IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 16, Issue 5 Ver. XII (May. 2017), PP 08-17
www.iosrjournals.org

Study of Mucin Histochemistry in Chronic Calculous Cholecysttits.

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Abstract: *Disorders of the biliary tract affect a significant portion of the world's population. Over 95% of the biliary tract disease is attributable to cholelithiasis.Gall stones afflict 10 to 20% of adult opulation in developed countries necessitating hospitalization and cholecystectomy. The bile content play crucial role in the formation of gallstones. Gall bladder secretes mucin which is a high molecular weight glycoprotein. . Gallbladder mucin shares with other epithelial mucins the ability to bind lipids and bile pigment.*
This gallbladder mucin has long been recognized as an important factor contributing to initiation and formation of gall stones .Many studie have been done to see if there is any relationship between pathological changes in diseased gall bladder and gall stones formation.
This study has been taken to see the histochemical alterations of mucin expressed in diseases gall bladder epithelium ,in cases of cholecystectomy done for chronic calculous cholecystitis .An attempt has also been made to correlate type of mucin expressed with histopathological features of chronic cholecystitis like inflammation and fibrosis.
Keywords: *Gall bladder , mucin , histochemistry , cholecystectomy, histopathology, inflammation, fibrosis*

I. Introduction

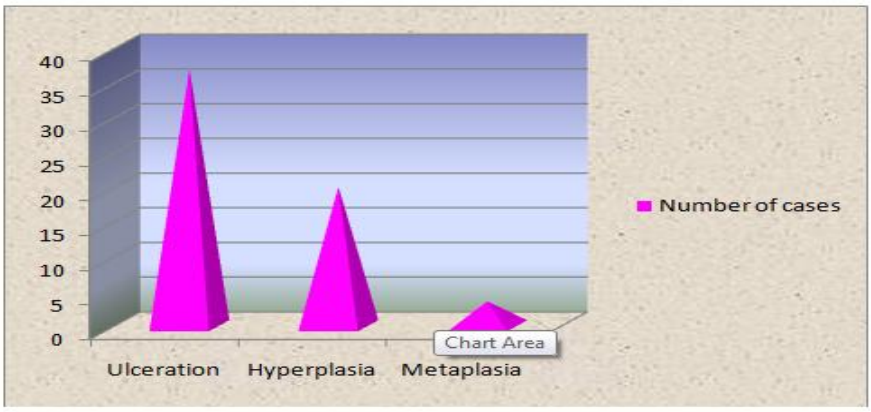
Gallstones are a major cause of morbidity throughout the world and one of the most common indications for cholecystectomy. The formation of gallstones is closely linked to bile and mucosal epithelial interaction.2 Apart from forming the nucleus for calculus, the mucins form a structural component of gallstones as shown by histochemical studies on calculi.3,4 Sulphated mucins are one the usually expressed by by normal gall bladder.4and are also found to be increased in diseased gall bladder. Before the appearance of gallstones there is always the formation of "biliary sludge" containing mucus gel, hydrophobic bile pigments, cholesterol, lecithin lipid crystals and cholesterol monohydrate crystals. The cholesterol crystal nucleation seems to occur in the mucus gel on the epithelial surface. Mucin secretion by gallbladder and formation of biliary sludge (of which mucus gel is a crucial component) are important factors in the pathogenesis of gallstone disease.6,7,8 Many studied are being done to see the corrlation between mucin expression, type of mucin expressed with histopatholgical features of diseased gall bladder, wheather they would give further insight onto pathogenesiss of gall stones.

II. Materials And Method

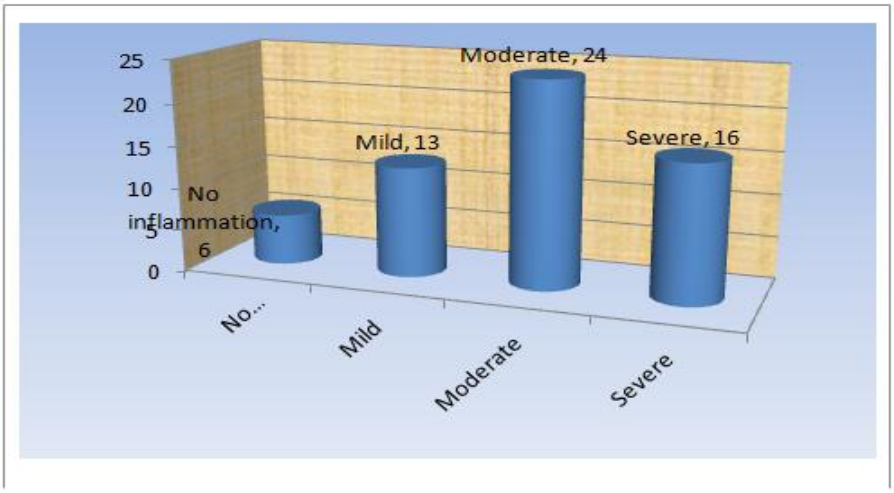
The study involved a total of 59 gallbladder specimen which were removed for chronic calculous cholecystitis and received at Department of histopatholgy Sree balaji medical college and hospital.The patient were aged between --- and included both males and females The specimen was received in 10% neutral buffered formalin .and fixed for 24 hours. Sections were taken from the fundus, body and neck of the gall bladder & additional sections were taken from grossly abnormal appearing mucosa if any. This was followed by processing with routine histological techniques for paraffin embedding and sectioning at 4 micron thickness. After deparafinisation, the sections were stained with Hematoxylin and Eosin for routine histopathology study , PAS and Alcian blue dual stains
were used for studying mucin histochemistry in the gall bladder epithelium.
Result –neutral mucin in epithelium stained magenta acid mucin in the epithelium stained -blue

II. Observations And Results

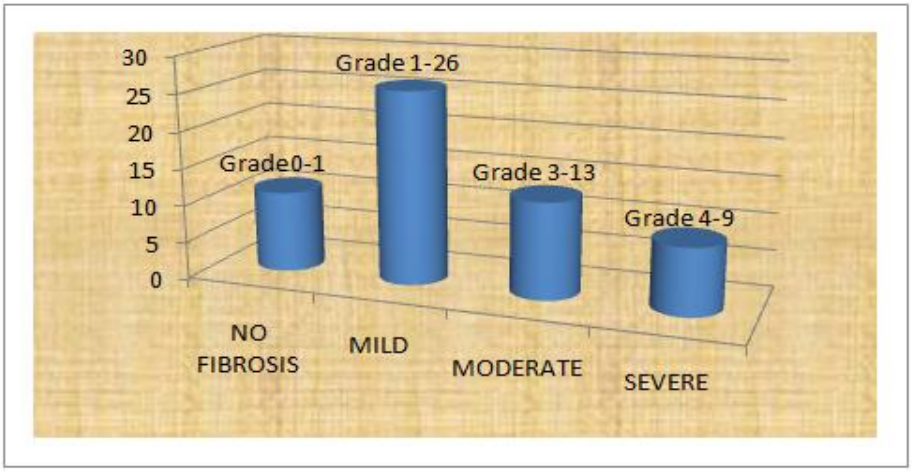
In this study conducted on cholecystectomy specimens of 59 patients ,the following were observed
*out of 59 cases ,36 were females, 23 males.
* Mucosal changes shows (fig 02.03)Ulceration was seen in 37 cases, hyperplasia in 20 cases and metaplstic changes in 3. Out of the 3, 1 case showed intestinal metaplasia and 2 showed gastric metaplasia.(Table 01)



*Grading of inflammation was done as per Zhou et al[82] ,based on number of inflammatory cells per field in any layer.(figs 04.05,06) The findings as per the grading system shows that out of the total 59 cases studied, 13 showed mild (Grade I) inflammation, 24 cases showed moderate (Grade II) inflammation and 16cases showed severe (Grade III) inflammation.(Table02) Grading of inflammation in the cases



*The distribution of fibrosis in the muscularis mucosae was graded as per the grading done in the study by Zhou et al82.
The grading was based on distributiom of collagen(fig 07,08).
Out of the 59 cases, 11 showed no fibrosis, 26 showed mild (Grade I) fibrosis, 13 showed moderate (Grade II) fibrosis and 9 showed severe (Grade III) fibrosis. (Table 03)



*Correlation of mucin score with grades of inflammation and fibrosis.

The sections were stained with Alcian Blue and PAS(.Fig16,17) The percentage of positive cells were scored and correlated with degree of inflammation and fibrosis. The mucin histochemistry scores obtained were obtained as per zhou et all , for the three grades of inflammation and fibrosis. An attempt was made to correlate the score with degree of inflammation, fibrosis .(figs11-15) There are progressive decrease in the mean scores for Alcian Blue positive (acid) mucins in the superficial and deep mucosal epithelium, with increasing grades of inflammation. Correlation of PAS stain (neutral mucin) with inflammation ,showed higher positivity/score in Grade III inflammation, than in Grade 1 inflammation..

Correlation of Alcian blue stain with Fibrosis Alcian Blue scores (acid mucins) are slightly lower in Grade III fibrosis, compared with Grade I.

Correlation of PAS stain with fibrosis Neutral mucins (PAS positive) shows higher scores in Grade II and decreases with increase in degree of fibrosis.

III. Discussion

Gall stones(fig01) are most common casuses of gall bladder disease most common being cholecystitis which causes considerable morbidity. Many mechanisms contribute to formation of gall stones. Nucleation of cholesterol plays an important role in formation of gall stones and gall bladder mucin plays a cruical role in this process.The mucin is synthesised and secreted by gall bladder epithelium and stains positive with PAS AB

The gall bladder mucosa is lined by columnar epithelium with lightly eosinophilic cytoplasm and basally located nuclei. Metaplasia is not seen in normal gallbladder, but is common in cholelithiasis and cholecystitis. Metaplasia can be gastric or intestinal in type.Normal gallbladder mucosa exhibited predominantly sulfomucin, traces of sialomucin and neutral mucin. Alteration of the expression pattern of mucins have been described in carcinomas as well as in their precursor lesions and also in the formation of gall stones. The major acid mucins secreted by gallbladder mucosa are sulphomucin.[9] Increase in the mucin content of bile in patients with gallstone disease, as against controls has been shown by many workers using biochemical techniques. Levy et al (1984) studied model bile and demonstrated the accelerating effect of mucins on nucleation of cholesterolmonohydrate crystals, an early step in lithogenesis.[8]

I.M.Ganesh et al (2007) observed in his study that in normal gastric and gallbladder mucosae, neutral mucins were predominant, whereas in intestinal metaplasia, gastric carcinoma and stone-containing gallbladder, there was a significant increase of acidic mucins. Dr J.P. Pani et al (2013) observed in his study that sulfomucin was a predominant variable for cholelithiasis and sialomucin was a predominant variable for cholecystitis. Womack et al as early as in the year 1963, demonstrated the presence of mucopolysaccharides in gallstones. Whole stones were sectioned and stained for mucins.[3] Subsequently other workers also showed presence of mucin as a structural component of cholesterol stone matrix.[11] The amount of mucins in cholesterol stones was found to be less, compared to pigment stones. It was also shown that the mucins in pigment stones are mostly sulphated. The bridging action of sulphomucins promoting solidification of the mucus gel during stone formation was suggested.[4]

Madrid et al (1988) studied epithelial mucins of gallbladder using conventional techniques and demonstrated the presence of sulphated and carboxylated mucins, the former predominating.In our present study the histochemical ananlysis of mucin , showed definite altered expressionin the diseased gall bladder epithelium in terms of amount and type of mucin expressed With increase in severity of inflammation and fibrosis, the total acid mucin content decreased while the neutral mucin content increased.(fig------) Similar changes were observed in cases of metaplasia too.

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.

Two cases of gastric metaplasia and one case of intestinal metaplasia were studied(fig09.10). The histochemical staining showed increase expression of sialomucin Fig 18,19). Studies conducted by Jyoti Prakash Pani et al (2013)[13] and Iniya Meenakshi Ganesh et al (2007)[12] also inferred the same in their study.

IV. Summary And Conclusion

In our study chronic calculous cholecystitis was commonly seen in the age group of 41 to 50 years with female preponderance and most cases were seen with moderate inflammation and mild fibrosis. There seems to be a definite altered mucin expresion in the diseased epithelium in cases of chronic calculous cholecystitis .The role of mucin has already been documented in the formation of gall stones.Studies like this involving the alteration in mucin expression in the epithelia of chronic cholecystitis may narrow down the role of specific type mucin in gall stone formation.Further studies may provide clue as to upgegulation and or inhibition of specific type of mucin in the epithelium can help to prevent gall stone formation.

Figure 1

Gallbladder with Stone

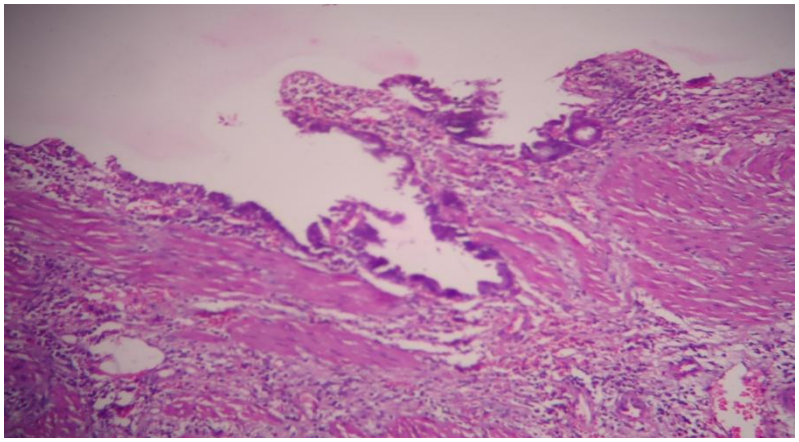


Figure 02: Ulceration of gallbladder mucosa H&E (100X)

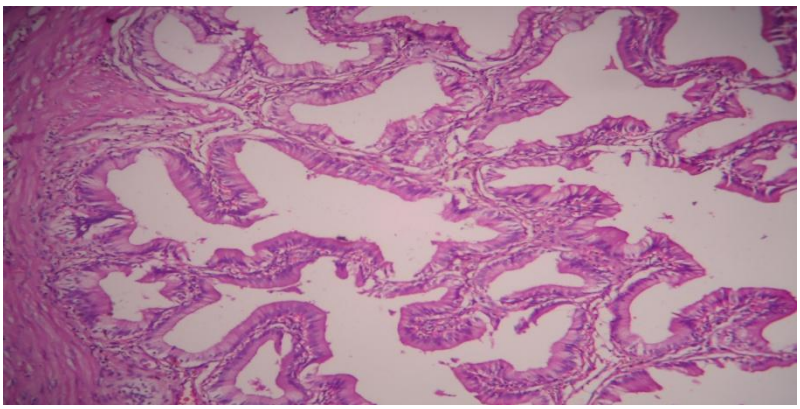


Figure 03: Hyperplastic Gall bladder mucosa H&E (100X)

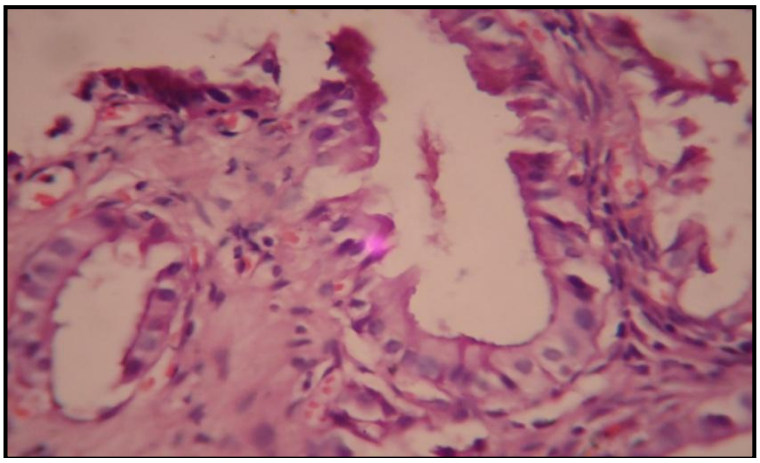


Figure 04

Mild (Grade-1) Inflammation-Gall Bladder.
A few lymphocytes are seen between glands. H & E (400X)

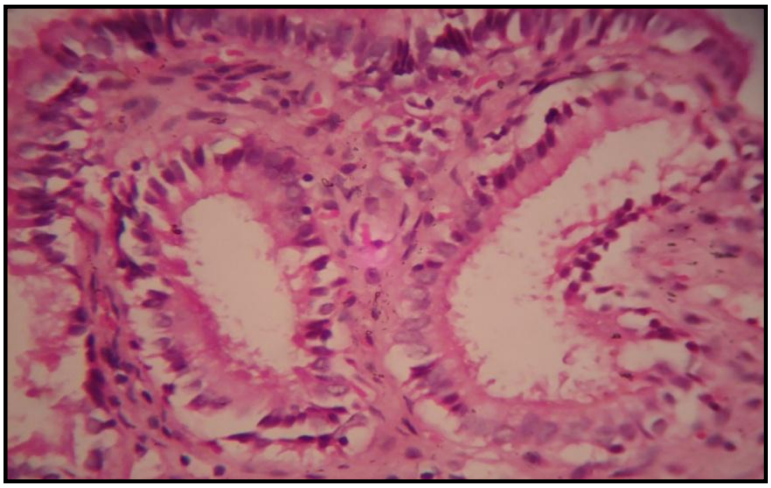


Figure 05

Moderate (Grade-2) Inflammation-Gall Bladder.
Plenty of Lymphocytes are seen .H & E (400X)

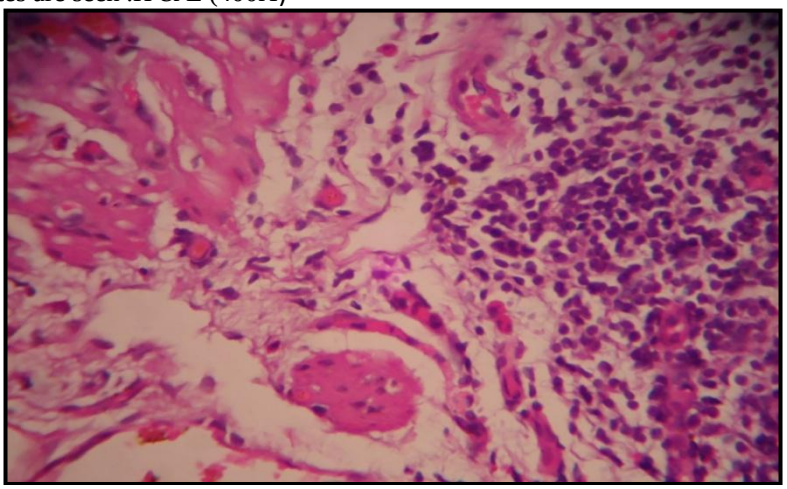


Figure 06

Severe (Grade-3) Inflammation-Gall Bladder.
Dense Sheets of Lymphocytes extended between smooth muscle bundles. H&E (400X)

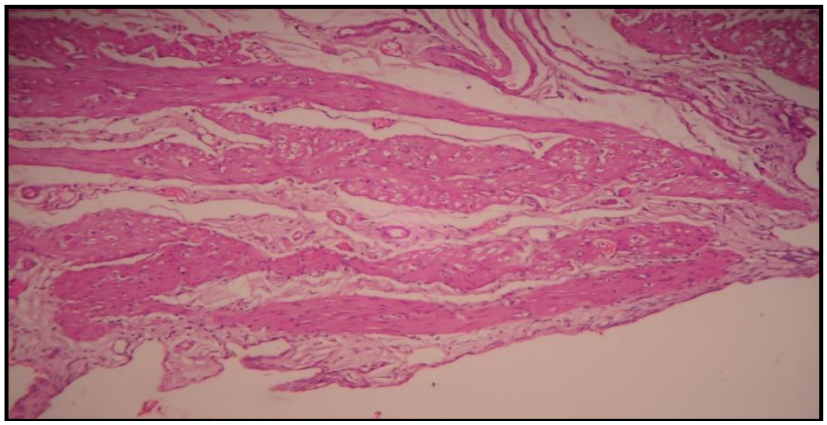


Figure 07

Moderate Fibrosis-Gall Bladder. H&E (100X)

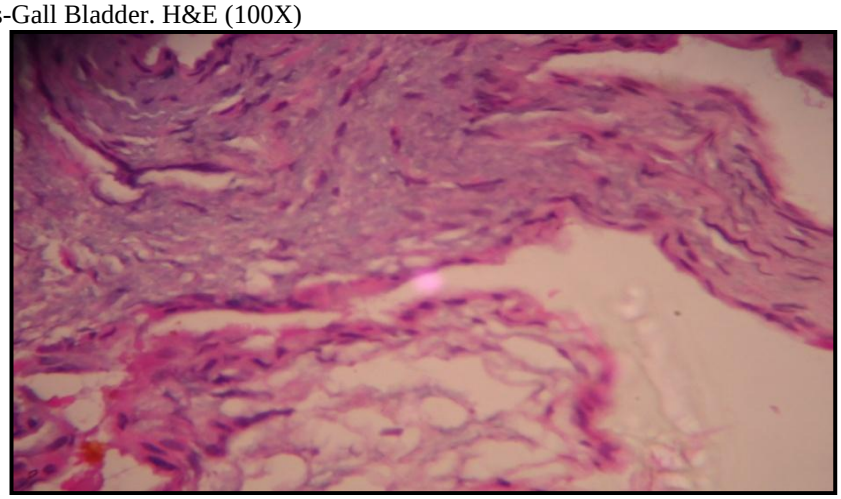


Figure 08

Severe Fibrosis-Gall Bladder. H&E (400X)

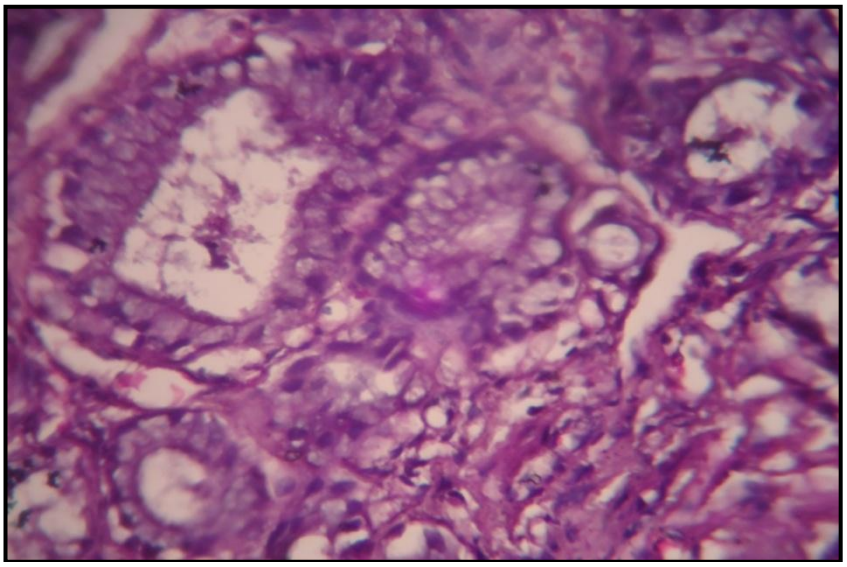


Figure 09

Photomicrograph shows Pyloric (Gastric) metaplasia in the deeper mucosa. H&E (100X)

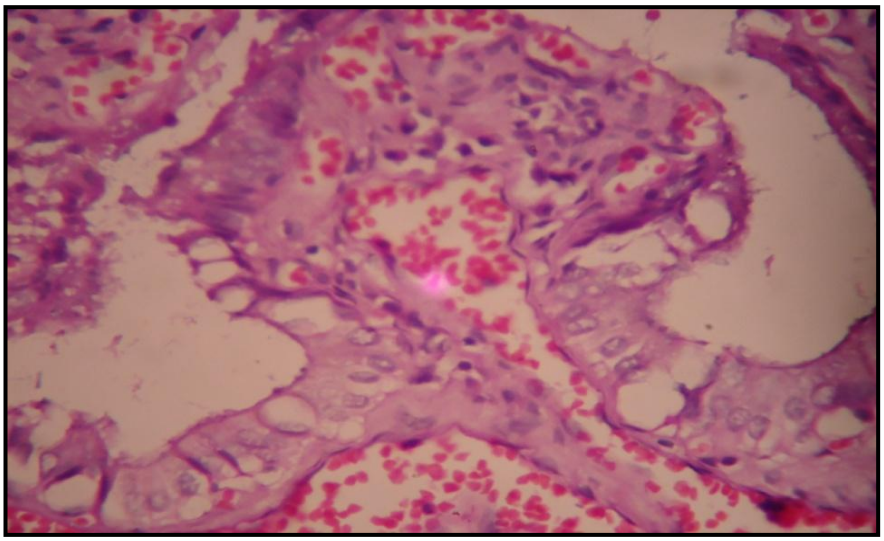


Figure 10

Intestinal metaplasia characterised by Goblet cells in the Gall Bladder mucosa. H&E (400X)

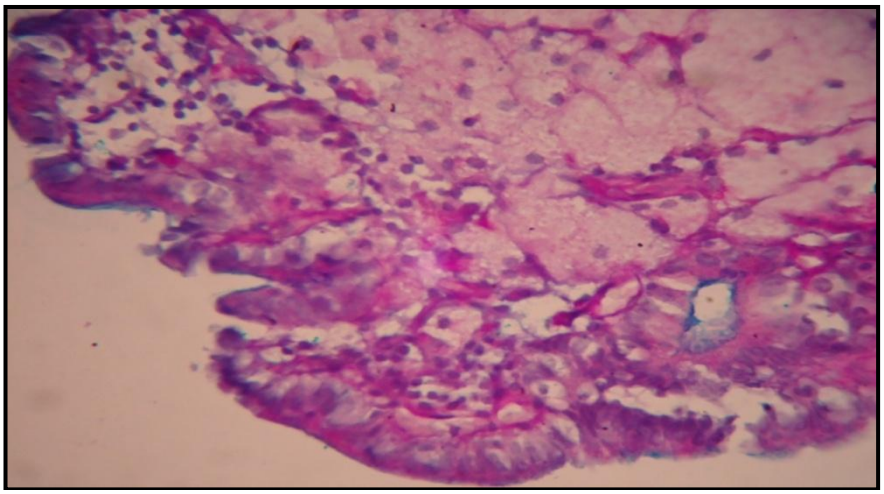


Figure 11

Score 1- About 5% of cells contain mucin in AB-PAS stain (100X)

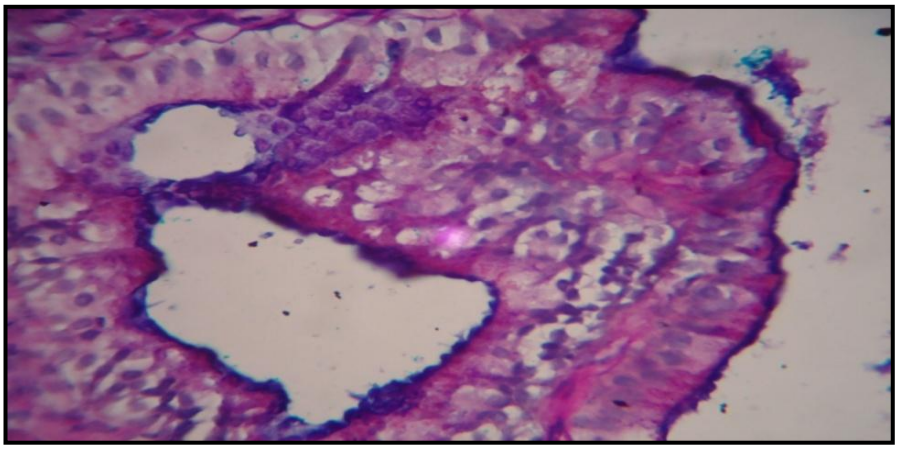


Figure 12

Score 2- About 25% of cells contain mucin in AB-PAS stain (100X)

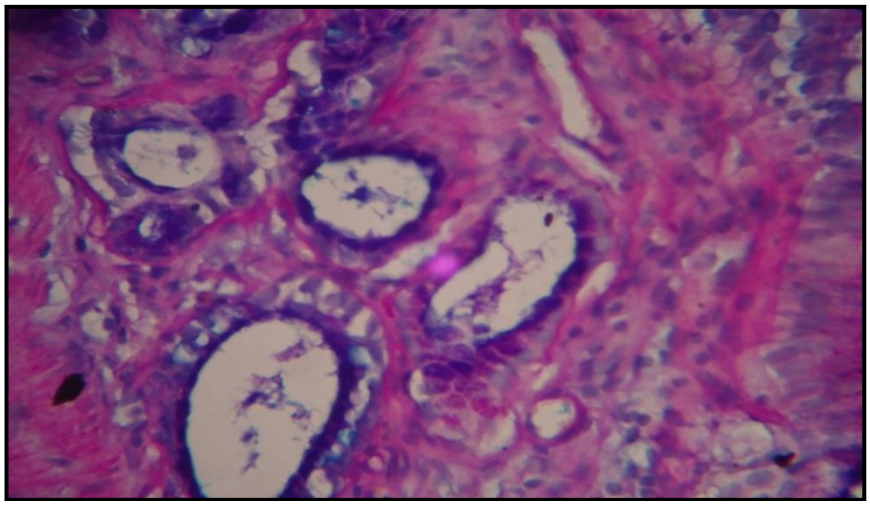


Figure 13

Score 3- About 50% of cells contain mucin in AB-PAS stain (100X)

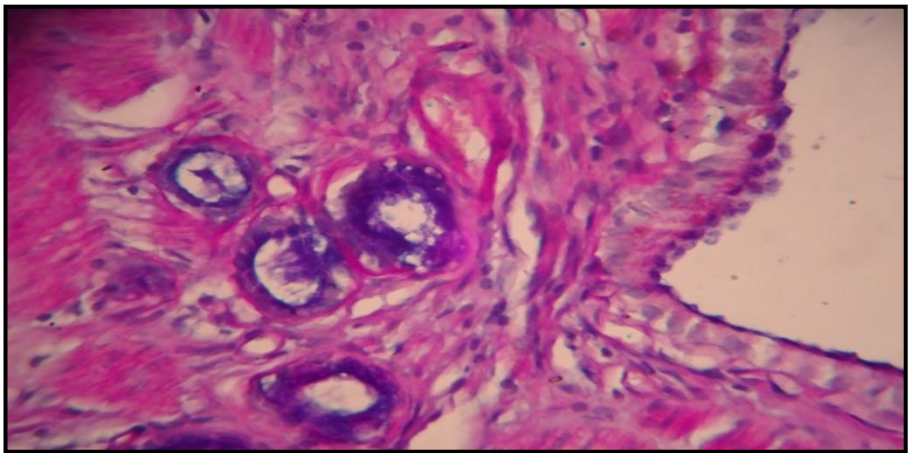


Figure 14

Score 4 - About 75% of cells contain mucin in AB-PAS stain (100X)

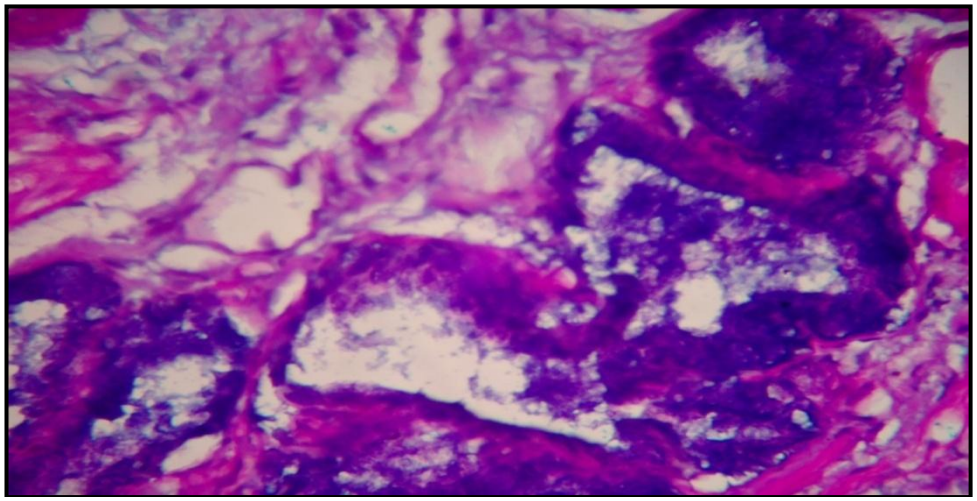


Figure 15

Score 5- About 100% of cells contain mucin in AB-PAS stain (100X)

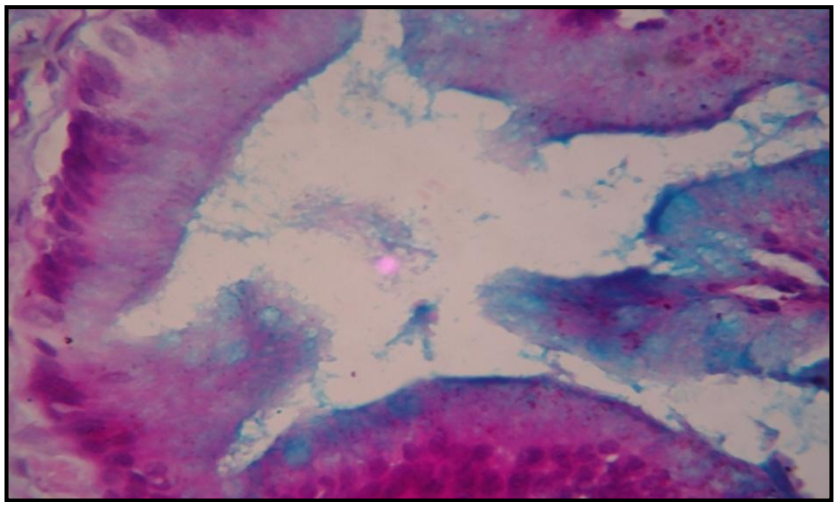


Figure 16

AB positive area denotes acid mucin in AB-PAS stain (400X)

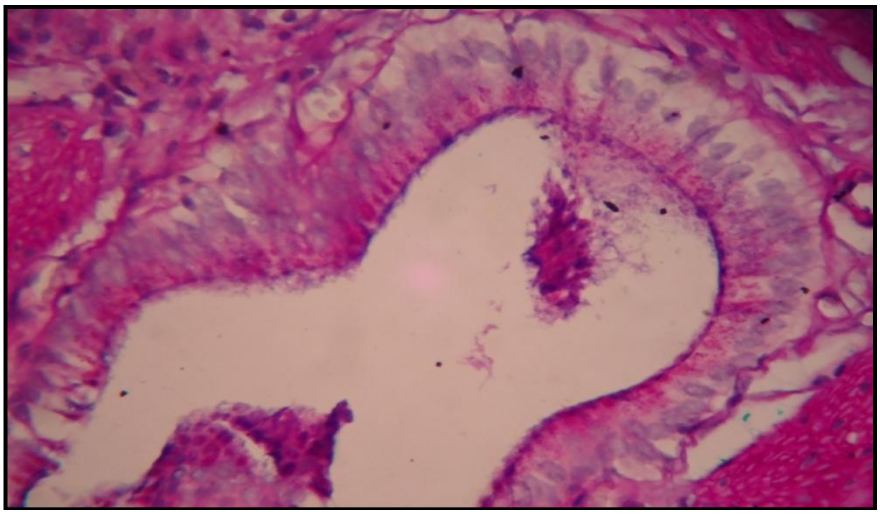


Figure 17

PAS positive area denotes neutral mucin in AB-PAS stain (400X)

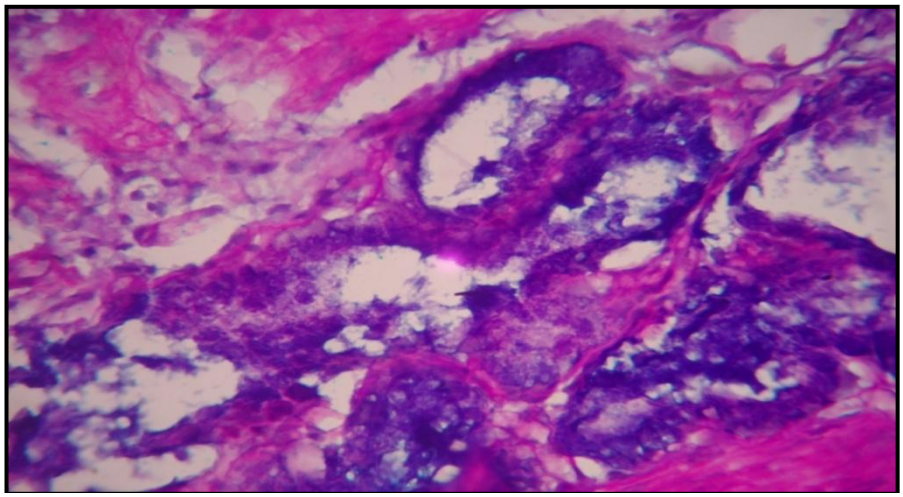


Figure 18

Gastric metaplasia showing PAS(neutral mucin) and AB (acid mucin) positive mucins in AB-PAS stain(100X)

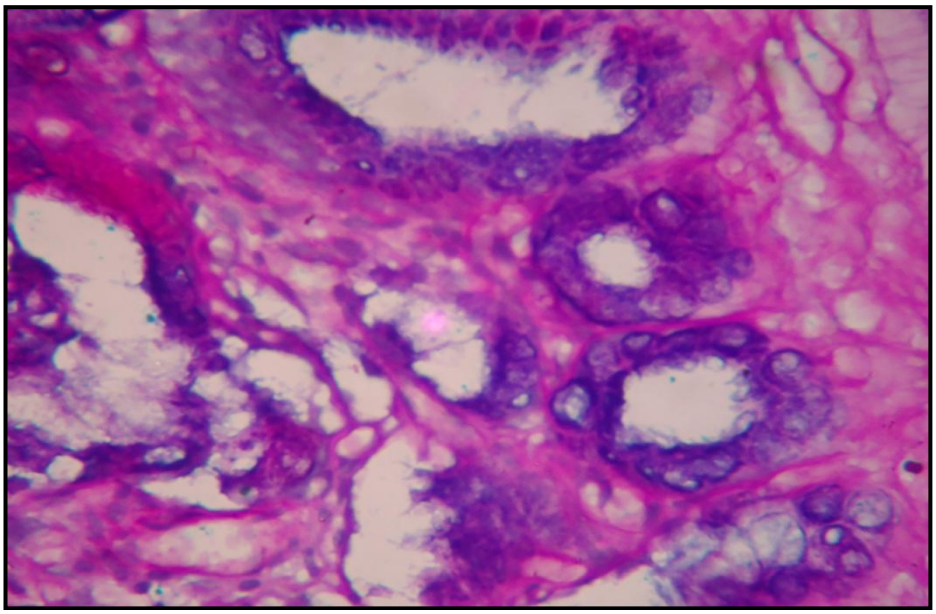


Figure 19

Intestinal metaplasia-Gall Bladder Goblet cells contain Alcian Blue(acid mucin) positive mucin in AB-PAS stain (400X)

List of abbrevations used

AB-Alcian blue

H&E-Haematoxylinand eosin

PAS-Periodic Acid Schiff

Bibilography

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