases and groups and implementation of possible therapeutic interventions.

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