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Short Running Title: A histopathological and histochemical study of cholecystitis

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TITLE: A histopathological and histochemical study of cholecystitis

ABSTRACT

Aims:
The present study mainly aimed at to assess the qualitative and quantitative assessment of gallbladder mucins in chronic calculous cholecystitis, correlating the mucin histochemistry and morphology of the gallbladder in chronic calculus cholecystitis with each other and with chemical composition of gallstones.

Methods:
Haematoxylin & Eosin stained sections of the selected specimens were screened and one to two sections with adequate amount of well preserved mucosa with lining epithelium were chosen. The corresponding paraffin blocks were selected and isolated. Four sections were cut on each of the selected blocks for special stains.

Results:
Comparison of inflammation and mucin histochemistry, inflammation and metaplasia, composition of calculi and grade of inflammation, composition of stones and mucin histochemistry, composition of calculi and metaplasia, fibrosis and mucin histochemistry, fibrosis and stone composition indicated a decrease in intraepithelial total acid mucin content in chronic calculous cholecystitis. Cases with severe inflammation showed the maximum decrease in sulphomucin, concomitant increase in sialomucin scores and a high incidence of gastric metaplasia. Intestinal metaplasia on other hand did not correlate with the degree of inflammation or sialomucin content.
Conclusion

This study concludes that normally gallbladder epithelium contains sulphated acid mucins with traces of neutral and sialomucins. The sulphomucin content decreases in chronic calculous cholecystitis and with severe inflammation, total acid mucin content decreases, neutral mucin increases, and there is a higher incidence of gastric metaplasia and pigment stones and correlating with pigment stones. This tends to have an association with severe inflammation, higher degree of fibrosis, gastric metaplasia and presence of sialomucin.

Keywords: Gall bladder stones, chronic calculous cholecystitis, mucin histochemistry, metaplasia.
TITLE: A histopathological and histochemical study of cholecystitis

INTRODUCTION

Million people’s are affected by gallstones and are common major factor of morbidity throughout the world, necessitating hospitalization and cholecystectomy. Cholesterol saturated as ‘lithogenic’ bile originating from the liver and considered an important factor in gallstones formation has been found in healthy individuals [1]. Lithogenic bile therefore cannot be the only factor involved in the process. Other factors such as super saturation of bile with calcium [2], gallbladder mucus, prostaglandins and functional failure of electrolyte absorption by gallbladder mucosa also may influence gallstone formation. Biliary calcium can reduce the solubility of cholesterol [3] rendering the bile lithogenic. Apart from being a critical initiating factor, calcium is physically incorporated into gallstones as well. Gallbladder mucus has long been recognized as an important factor contributing to gall stone development [4, 5]. The implication of mucin in gallstone formation has been widely studied. Hypersecretion of mucus occurs during gallstone formation in humans [6-9], and experimental animals [10]. Apart from forming the nucleus for calculus, the mucins form a structural component of gallstones as shown by histochemical studies on calculi [11, 12]. Calcium and prostaglandins can stimulate mucus secretion by gallbladder mucosa [13-15]. Normal human gallbladder contains predominantly sulphated acid mucin [16]. It is this sulphated mucin content that is increased in gallstone disease. Metaplastic and neoplastic gallbladder epithelium on the other hand shows an increase in sialomucins and decrease in sulphomucins. Several studies have suggested progression from metaplasia, through dysplasia, to adenocarcinoma of gallbladder [17]. The existence of such a pathway has not been definitely proven.

Mucins

Mucins are the chemical components of the secretion delivered by certain types of epithelial and connective tissue cells. The original term mucin was coined by Carpenter as early as in the year 1846 [18]. Reid and Clamp in 1978 suggested glycoconjugates as general term, which could be subdivided into ‘proteoglycans’ and ‘glycoproteins’ [19].
Free hexose groups are often available, together with certain acidic moieties, the presence of which will markedly influence histochemical reactivity. The different mucins may be present as a single type within a given tissue unit, or more usually as a mixture of different types. The synthesis of mucin is initiated in the rough endoplasmic reticulum of the producing cells and is completed in the golgi apparatus. Sulphation of the hexosamine molecule occurs in the golgi region [8, 20]. Scheme of an easy method to classify the mucin like a proteins the only two classical techniques [8].

The different types of mucins which can be distinguished histochemically are as follows [21].

**ACID MUCINS**

1. Strongly sulphated
   a. connective tissue
   b. epithelial

2. Weakly sulphated
   Sulphated; histochemically atypical

3. Carboxylated; sialomucin
   a. enzyme – labile (N-acetyl form)
   b. enzyme – Resistant (N-acetyl O-acetyl form)

4. Sulfated sialomucin

5. Carboxylated; nonsulphated uronic acid (Hyaluronic acid)

**NEUTRAL MUCINS**

There are no subdivisions to this group

**MATERIALS AND METHODS**

A total number of 40 specimens were selected from gallbladders with clinical and histopathological diagnosis of chronic calculous cholecystitis received in the department of pathology PSG INSTITUTE OF MEDICAL SCIENCES AND RESEARCH during the period of 2005 to 2007. Criteria for selection were 1) Histopathological confirmation of chronic calculous cholecystitis. 2) Presence of calculous accompanying the specimen. 3) Availability of sufficient mucosa and well preserved lining epithelium in sections. 4) Availability of corresponding paraffin
blocks. While 36 specimens fulfilled the above criteria, 4 cases had only biliary sand in the container. 3 gallbladders resected for choledochal cysts, were taken as controls. Haematoxylin & Eosin stained sections of the selected specimens were screened and one to two sections with adequate amount of well preserved mucosa with lining epithelium were chosen. The corresponding paraffin blocks were selected and isolated. Four sections were cut on each of the selected blocks for special stains. A proforma was prepared for assessment. The slides were assessed according to the proforma.

**Haematoxylin & Eosin Stain**

Sections stained with Haematoxylin & Eosin were assessed for the intensity of inflammation and degree of fibrosis, which in turn were graded as mild, moderate and severe (1+, 2+, 3+) (Figure 1, 2). The number of Rokitansky Aschoff sinuses was indicated as many, few and nil (Figure 6). Gastric metaplasia and intestinal metaplasia were also noted and indicated as present or absent (Figure 3, 4).

**Mucin Histochemistry**

The following special stains for mucin were done with a view to assess the quantity and quality of mucins in the superficial and deep parts of gallbladder mucosa.

1. High Iron Diamine – Alcian Blue Stain (HID-AB)
2. Alcian Blue – Periodic Acid Schiff (AB-PAS)

A scoring system was devised, based on the percentage positivity of cells in each field under low power examination (10X), as shown below:

<table>
<thead>
<tr>
<th>Percentage Positivity</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>75% -100 %</td>
<td>5+</td>
</tr>
<tr>
<td>50% - 75%</td>
<td>4+</td>
</tr>
<tr>
<td>25% - 50%</td>
<td>3+</td>
</tr>
<tr>
<td>5% - 25%</td>
<td>2+</td>
</tr>
<tr>
<td>0% - 5%</td>
<td>1+</td>
</tr>
</tbody>
</table>

The values were tabulated and statistically assessed using ANOVA, t-test, $\chi^2$ (Chi$^2$) test.

Biochemical analysis of calculi, wherever available (36 samples) was performed to determine the chemical composition of calculi. Gross parameters such as external appearance, colour, number of stones and weight were noted. The following biochemical procedures were carried out on the 36 samples of calculi.
Biochemical analysis [22]

Cholesterol

Wash the gallstones with water and dry. Powder the stone and heat some with successive small portions of ether in a test tube by inserting the tube in some warm water and filter. Dissolve a little of residue obtained on evaporation of the ether in chloroform and add a little of mixture of acetic anhydride and sulphuric acid (in the proportion of 10 ml to 0.1 ml). A dark green colour develops rapidly.

Phosphate And Calcium Oxalate

Treat the remaining residue after ether extraction, with dilute hydrochloric acid (25%). This dissolves the inorganic salts present. Filter and test the filtrate for phosphate with molybdate. Make some of the solution alkaline with ammonia and add acetic acid and ammonium oxalate solution. If calcium is present a precipitate of calcium oxalate is formed.

Bile Pigment

Test the precipitate remaining after treatment with hydrochloric acid for bile pigments. Wash the material remaining on the filter paper and extract with warm chloroform. Examine the chloroform extract for bilirubin by means of diazo reagent. Change to pink colour indicates presence of bile pigment.

The mucin histochemistry scores obtained were tabulated, separately for the three grades of inflammation and fibrosis. An attempt was made to correlate the score with degree of inflammation, fibrosis, presence of metaplasia (intestinal and pyloric) and type of stone. Statistical assessment of the results was also performed.

RESULTS

The three specimens of gall bladder used as a control in the present study showed predominantly alcian blue (AB) positive mucins in the surface and deep mucosal epithelium. Periodic Acid Schiff (PAS) positivity was seen in traces. Comparison of inflammation and mucin histochemistry, inflammation and metaplasia, composition of calculi and grade of inflammation, composition of stones and mucin histochemistry, composition of calculi and metaplasia, fibrosis and mucin histochemistry, fibrosis and stone composition was done.
High Iron diamine and alcian blue (HID-AB) stain was done in the three controls and it showed strong HID-AB positivity indicating the predominance of sulphomucin in superficial and deep mucosa and evaluated by mean score which is normally observed in the normal gall bladder mucosa (Table 1).

Comparison of inflammation and mucin histochemistry (Tables 2 & 3) showed that out of the total 36 cases studied, 9 (25%) cases showed mild (Grade I) inflammation, 16 (44.4%) cases showed moderate (Grade II) inflammation and 11 (30.5%) cases showed severe (Grade III) inflammation. The mean scores for AB positive (acid mucins) in the superficial and deep mucosa, in the three grades of inflammation showed a progressive decrease for Alcian Blue positive (acid) mucins in the superficial and deep mucosal epithelium, with increasing grades of inflammation (Table 2). The mean scores for PAS positive mucins in the superficial and deep mucosa were higher in Grade III inflammation, than in Grade I inflammation. Grade II inflammation however shows random scores, not conforming to any pattern (Table 2). The mean scores for HID (sulfomucin) positive are the lowest for Grade III inflammation. A progressive decrease in HID scores was observed in the superficial mucosal epithelium (Table 3). Alcian Blue (sialomucin) scores were seen to be highest in Grade III inflammation (Table 3).

Comparison of grades of inflammation and metaplasia (Table 4) showed that out of the 36 cases, 13 (36.1%) showed intestinal metaplasia, 12 (33.3%) showed gastric metaplasia and 11 (30.5%) did not show any metaplasia. Metaplastic epithelium is characterized by presence of PAS positive mucins and / or goblets cells containing AB positive mucins in AB – PAS stains sections (Figures 9-11). Intestinal metaplasia has the highest incidence in Grade I inflammation (56%), and is lowest in Grade II inflammation (19%). The reverse is seen with gastric metaplasia, which has the highest incidence in Grade III inflammation (46%), and a considerably lower incidence in Grade I inflammation (11%).

Comparison of composition of calculi and grade of inflammation (Table 5), 3 cases out of 36 cases did not show calculi. 13 (39.3%) showed cholesterol calculi and 20 (60.6%) showed pigment stone calculi. The presence of pigment stones appears to correlate with severity of inflammation [(3/8 (37.5%) in Grade I inflammation,
9/11 (81.8%) in Grade III inflammation) as against cholesterol stones [(5/8 (62.5%) in Grade I inflammation, 2/11 (18.1%) in Grade III inflammation].

Comparison of stones and mucin histochemistry (Table 6) showed that the mean scores of sialomucins in the superficial and deep mucosal epithelium are higher in cases with pigment stones (0.85, 0.72), when compared with those having cholesterol stones (0.24, 0.58). The number of cases that show presence of sialomucins is low with both types of calculi (5/20 cases with pigment stones and 6/13 cases with cholesterol stones).

Comparison of composition of calculi and metaplasia (Table 7) showed that intestinal metaplasia is more or less equally associated with pigment and cholesterol calculi (35% and 30% incidence). Gastric metaplasia shows a considerably higher incidence (40%) in association with pigment stones, when compared with cholesterol stones (23%).

Comparison of fibrosis and mucin histochemistry (Table 8), out of 40 cases, 33 (82.5%) cases showed fibrosis. Alcian Blue scores (acid mucins) are slightly lower in Grade III fibrosis, compared with Grade I. Neutral mucins (PAS positive) on the other hand shows higher scores in Grade III as against Grade I fibrosis. No correlation is seen between HID scores (for sulphomucins) and degree for fibrosis. Alcian Blue scores (for sialomucins) are the lowest with Grade III fibrosis (Table 8).

Comparison of fibrosis and stone composition (Table 9) showed that out of the 33 cases pigment stones have a higher incidence in Grade III fibrosis 80% (4/5) as against Grade I fibrosis 52.6% (10/19).

**DISCUSSION**

Attempts have been made in the past to correlate gallbladder morphology, mucin histochemistry, and composition of calculi in gallstone disease [16, 23, 24]. Most of the previous studies however have combined any two of the three aspects, that is, either morphology with mucin histochemistry, mucin histochemistry with composition of calculi or composition of calculi with morphology. Few studies correlating all the three with one another have been recorded in literature. Purpose of the present study was to determine whether qualitative and/or quantitative variations in gallbladder mucins occurs in chronic calculous cholecystitis and whether the
alterations, if any, correlate with morphological changes in the gallbladder and/or with the type of calculous present. In short, an attempt has been made through this study, to correlate gallbladder morphology, mucin histochemistry and composition of calculi to one another, in chronic calculous cholecystitis specimens. Gallbladders removed for choledochal cysts were the control in the present study. The mucosal histology was normal and there was no inflammation, fibrosis or metaplasia. AB-PAS and HID-AB staining of sections showed predominantly HID positive sulphated acid mucins throughout the mucosa, with traces of sialomucins and neutral mucins in foci. Normal gallbladder mucosa is known to contain predominantly sulphomucins. Traces of sialomucins and neutral mucins also may be present [24]. Our observations on control samples conform to this well-established normal pattern of mucin histochemistry of gallbladder mucosa. Our results indicate a decrease in intraepithelial total acid mucin content in chronic calculous cholecystitis. Mucin depletion in mucosal epithelial cells is well known in inflammatory conditions of the gastrointestinal tract. In various forms of colitis presenting with mucus diarrhoea or dysentery and showing active inflammation of the mucosa, mucin depletion is a constant finding. Increased mucin secretion by gallbladder mucosa during gallstone formation has been described in literature [6-9, 24]. Earlier investigators have shown that mucins, in addition to being a structural component of gallstones [12], also play an acceleratory role in lithogenesis [25]. Our observation of decreased intraepithelial mucins in inflamed gallbladder mucosa is likely to be a reflection of increased secretion of mucin into bile which is known to occur in calculous disease. The decrease in the intraepithelial mucins in chronic cholecystitis, we found, was due to decrease in sulphomucin which is the predominant type of mucin in gallbladder mucosa. Further, it was observed that cases with severe inflammation showed the maximum decrease in sulphomucin. This was associated with a concomitant increase in sialomucin scores and a high incidence of gastric metaplasia. Intestinal metaplasia on the other hand, did not correlate with the degree of inflammation or sialomucin content. No qualitative changes in gallbladder epithelial mucins have been observed in the earlier studies on chronic cholecystitis [26]. Sialomucins are known to occur in traces in normal gallbladder mucosa and in considerable quantities in metaplastic mucosa. In the present study, an increase in
sialomucins was observed in gallbladders showing severe inflammation. Interestingly, it was in this group that gastric metaplasia had the highest incidence (Figure 12). It therefore follows that, sialomucins in significant quantities tend to appear in the areas of gastric metaplasia in the gallbladder mucosa. Their presence is not confined to the goblet cells of intestinal metaplasia which had the lowest percentage of incidence in severe inflammation, in the cases assessed (Figure 13). It has been suggested that antral and intestinal metaplasia in the gallbladder are histogenetically related having the same progenitor cell, and could therefore be parts of a morphological spectrum [27]. Transition from gastric to intestinal metaplasia is a likely possibility [17]. Pre-neoplastic role of intestinal metaplasia in the gallbladder and the metaplasia → dysplasia → neoplasia sequence have received wide attention among workers [11, 17, 28]. The mucin profile changes with progressive transformation to neoplasia, from normal with sulphomucin predominating through metaplastic and dysplastic showing increasing amounts of sialomucin, to full fledged neoplastic with sialomucins predominating. The high incidence of gastric metaplasia in severe inflammation and its association with increased expression of sialomucins with the concomitant reduction in sulphomucins would point, perhaps tentatively, towards a role for gastric metaplasia in the proposed chain of events stated above. Basu et al studied the morphological changes in chronic calculous cholecystitis in relation to the type of stones [29]. They found that inflammation was more severe with pigment calculi while fibrosis and related complications were more frequent with cholesterol calculi. The present study supports the association between pigment stones and severe inflammation. Fibrosis also was more in cases with pigment calculi in our study. Mucins have been shown to be a structural component of gallstones [11, 30]. Histochemical studies carried out on calculi have demonstrated presence of sulphomucins in them, especially in pigment stones [12]. However no correlation between mucin histochemistry of mucosal epithelium and the type of stone has been recorded in literature. In the present study, pigment stones were found more often in association with severe inflammation, gastric metaplasia and increased expression of sialomucins, as against cholesterol stones. We were unable to establish a statistical significance to the above observations, as the number of cases studied was small, especially in the sialomucin expressing group (even though
the scores were high.) But the scores and percentage values did show a distinct pattern indicating a correlation between a severe inflammation, gastric metaplasia, sialomucins and pigment calculi (Refer tables 3 to 7).

Considering the proposed pathway of Gastric metaplasia → Intestinal metaplasia → Dysplasia → Adenocarcinoma of gallbladder and the proven presence of sialomucins in considerable amounts in dysplastic and neoplastic gallbladder mucosa, it is reasonable to speculate on a pigment stone → severe inflammation → gastric metaplasia → sialomucin link up, with possible transition to dysplasia, with or without the intervention of intestinal metaplasia. Further studies on large series are required to enable us to draw definite conclusions. If such a high risk group emerges, it will be of significance from the preventive, prognostic and therapeutic point of view.

CONCLUSION

I. The normal gallbladder epithelium contains sulphated acid mucins with traces of neutral and sialomucins.

The sulphomucin content decreases in chronic calculus cholecystitis

II. In chronic calculus cholecystitis with severe (Grade III) inflammation (as against mild inflammation):

1) Total acid mucin content is decreased.
2) This decrease is due to HID positive (sulpho) mucin.
3) Neutral mucin and sialomucin contents are increased.
4) There is a higher incidence of Gastric metaplasia and pigment stones.

III. Pigment gallstones tend to have an association with:

1) Severe inflammation
2) Higher degree of fibrosis
3) Gastric metaplasia
4) Presence of sialomucins

More number of cases need to be studied to see whether high risk group consisting of pigment stones → severe inflammation → gastric metaplasia → sialomucin emerges. If it does, will be of therapeutic and prognostic significance.
CONFLICT OF INTEREST

The authors declare no conflict of interests.

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prostaglandins, E2 in vivo on secretory behaviour and ultrastructural changes

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**TABLES**

Table 1: HID–AB stain showed strong HID positivity indicating predominance of sulphomucins.

<table>
<thead>
<tr>
<th>Techniques</th>
<th>AB-PAS</th>
<th>HID-AB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Superficial</td>
<td>Deep</td>
</tr>
<tr>
<td>AB-PAS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>4.7</td>
<td>2.6</td>
</tr>
<tr>
<td>PAS</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>HID-AB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HID</td>
<td>4.5</td>
<td>2.8</td>
</tr>
<tr>
<td>AB</td>
<td>0</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*HID- High iron diamine AB- Alcian blue PAS- Periodic Acid Schiff
Table 2: Comparison of inflammation and mucin histochemistry - AB PAS STAIN.

<table>
<thead>
<tr>
<th>Inflammation Grade</th>
<th>Alcian blue [Acid Mucin] Mean Score</th>
<th>Pas [Neutral Mucin] Mean Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Superficial</td>
<td>Deep</td>
</tr>
<tr>
<td>I (9 cases)</td>
<td>2.7</td>
<td>3.4</td>
</tr>
<tr>
<td>II (16 cases)</td>
<td>2.2</td>
<td>3.1</td>
</tr>
<tr>
<td>III (11 cases)</td>
<td>1.95</td>
<td>2.5</td>
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</table>
Table 3: Comparison of inflammation and mucin histochemistry: HID-AB.

<table>
<thead>
<tr>
<th>Inflammation Grade</th>
<th>HID – [Sulphomucin] Mean Score</th>
<th>Inflammation Grade</th>
<th>Alcian Blue –[Sialomucin] Mean Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Superficial</td>
<td>Deep</td>
<td>Superficial</td>
</tr>
<tr>
<td>I (9 cases)</td>
<td>3.9</td>
<td>3.6</td>
<td>I (9 cases)</td>
</tr>
<tr>
<td>II (16 cases)</td>
<td>2.5</td>
<td>3.9</td>
<td>II (16 cases)</td>
</tr>
<tr>
<td>III (11 cases)</td>
<td>2.1</td>
<td>2.8</td>
<td>III (11 cases)</td>
</tr>
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Table 4: Comparison of grades of inflammation and metaplasia.

<table>
<thead>
<tr>
<th>Type of Metaplasia</th>
<th>Inflammation Grade</th>
<th></th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I (9 cases)</td>
<td>II (16 cases)</td>
<td>III (11 cases)</td>
<td></td>
<td>36</td>
</tr>
<tr>
<td>IM (Intestinal metaplasia)</td>
<td>5 (56%)</td>
<td>3 (19%)</td>
<td>5 (22%)</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>GM (Gastric Metaplasia)</td>
<td>1 (11%)</td>
<td>6 (38%)</td>
<td>5 (46%)</td>
<td></td>
<td>12</td>
</tr>
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Table 5: Comparison of calculi and grade of inflammation.

<table>
<thead>
<tr>
<th>Grade of Inflammation</th>
<th>Pigment Stone</th>
<th>Cholesterol Stone</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (8 cases)</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>II (14 cases)</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>III (11 cases)</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL - 33</td>
<td>TOTAL - 20</td>
<td>TOTAL - 13</td>
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</table>
Table 6: Comparison of stones and mucin histochemistry.

<table>
<thead>
<tr>
<th></th>
<th>Pigment Stones</th>
<th>Cholesterol Stones</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Number</strong></td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td><strong>Sialomucins Present In</strong></td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td><strong>Sialomucin Score</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superficial</td>
<td>0.85</td>
<td>0.72</td>
</tr>
<tr>
<td>Deep</td>
<td>0.24</td>
<td>0.58</td>
</tr>
</tbody>
</table>
Table 7: Comparison of composition of calculi and metaplasia.

<table>
<thead>
<tr>
<th>Types of Metaplasia</th>
<th>Pigment Stones</th>
<th>Cholesterol Stones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal Metaplasia</td>
<td>35%</td>
<td>30%</td>
</tr>
<tr>
<td>Gastric Metaplasia</td>
<td>40%</td>
<td>23%</td>
</tr>
</tbody>
</table>
Table 8: Fibrosis and mucin histochemistry AB-PAS & HID-AB stain.

<table>
<thead>
<tr>
<th>Fibrosis Grade</th>
<th>AB [Acid Mucin] Mean Score</th>
<th>PAS [Neutral Mucin] Mean Score</th>
<th>HID [Sulphomucin] Mean Score</th>
<th>AB [Sialo Mucin] Mean Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Superficial</td>
<td>Deep</td>
<td>Superficial</td>
<td>Deep</td>
</tr>
<tr>
<td>I (19 cases)</td>
<td>2.46</td>
<td>2.81</td>
<td>0.13</td>
<td>0.22</td>
</tr>
<tr>
<td>II (9 cases)</td>
<td>3.0</td>
<td>3.1</td>
<td>0.96</td>
<td>0.45</td>
</tr>
<tr>
<td>III (5 cases)</td>
<td>2.0</td>
<td>1.7</td>
<td>0.85</td>
<td>0.32</td>
</tr>
</tbody>
</table>
Table 9: Comparison of fibrosis and stone composition.

<table>
<thead>
<tr>
<th>Grade of Fibrosis</th>
<th>Pigment Stone</th>
<th>Cholesterol Stone</th>
<th>No Stones</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>III</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Total (33)</td>
<td>23</td>
<td>13</td>
<td>4</td>
<td>33</td>
</tr>
<tr>
<td>Mucins</td>
<td>AB-PAS</td>
<td>HID-AB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>----------</td>
<td>---------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutral Mucins</td>
<td>Red</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulphomucins</td>
<td>Red-blue</td>
<td>Brown-black</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sialomucins</td>
<td>Red-blue</td>
<td>Blue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulpho-Sialomucins</td>
<td>Red-blue</td>
<td>Brown-blue</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AB-PAS, sequential staining with AB(2.5) (blue) and PAS (red); HID-AB, sequential staining with HID(brown to black) and AB(2.5) (blue).

**FIGURE LEGENDS**

Figure 1: Severe (Grade III) Inflammation - Gall Bladder. Dense Sheets of Lymphocytes extended between smooth muscle bundles. H & E. (X 100).

Figure 2: severe Fibrosis - Gall Bladder. H & E. (X 100).

Figure 3: Photomicrograph shows Pyloric Metaplasia in the deeper mucosa. H & E. (X 100).

Figure 4: Intestinal Metaplasia characterised by Goblet cells in the Gall Bladder mucosa. H & E. (X 100).

Figure 5: Score I - About 5% of cells contain Mucin AB-PAS (X 100).

Figure 6: Score 3 - About 50% of cells contain Mucin AB-PAS (X 100).

Figure 7: Score 4 - About 75% of cells contain Mucin AB-PAS (X 100).

Figure 8: Score 5 - About 75-100% of cells contain Mucin HID-AB (X 100).

Figure 9: This field shows Gastric and Intestinal Metaplasia. The Gastric Metaplastic epithelium shows PAS and Alcian Blue Positive Mucins. Goblet cells of Intestinal Metaplasia are Alcian Blue positive Mucins. AB-PAS (X 100).

Figure 10: Intestinal Metaplasia - Gall Bladder Goblet cells contain Alcian Blue positive mucin AB-PAS (X 100).

Figure 11: Gastric Metaplasia - Gall Bladder. The Non - Neoplastic epithelium is HID positive (Brown) Metaplastic epithelium Alcian Blue positive (Blue) HID-AB (X 100).

Figure 12: Intestinal Metaplasia - Gall Bladder Goblet cells contain Alcian Blue...
positive mucin. The columnar cells are HID positive (Brown) HID-AB (X 400).

Figure 13: Intestinal Metaplasia - Gall Bladder HID-AB (X 400).

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