

Histomorphology Spectrum of Gall Bladder Pathology in Cholecystectomy Specimens with Clinical Diagnosis of Chronic Cholecystitis

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ABSTRACT

Introduction: Chronic cholecystitis is a common disease in surgery where majority of cholecystectomy are performed. This study was carried out to see histomorphological pattern of gallbladder diseases, staining pattern with various stains and prevalence of carcinoma.

Methods: This prospective study was conducted in the department of Pathology, B.P. Koirala Institute of Health Sciences, Dharan, Nepal from January to December 2008. A minimum of fifty random samples were included in study. Clinical details and pathological data were retrieved from case sheets and patient's history. All specimens were fixed in 10% formalin. Three sections each from neck, body and fundus were taken. Tissues were processed in an automated tissue processor and paraffin blocks made. The tissue sections were examined under light microscope after hematoxylin-eosin, neutral mucin, sulfated mucin and sialomucin stain.

Results: Chronic cholecystitis without cholelithiasis constituted majority of 25 (50.0%) cases with M: F of 1: 7.1. The gastric and intestinal metaplasias were present in 33.0% and 8.0% of the cases respectively. The neutral, sulfated and sialomucin were positive in 35.0%, 21.0 %, 1.0% and 34.0%, 21.0%, 1.0% cases respectively in gastric and intestinal metaplasia. Both gastric and intestinal metaplasia showed significant p-value on sulfated mucin.

Conclusions: The increase risk factors for developing of chronic cholecystitis were seen in female gender (86%). The gastric and intestinal metaplasia showed positivity more with neutral followed by sulfated mucin.

Keywords: *chronic cholecystitis; metaplasia; mucins.*

INTRODUCTION

The incidence of chronic cholecystitis and carcinoma gallbladder in Eastern Nepal is 12.2% and 1.2% respectively, of which 63.63% females.¹ Chronic cholecystitis is associated with cholelithiasis in > 90% of cases. It produces diverse histopathological changes in gallbladder mucosa like acute - chronic inflammation, xanthogranulomatous cholecystitis, glandular hyperplasia, cholesterosis, and metaplasias:

gastric, intestinal, dysplasia and carcinoma.

The lining cells and neck mucus glands contain mainly sulfated acid mucin with very small amount of non-sulfated acid mucin. Similarly metaplastic cells contain non-sulfated acid mucin and neutral mucin but little

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sulfated acid mucin. These metaplastic changes increase steadily with the age.²

The population incidence rates of gallbladder cancer vary widely among various races and different geographic regions.³

The mucin histochemistry of stone-containing gallbladder

The intense blue staining of sialomucins along with the weak staining intensity of neutral mucins in goblet cells is observed by AB-PAS technique.⁴

METHODS

Fifty random samples received for histopathological examination during the period were included in the study. Clinical details and pathological data were retrieved from the case sheets as well as patient's history. Three sections were taken from each gallbladder from neck, body and fundus. After sectioning, tissues were processed in an automated tissue processor and paraffin blocks were made. The tissue sections of 5mm were cut and examined under light microscope after staining the slides with hematoxylin-eosin stain and other three slides with neutral mucin, sulfated mucin and sialomucin.

The following special staining techniques were used:

- Phenylhydrazine- PAS technique for neutral mucin
- "Mild " methylation – alcian blue method for sulfated mucin
- Mild PAS – technique for sialomucin

INCLUSION CRITERIA

All the cases with a clinical diagnosis of chronic cholecystitis, irrespective of age and sex were included in this study.

EXCLUSION CRITERIA

All the cases of malignant gallbladder lesions were excluded from this study.

DATA PROCESSING AND ANALYSIS:

Useful data were entered in database for statistical analysis. The SPSS version 10.0 software was used. A "p" value of less than 0.05 was considered significant.

RESULTS

Socio-demographic characteristic of patient:

Age group and sex: The age group of patient varied from 10 – 69 years, with maximum and minimum frequency of 17 (34%) and 3 (6.0%) in age group of 30 – 39 years and 60 – 69 years respectively. Interestingly, two female patients of paediatric age group of 13 and 14 years were also seen. The mean age being 34.74 years with standard deviation of 13.29. The male patient was 7 and the remaining 43 were female, with M:F ratio of 1 : 7.16.

Address: Patient in the study were usually from the district around the BPKIHS hospital which is located at Sunsari District. In this study, maximum patients 22 (44%) were from Sunsari district followed by Jhapa, Dhankuta, Morang and Siraha.

Occupation : The occupation of the maximum number of patients 35 (70%) in this study group was found to be housewife followed by shopkeeper, student, teacher, farmer and unemployed.

Personal habit of patient: In this study, 44 patients (88.0%) were non-smoker and 40 patients (80.0%) were found to be non-alcoholic. The non-vegetarian with red meat dietary habit were found to be in 28 patients (56.0%) followed by the patient spending sedentary life style among 38 patients (72.0%) (Table 3).

Table 1. Personal habit of patient.

Characteristic	Categories	n (%)
Smoking	Non-smoker	44 (88.0)
	Occasional	5 (10.0)
	Regular	1 (2.0)
Alcohol	Habitual	2 (4.0)
	Non-alcoholic	40 (80.0)
	Occasional	8 (16.0)
Dietary habit	Mixed	17 (34.0)
	Non-veg red meat	28 (56.0)
	Vegetarian	5 (10.0)
Life style	Healthy	12 (24.0)
	Sedentary	38 (76.0)

Histomorphological spectrum of cholecystectomy specimens: Out of total 50 cases in the study, chronic cholecystitis without cholelithiasis constituted majority of the histomorphological spectrum of gallbladder pathology. Rest of the spectrum are shown in (Table 2).

Malignancy was not found.

Table 2. Histomorphological spectrum.

Histomorphological spectrum	n (%)
Acute on chronic cholecystitis. No stones received.	4 (8.0)
Chronic cholecystitis with cholesterosis. No stones received.	11 (22.0)
Chronic cholecystitis with cholelithiasis.	4 (8.0)
Chronic cholecystitis. No stones received.	25 (50.0)
Follicular cholecystitis. No stones received.	2 (4.0)
Chronic cholecystitis with xanthogranulomatous change. No stones received.	3 (6.0)
Xanthogranulomatous cholecystitis with cholelithiasis.	1 (2.0)

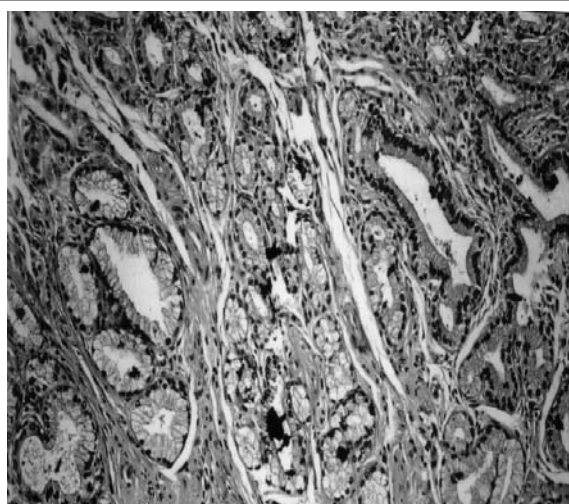


Figure 1. Gastric metaplasia in chronic cholecystitis (H&E X100).

Additional histopathological findings:

The gastric and intestinal metaplasia (Figure 1,2) were the additional histo-pathological findings in this study. Of which, the gastric and intestinal metaplasia were present in 33.0 % and 8.0% of the cases respectively.

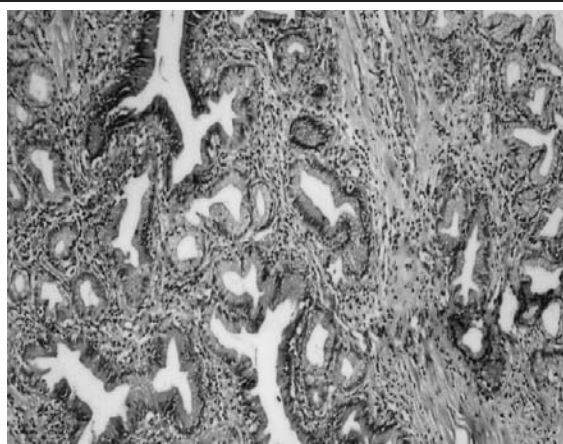


Figure 2. Intestinal metaplasia in chronic cholecystitis (H&E X100).

Likewise, the different special stain regarding the mucin demonstration were performed in this study. It revealed that the neutral mucin, sulfated mucin and sailomucin were positive in 35.0%, 21.0% and 1.0% cases respectively in gastric metaplasia of gallbladder. The overall findings are shown below (Figure 3,4,5 and 6).

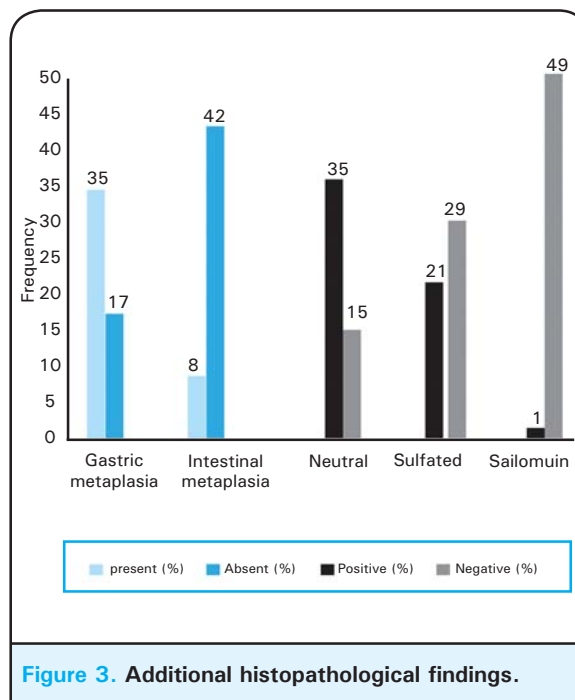


Figure 3. Additional histopathological findings.

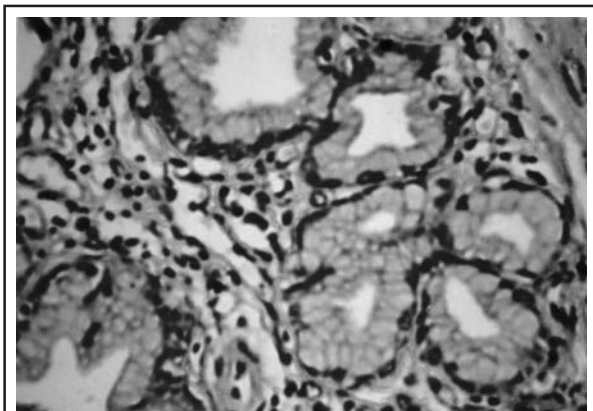


Figure 4. Metaplasia in chronic cholecystitis showing neutral mucin positivity (PAS-stain x 200).

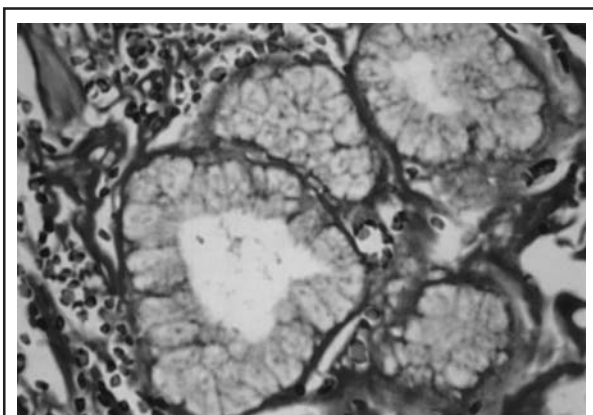


Figure 5. Metaplasia in chronic cholecystitis showing sulfated mucin positivity (Mild Methylation-AlcianBlue stain x 200).

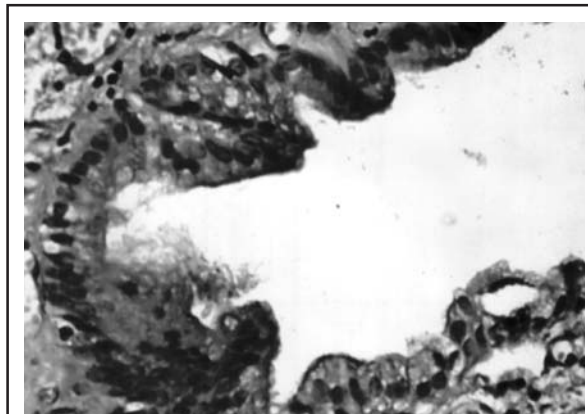


Figure 6. Metaplasia in chronic cholecystitis showing Sialomucin positivity (Mild PAS-Stain x 200).

Among all the cross tabulations between neutral, sulfated and sailo mucins with gastric metaplasia in this study, the sulfated mucin with gastric metaplasia showed a very high significant P-value of 0.001. This was followed by neutral mucin with significant p-value of 0.05 and of sailomucin with non significant p-value of 0.666 (Table 3).

Among all the cross tabulations between neutral, sulfated and sailo mucins with intestinal metaplasia in this study revealed positivity in 34.0%, 21.0% and 1.0% cases respectively. The sulfated mucin with intestinal metaplasia showed a very high significant p-value of 0.006. This was followed by neutral mucin with p-value of 0.19 and of sailomucin with p-value of 0.840. The different categories, its positivity and negativity, p-value and remarks (Table 4).

Table 3. Cross tabulations between different types of mucins and gastric metaplasia in gallbladder.

Categories		Gastric metaplasia		P-value	Remarks
		Negative	Positive		
Neutral mucin	Negative	8	7	0.05	Significant
	Positive	9	26		
Sulfated mucin	Negative	16	13	0.001	Significant
	Positive	1	20		
Sailomucin	Negative	17	32	0.666	Not significant
	Positive	0	1		

Table 4. Crosstabulations between mucins with intestinal metaplasia in gallbladder.

	Categories	Intestinal metaplasia		P-value	Remarks
		Negative	Positive		
Neutral mucin	Negative	15	1	0.194	Not significant
	Positive	27	7		
Sulfated mucin	Negative	28	1	0.006	Significant
	Positive	14	7		
Sialomucin	Negative	41	8	0.840	Not significant
	Positive	1	0		

DISCUSSION

Chronic cholecystitis has various histomorphological spectrum like follicular cholecystitis, xanthogranulomatous cholecystitis, acute on chronic cholecystitis, cholesterosis and porcelain gall bladder. In the present study, the histomorphological spectrum of 50 clinically diagnosed case of chronic cholecystitis revealed the mucosal inflammatory changes in all cases. Among which majority were related to gallstones. Malignancy was not detected in the study.

In a study done at University of Calgary,⁵ Dr S W states that more than 7 lakh cholecystectomies are done in USA. The burden of disease is epidemic among the American Indians (60-70%); a corresponding decrease occurs in Hispanics of mixed Indian origin. 10 – 15% of white adults in developed countries harbour gallstones. Certain risk factors for gallstones are immutable: female gender, increasing age. Others are modifiable like obesity, rapid weight loss, gallbladder stasis. The only established dietary risk is a high caloric intake. Protective factors include diets containing fibre, vegetable protein, nuts plus physical activity.

In this study, female gender predominated the male by more than seven times. And the frequency of disease onset was more among the early forties of age group. Similarly, maximum number of the patients were 70 kg in weight, spending sedentary life style throughout. Also, maximum number of patients in this study had non-vegetarian dietary habit with red meat consumption. So, protective factors among the patients were almost nil in the study.

Tyagi SP et al⁶ studied the morphological changes of gall bladder in 415 cholecystectomy specimens. There was a preponderance of females (male to female ratio - 1: 6.5). The mean age was 43.6 years. Most

of the cases (63.4%) were seen in the 4th and 5th decades of life. Associated cholelithiasis was present in 85.3% cases. Chronic cholecystitis was the main histological diagnosis (50.8%). Other lesions observed were adenomyomatosis (8.2%), adenomatous hyperplasia (10.1%), granulomatous cholecystitis (4.1%), cholesterosis (2.7%), acute cholecystitis (4.1%), acute on chronic infection (10.8%), sub-acute cholecystitis (2.4%) and carcinoma gall bladder (6.8%). In 13 cases (6.2%) the diagnosis of chronic follicular cholecystitis was made. All the cases of cholesterosis were multiparous women of younger age.

In the present study, there was also a female preponderance with male to female ratio of 1:7.16. The mean age of the patients was 34.74 years with the standard deviation of 13.29. Most of the cases (34.0%) were in the 3rd decades of life. The other lesions observed were acute on chronic cholecystitis without cholelithiasis (8.0%), chronic cholecystitis with cholesterosis and without cholelithiasis (22.0%), chronic cholecystitis with cholelithiasis (8.0%) and chronic cholecystitis without cholelithiasis (50.0%), follicular cholecystitis (4.0%), xanthogranulomatous cholecystitis with cholelithiasis (2.0%) and without stones (6.0%). The mean age of the patient with cholesterosis and xanthogranulomatous changes were 36 years and 26 years respectively.

Liew L P et al⁷ in Taiwan declared obesity to be an important risk factor for gallbladder disease. In their study, the prevalence and clinicopathology of gallbladder disease among obese patients was considered. For which prevalence and various clinical factors associated with cholelithiasis were studied in 199 patients. Of the 199 patients, 91% were females and 9% were males, mean age being 34.26±8.41 years. The prevalence

of cholelithiasis was 10.1%. All obese patients in the study presented with variable degrees of chronic mononuclear cell infiltration in the gallbladder mucosa. Cholesterolosis was present in 100 patients (50.3%), followed by gastric metaplasia (27.1%), cholesterol polyp (16.1%) and acute inflammation (9.5%). They concluded that the mucosal mononuclear cell infiltration were common in obese patients, which was related to cholelithiasis.

In the present study, weight of the majority of patient ranged from 60 – 69 kg, with mean of 57.66 kg \pm 10.30 SD. Among the patients, 22.0% had cholesterosis, 4.0% had acute on chronic inflammation, 8.0% had xanthogranulomatous changes, 33.0% had gastric metaplasia and 8.0% had intestinal metaplasia. As in the study above, the present study also showed the majority of mucosal chronic inflammatory cell among high weight group patients.

Dittrick et al⁸ in their study concluded that the obese patients had an increased incidence of benign gallbladder disease. Similarly, the two prospective studies by Csendes et al⁹ suggested that the chronic inflammatory changes could occur prior to the appearance of stones. Morbidly obese patients not only have a high frequency of gallstones, but also have a high proportion of abnormal histologic findings in the gallbladder mucosa.

Katsika D. et al¹⁰ conducted a study with the aim to examine the association between symptomatic gallstone disease and overweight, obesity, alcohol, smoking by analyzing a large twin population. The result showed that the overweight and obesity were associated with a significantly higher risk for symptomatic gallstones disease in the whole study population. High alcohol consumption was associated with a lower risk for gallstones disease in the whole population. Smoking was not correlated with gallstones disease.

In the present study, majority of the patients were non-smoker (88%), occasional smoker (10%) and regular smoker (2%). Likewise, the non-alcoholic group of patients were in maximum number (80.0%) followed by occasional (8.0%) and habitual alcoholic (4.0%).

Among the personal habit of patient in the present study, the life style included two criteria. One being the patient spending healthy life style through different physical activity, whereas the other group spending sedentary life style with lack of any physical activity. The latter constituted 72.0%, followed by the prior about 24.0%. Hence, the group spending sedentary life style without any physical activity had more gallbladder stone disease.

Similar study done by Kriska et al,¹¹ revealed that the physical activity seemed to be significantly and inversely related to the development of gallbladder disease as assessed by ultrasonography in a population at high risk for gallbladder disease. Hence, a causal link between physical activity levels and a decreased risk of gallbladder disease was established.

Pannwitz H et al¹² in their study concluded that the gallstones were infrequently present in nulliparous women. The prevalence of gallstones increased with number of births and with age. Hence, intake of hormonal contraceptives had no or only a marginal influence on prevalence of gallbladder stones.

Out of 43 female patients in the present study, 38 were married females. Of which 55.26% had parity > 2, followed by 44.74% had parity \leq 2. Among them, oral contraceptive pills user were 13.15%, inj. Depo. user were 10.52%. With this statistic, the multiparous women in this study had more gallstone disease.

Similar type of study done by Khan MK et al¹³ in Bangladesh showed that the significant higher incidence of gallstones were found in younger patient taking oral contraceptives, than without contraceptives. The reverse findings were obtained in the older age group patients.

Xanthogranulomatous cholecystitis (XGC) is referred to the presence of diffuse or nodular collections of macrophages containing neutral fat and lipofuscin pigments. They are due to the rupture of Rokitansky–Aschoff sinuses. XGC is an uncommon, benign, destructive inflammatory process of the gallbladder that is believed to be a variant of chronic cholecystitis. It is characterized by xanthogranulomatous inflammation within the gallbladder wall, which often infiltrates the surrounding structures, such as the liver, small and large intestine and omentum.¹⁴

In the present study, xanthogranulomatous cholecystitis without stones were 6% and with cholelithiasis were 2%. The mean age of the patient were 26 years and 27 years respectively.

In a study by Franco V et al¹⁵ the retrospectively estimated frequency of XGC varied from 0.7 to 5.2% in surgically resected gallbladders. There was a female preponderance in XGC and patients tend to present in the sixth and seventh decades of life.^{14,16}

Xanthogranulomatous cholecystitis are always misdiagnosed grossly as malignant due to the appearances and thickness of the wall. Studies have been carried out to see the association or risk of

developing carcinoma in them. In a study by Saxena et al,¹⁷ they observed that xanthogranulomatous cholecystitis was neither associated nor does it increase risk of developing into carcinoma gallbladder.

Carcinoma of gallbladder is a rare but lethal disease. Early diagnosis is difficult due to the non-specific symptoms and lack of awareness.

In the present study, carcinoma gallbladder was not detected. All the cases were of benign nature. Pradhan et al¹⁸ observed the incidence of carcinoma gallbladder to be 2.63%, with M:F of 1:4. Similarly in the study conducted by Shukla V K et al¹⁹ observed 1.2/100000 for males and 0.9/100000 females in South India and 4.5/100000 males and 10.1/100000 females in North India.

In a similar study carried out by Lakhey et al¹ in 2001 in the eastern region of Nepal, reported 11 (1.2%) cases of carcinoma gallbladder, of which 7 (63.63%) were females. They also observed that the majority of the patients

Acute cholecystitis is a rarely encountered variant of cholecystitis. Microscopically, it is characterized by the presence of edema, hyperemia, extravasations of red blood cells and widespread fibroblastic proliferation. Acute on chronic cholecystitis with no stones in this study was seen in 4 cases (8%). The range of age group was from 13 – 65 years, with mean age of 38 years.

Similar study carried out by Shrestha et al²⁰ in Nepal observed 4.7 % of acute cholecystitis whereas Baig et al⁶ in India did not find any case of acute cholecystitis.

In the study by Gupta SC et al,²² 150 surgically resected gallbladder specimens were included to evaluate the relationship between the prevalence of gall stones and histochemical alteration in sequential changes of metaplasia, dysplasia and neoplasia in gallbladder epithelium. Different mucin stains were used. Result revealed that prevalence of gallstones in gallbladders

with metaplastic, dysplastic and neoplastic mucosal changes was significantly higher than those gallbladders which had no epithelial changes. Increase in sialomucin with a corresponding decrease in sulfated mucin was observed from metaplasia to malignancy. Neutral mucin was increased in metaplastic cells but was significantly reduced in neoplastic cells.

Similar type of study done by Ganesh I M et al²³ suggested that the sulfated mucins have a greater role in gallstone formation than the neutral mucins. Also, the sialomucins and sulfomucins play an important role in cancer progression and metastasis.

In the present study, the gastric metaplasia was present in 33.0% of cases and intestinal metaplasia in 8.0%. Maximum positivity among the three mucins (neutral, sulfated and sialo) was of neutral mucins (35.0%), followed by sulfated mucins (21.0%) and sialomucins (1.0%).

The sulfated mucin positivity with both of the gastric and intestinal metaplasia yielded a significant p-value of 0.001 and 0.006 respectively. Similarly, the neutral mucin yielded a significant p-value of 0.05 with gastric metaplasia. The p-value of neutral mucin with intestinal metaplasia was not significant. Also, the p-value of sialomucin with both the gastric and intestinal metaplasia was not significant.

CONCLUSIONS

Chronic cholecystitis is a common disease in surgery. It has a wide histomorphological spectrum. Acute on chronic cholecystitis, chronic cholecystitis with cholesterosis, xanthogranulomatous cholecystitis, follicular cholecystitis are some of them. Most of them are associated with cholelithiasis, at all ages. Also, gastric and intestinal metaplasias are additional histopathological changes in chronic cholecystitis. Involvement of younger age groups was interesting observation in this study.

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