
Histological Changes of Gall Bladder Mucosa: Correlation with Various Types of Cholelithiasis

Muna Zuhair Rana Mumtaz

MBChB, MSc. * MBChB, MSc. **

Khalida Shaya

MBChB, MSc. ***

Abstract:

Background & Objective: The aim of this study is to investigate the histological changes of gall bladder mucosa in relation to different gallstones whether it was cholesterol, pigment or mixed stone.

Patients and Methods: Thirty specimens of gallbladders collected from patients who underwent cholecystectomy, Paraffin sections were stained with haematoxylin and eosin to demonstrate the general histology while Periodic acid-Schiff and Alcian blue were utilized to evaluate the intraepithelial mucin content.

Results: The histological changes were found to be more obvious in gall bladders with cholesterol stones, except the muscular hypertrophy which was more prominent in gallbladder with pigment stones. The mucosal hyperplasia in gall bladders with cholesterol stones was found in 26.6% of cases, Rokitansky-Aschoff sinuses in 13.3% of cases, distended columnar cells were found in 23.3%, mucous gland metaplasia was found in 10% of cases while lymphatic cells infiltration and haemorrhage with congestion both were found in 26.6% of cases. The neutral mucopolysaccharides and sulfate acid mucopolysaccharides were increased in the basement membrane and found to be more prominent in gall bladders with cholesterol stones than in pigment and mixed stones.

Conclusion: Gallstones are accompanied by major changes in the gallbladder epithelium. These changes were clearer in gallbladder mucosa with cholesterol stones may be due to the large size stones leading to more irritation to the mucosa in addition to the toxic effect of the lithogenic bile which produce chemical injury to the mucosa.

Key Words: Gallbladders, Cholesterol stone, Rokitansky-Aschoff sinuses.

Introduction:

Gallstone disease is a common health problem worldwide forming about 95% of the biliary tract disorders. The function of gallbladder is not only to store bile, but also to concentrate it during the interdigestive phase by means of salt-dependent water reabsorption^[1]. There are three main types of gallstones, about 80% are cholesterol stones containing crystalline cholesterol monohydrate; the remainder are either pigment stones composed predominantly of bilirubin calcium salts or mixed stones which are mixture of the components of the both other stones. Cholesterol stones are usually yellow-green while pigment stones are small dark stones made of bilirubin^[2].

Epithelium of the gallbladder and biliary tract is exposed to high concentrations of potentially harmful exogenous and endogenous compounds excreted into the primary bile^[3]. Bile stasis caused by gallbladder dyskinesia is the prime factor for gall stone formation^[4].

All columnar epithelial cells are lined by a blanket of mucus, a native physiological gel-like secretion which plays a regulatory role in cholelithiasis as it promotes the nucleation of stones^[5]. Morphological changes of gallbladder before stone formation in mice revealed an early and rapid increase in mucous production with early focal pseudostratification and large number of degenerative cells thus mucin, calcium and lipids are key factors in gallstone formation^[6]. However, there is little information about the diversity of mucin secretion according to the stone composition^[7].

The most common complications of the presence of gallstones are cholecystitis (inflammation of the gallbladder) whether acute or chronic it may be associated with the appearance of hyperplasia, metaplasia and even carcinoma of the gallbladder^[8,9] those changes in the gallbladder are mainly provoked by the inflammation, chronic trauma and infection.

It has been suggested that the metaplastic epithelium is more susceptible to malignant transformation than the normal mucosa and the intestinal metaplasia-dysplasia carcinoma sequence exists in the gall bladder^[10,11].

In the present study, we examined the morphological changes of the gallbladder mucosa and the relation of those changes to the type of the gallstones.

Materials and Methods:

Gallbladders of 30 patients aged between 27 - 35 years who underwent cholecystectomy whether open or laparoscopic for gallstone with chronic cholecystitis were collected from Al-Jumhuri Teaching Hospital in Mosul between January 2010 to the end of June 2010, the gallbladders were divided into 3 groups depending on the type of gallstones found whether cholesterol, pigment or mixed stones.

The gallbladder specimens were immediately fixed in 10% neutral formalin, after fixation they were opened longitudinally, bisected then a piece of tissue of about (1 cm x 0.5 cm) were taken from the fundus region, the specimens were dehydrated, cleared and embedded in paraffin, sectioned serially

at a thickness of about 4 micrometers and finally stained by Haematoxyline and Eosin stain for studying the general histology and Periodic Acid Schiff's stain and Alcian Blue stain for evaluating the intraepithelial mucin content.

Staining methods and techniques were done on the basis of Druray and Willington (1980)^[12].

Results:

This study showed the presence of many mucosal changes in the wall of the gallbladder, the type and frequency of the histological findings were closely related to the type of the stone whether it was cholesterol, pigment or mixed stones.

I-Histological findings:

1-Disrupted epithelium with mucosal hyperplasia:

The epithelium appeared disrupted with discontinuous, irregular surface and vacuolated cytoplasm (Figs. 1) the cells looked crowded, longer than normal with a pseudostratified arrangement (Fig. 2). This finding was observed in 26.6 % of the gallbladders with cholesterol stone; however this disruption and hyperplastic activity of the epithelium were less obvious in the mucosa of the gallbladders with pigment 6.6% and mixed 13.3% stones (Table 1).

2- Rokitansky- Aschoff Sinuses:

Numerous extensions of the surface epithelium into the thickened gallbladder muscular layer leads to the formation of well developed outpouching of the mucosal epithelium through the wall (Rokitansky- Aschoff sinuses) were found in 13.3% of the gallbladders with cholesterol stone and in 6.6 % of the gallbladders with pigment stone and 13.3% with mixed stone (Fig. 3) (Table 1).

3-Distended Columnar Cells:

Appeared scattered in between the columnar cells of the gallbladder epithelium, they looked pale due to the accumulation of mucous secretion in the apical portion of the cells, those cells were observed mainly in the gallbladders with cholesterol stones 23.3% while in the gallbladders with pigment or mixed stone, this observation was only found in 13.3% and 20 % respectively (Fig.4) (Table 1).

4- Mucous Gland Metaplasia:

Were found in 20 % of the gallbladder specimens having cholesterol stone (Fig. 5, Fig 6) however the metaplastic changes of the epithelium were less obvious in the gallbladders with pigment 6.6 % and mixed 13.3% stones (Table 1), the glands appeared as an oval shaped acini within the lamina propria

lined by pyramidal cells with flat basally located nuclei.

5- Lymphocytic infiltration:

Lymphocytic infiltration of the lamina propria was clearly obvious in most of the specimens with cholesterol stones 26.6%, pigment stone 6.6 % and mixed stone 20 % (Table 1), some of the specimens showed formation of lymphatic nodule in the lamina propria extending to the muscle layer, this finding was observed only in 3.3% of the gall bladder specimens with cholesterol stone (Fig.7).

6- Haemorrhage and Congestion:

Haemorrhage and Congestion of the mucosal blood vessels was a very frequent change in most of the gallbladders with cholesterol 26.6% and mixed 23.3% stones (Table 1), the blood vessels were seen congested filling the lamina propria and extending throughout the mucosa to the surface epithelium (Fig. 8) some of the specimens showed hemorrhage and extravasations of the blood within the lamina propria.

7- Muscular hypertrophy:

Flattening of the mucosal folds with muscular hypertrophy and thickening of the muscle coat was observed mainly in the gallbladders with pigment stone 26.6% the surface epithelium appeared flat with low columnar or cuboidal cells with extension of the muscular layer to the surface epithelial cells (Fig. 9), this change was less obvious in the gall bladders with cholesterol 6.6% and mixed 16.6 % stones (Table 1).

II-Histochemical findings:

a- Neutral Mucopolysaccharides (using PAS stain):

All the sections of the gallbladder mucosa showed a positive reaction to PAS stain but the intensity of the reaction differed according to the type of the stone being strongly positive in the gallbladders with cholesterol stone (Fig. 10), (Table 2) appeared on the brush border and apical portion of the mucosal epithelial cells and the degree of the reactivity gradually decreased towards the nucleus.

B-Sulfate Acid Mucopolysaccharides (using Alcian Blue PH 2.5 stain):

The reaction of the epithelial cells to this stain in the gallbladder specimens with cholesterol stone was strong in the apical portion of the surface epithelial cells (Fig. 11), (Table 2).

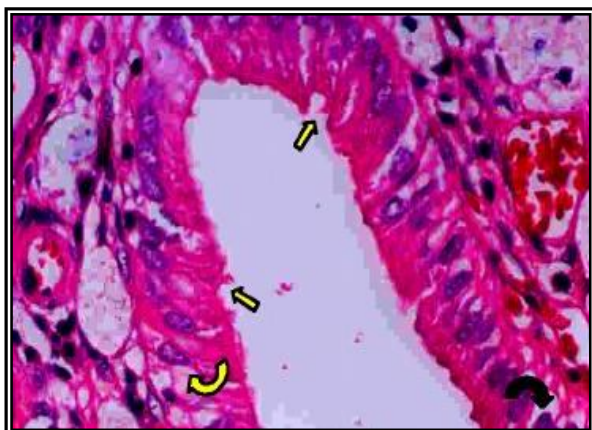


Fig.1: Photomicrograph of gallbladder mucosa with cholesterol stone showing disrupted mucosal epithelium with loss of continuity (arrows), (H&E X400).

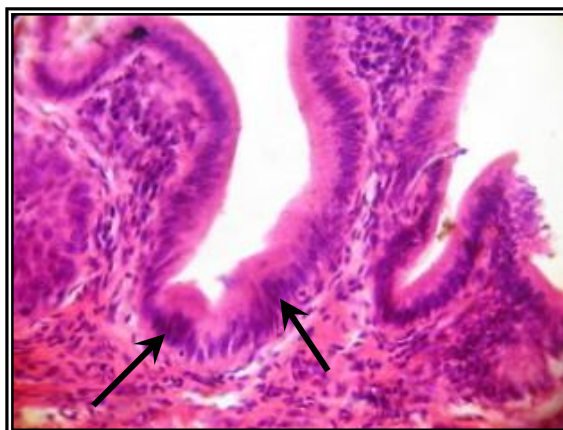


Fig.2: Photomicrograph of gallbladder mucosa with cholesterol stone showing hyperplasia of the epithelial cells (arrows), (H&E X400).

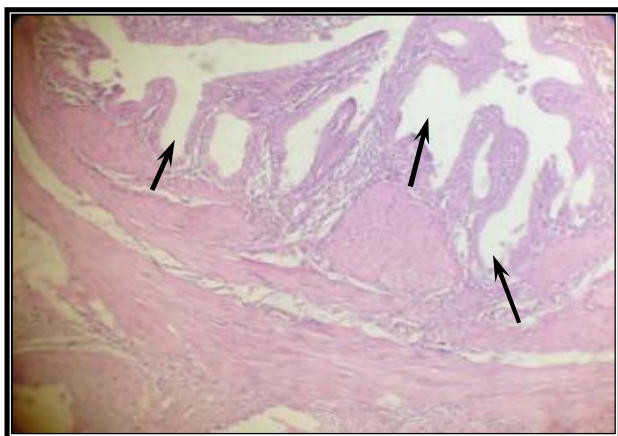


Fig. 3: Photomicrograph of gallbladder mucosa with cholesterol stone showing Rokitansky-Aschoff sinuses (arrows),(H&E X100).

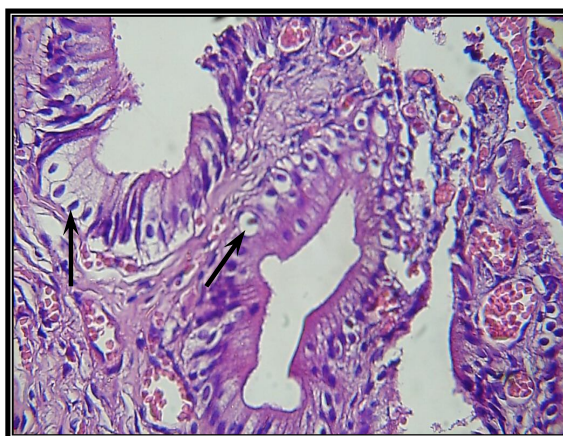


Fig. 4: Photomicrograph of gallbladder mucosa with cholesterol stone showing distended columnar cells with excessive mucoid secretion scattered in between the mucosal epithelial cells (arrows), (H&E X100).

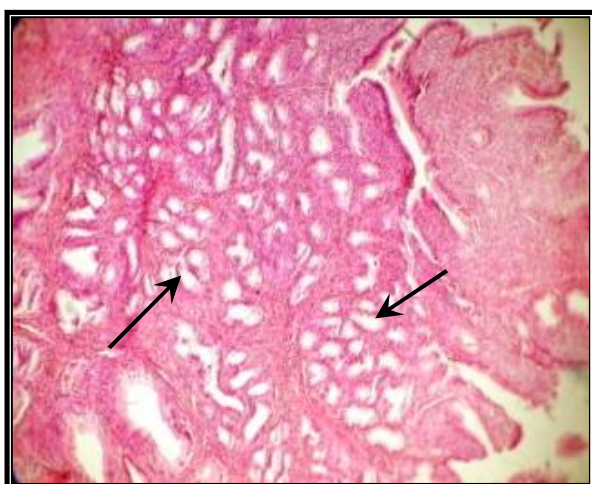


Fig. 5: Photomicrograph of gallbladder mucosa with cholesterol stone showing mucous gland metaplasia (arrows), (H&E X100).

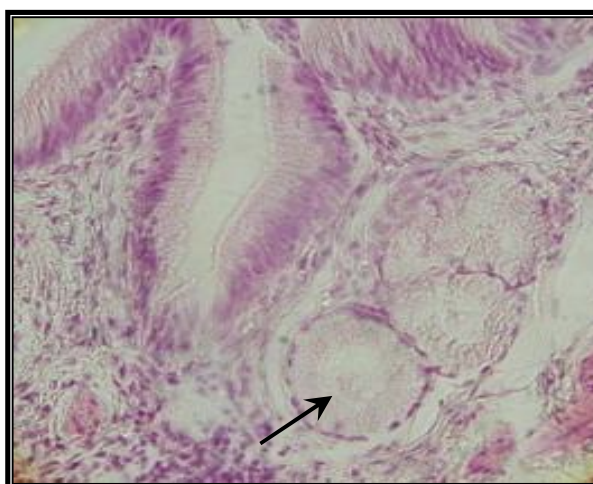


Fig. 6: Photomicrograph of gallbladder mucosa with cholesterol stone showing mucous gland metaplasia in the lamina propria (arrow), (H&E X400).

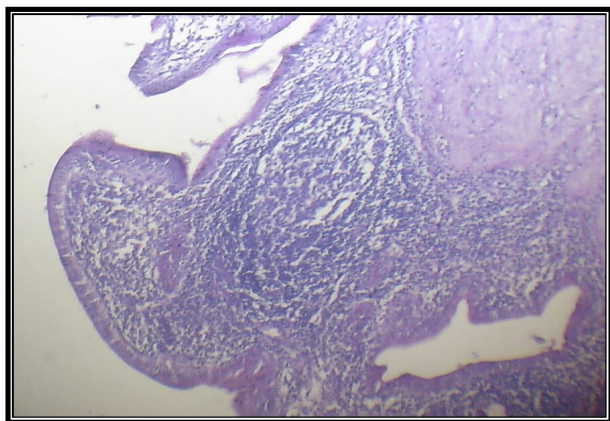


Fig. 7 : Photomicrograph of gallbladder mucosa with cholesterol stone showing lymphocytic infiltration within the lamina propria forming a lymphatic nodule (H&E X100).

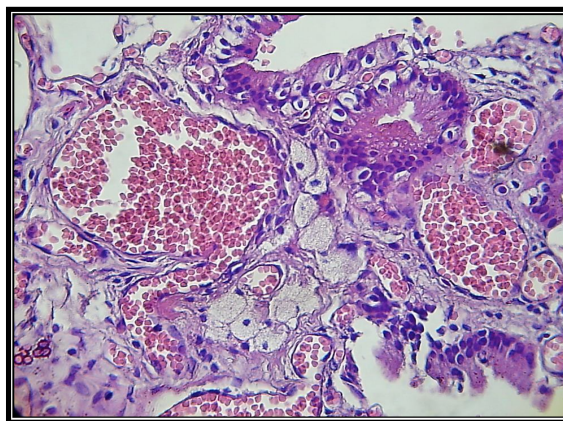


Fig. 8: Photomicrograph of gallbladder mucosa with cholesterol stone showing hemorrhage and congestion of the mucosal blood vessels (H&E X100).

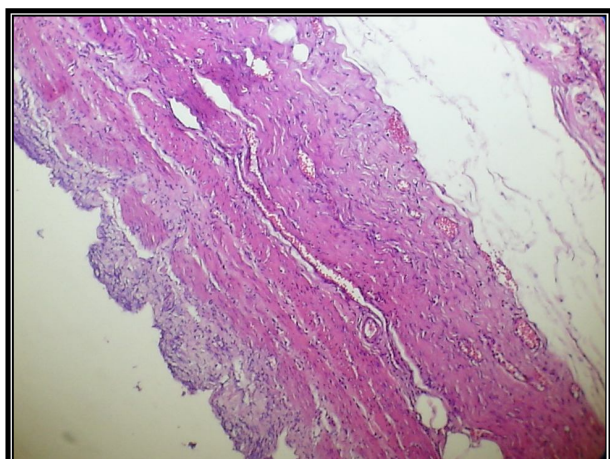


Fig. 9: Photomicrograph of gallbladder mucosa with pigment stone showing flattening of the mucosal folds with muscular hypertrophy extending to the lamina propria (H&E X100).

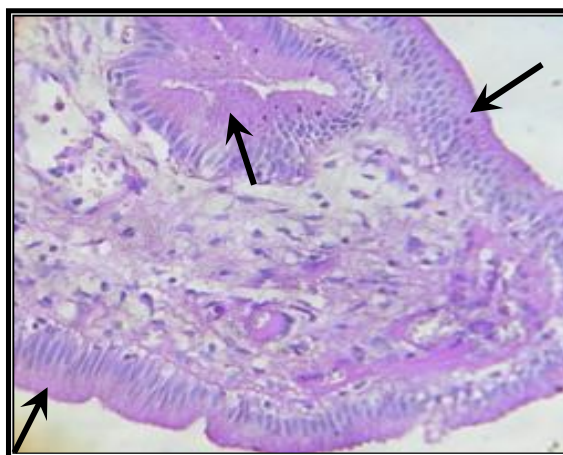


Fig. 10: Photomicrograph of gallbladder mucosa with cholesterol stone showing strong reaction to PAS in the surface epithelial cells (arrows), (PAS X100).

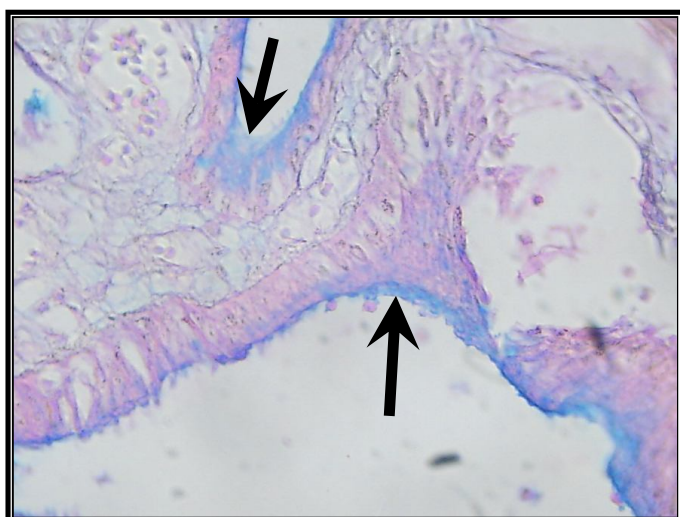


Fig. 11: Photomicrograph of gallbladder mucosa with cholesterol stone showing strong reaction to Alcian Blue stain in the surface epithelial cells (arrows), (Alcian Blue X100)

Table 1: Histological changes of gallbladder mucosa in various types of gallstones.

Histological Changes	Cholesterol stone N=30(%)	Pigment stone N=30(%)	Mixed stone N=30(%)
1. Mucosal Hyperplasia	8 (26.6)	2 (6.6)	4 (13.3)
2 Rokitansky-Aschoff Sinuses	4 (13.3)	2 (6.6)	4 (13.3)
3 Distended Columnar Cells	7 (23.3)	4 (13.3)	6 (20)
4. Mucous Gland Metaplasia	6 (20)	2 (6.6)	4 (13.3)
5.Lymphocytic Infiltration	8 (26.6)	2 (6.6)	6 (20)
6. Haemorrhage & Congestion	8 (26.6)	6 (20)	7 (23.3)
7. Muscular Hypertrophy	2 (6.6)	8 (26.6)	5 (16.6)

Table 2: Histochemical reactions of the gallbladder mucosa in various types of gallstones.

Type of the stone	PAS	Alcian Blue
Cholesterol stone	+++	+++
Pigment stone	++	++
Mixed stone	++	++

Discussion:

Cholelithiasis is the most prevalent disorder of the biliary tract, it produces a series of epithelial pathological changes which might be precursor lesions of gallbladder cancer, these changes include hyperplasia, metaplasia [13,14]. The pathogenesis of all these changes were mainly due to the chronic irritation and chemical injury of the gall bladder mucosa [15] even such changes in the wall of the gall bladder may start before the formation of the stones [16].

Bile stasis secondary to dyskinesia which may be the result of gallbladder pathology is the most widely accepted theory for stone formation [17]. Other studies related stone formation to alteration in the bile composition resulting from oxidative stress and the effect of oxygen free radicals in the gallbladder mucosa, such condition might lead to an altered absorption and concentration of bile with resultant saturation followed eventually by stone formation [18].

In the present work we tried to evaluate the histological changes in the gallbladder mucosa in gallstone patients, and to correlate those changes with the chemical composition of the stone. Many changes were found such as hyperplasia, Rokitansky- Aschoff sinuses, metaplasia, muscular hypertrophy and lymphatic infiltration. The incidence of each change differs according to the type of the stone found.

Regarding the histological findings, epithelial hyperplasia with disruption, irregular surfaces and vacuolated cytoplasm were the most frequent finding in the gall bladder mucosa being more marked in gall bladders with cholesterol stones. This hyperplastic activity is explained to be a reactive process related to mucosal irritation and regeneration [19]. This finding was also reported by others who stated that gall bladder mucosa is exposed to concentrated biliary solutes which influence muscle contraction [20] Putz and Willens (2002) [21] stated that cholelithiasis induces active

proliferation of the epithelium in response to chronic irritation so the surface epithelial cells look more crowded, taller than normal and have a pseudostratified appearance.

Prominent Rokitansky-Achoff sinuses were another important change found mainly in the gallbladders with cholesterol stones. Formation of gall stones is usually associated with inward proliferation of the mucosa due to increase in the intraluminal pressure and weakening of the wall by distension leading to the formation of Rokitansky-Achoff sinuses [22]. In our study formation of Rokitansky-Achoff sinuses was less obvious in mixed stones and relatively rare in gallbladders with pigment stones, the explanation was that the cholesterol might be a more potent stimulus for Rokitansky-Achoff sinuses formation.

Mucin is a high molecular weight glycoprotein plays an important role in protecting gall bladder mucosa from detergent effects of bile [23]. In our study many mucous cells distended with mucinous substance were observed between the columnar epithelial cells of the gallbladder especially those containing cholesterol gallstones. Gall bladder epithelium demonstrates a unique and diverse pattern of mucin core proteins that becomes altered with increasing degrees of inflammation [24].

Chang HG (1999) [25] in his study mentioned that initially the mucous cells may enhance stone formation by mucous hypersecretion then the stone itself will enhance metaplastic changes along with inflammation and physical injury to the epithelium also he observed pseudopyloric mucous glands similar to the pyloric glands of the stomach within the lamina propria of the gallbladder with cholesterol stones and this may be due to the toxic effect the lithogenic bile on the gall bladder mucosa. Intestinal metaplasia found by other workers is regarded as a precancerous lesion in contrast to the pyloric gland metaplasia which is regarded as a benign condition [26].

Lymphocytic infiltration is a common finding in the gallbladders with the three types of stones and more common with cholesterol stones is regarded as a part of the inflammatory process induced by the stones associated with the hemorrhage and congestion of the mucosal blood vessels, those changes were also reported by many other studies [27].

All the above histological changes were clearer in gallbladders having cholesterol stones due to their larger size leading to more irritation to the gall bladder mucosa, together with the toxic effect of the lithogenic bile which produces chemical injury to the mucosa where as pigment stones are smaller and produce less chronic reaction. The only important with pigment stones was the absence of the mucosal folds with flattening of the surface epithelium and thickening of the gall bladder wall. This was also observed by Baig SJ et.al. (2002) [22]

Concerning the histochemical, this study revealed that the reaction of the epithelial cells to the Periodic Acid-Schiff and Alcian Blue stains were generally stronger in the gallbladders with cholesterol stones than in the other types of stones. The neutral and the acid sulfated mucopolysaccharides were found to be concentrated in the apical part of the epithelial cells, this might be due to the effect of lithogenic bile also could be due to the larger size of cholesterol stones producing more injury to the epithelial cells. Those results were in agreement with many other studies done in this field [28].

Conclusion:

We conclude that gallstones are accompanied by major changes in the gallbladder epithelium. These changes were clearer in gallbladder mucosa with cholesterol stones may be due to the large size stones leading to more irritation to the mucosa in addition to the toxic effect of the lithogenic bile which produce chemical injury to the mucosa.

References:

1. Meyer G, Guizzardi F, Rodighiero S, Manfredi R, Saino S, Sironi C. Ion transport across the gallbladder epithelium. *Curr Drug Targets Immune Endocr Metabolic Disorders* 2005; 5:143-151.
2. Vinay K., Ramzis C. and Stanley R. The biliary tract. In: Robbins Basic Pathology, 7th edition. Saunders Company 2003, 628-633.
3. Aust S, Obrist P, Jaeger W, Klimpfinger M, Tucek G, Wrba F, et al. Thalhammer T. Subcellular localization of the ABCG2 transporter in normal and malignant human gallbladder epithelium. *Lab Invest* 2004; 84:1024-1036.
4. Velanovich VF. Biliary dyskinesia and biliary crystals: a prospective study. *Am Surg* 2000; 63:69-73?

5. Apstein MD. And Carey MC. Cholesterol gallstones: Aparsimonious hypothesis. *Eur J Clin Invest* 1999; 26: 343.
6. Kim HJ, Kim JS, Kim KO, Park KH, Yim HJ, Kim JY. Expression of MUC3, MUC5AC, MUC6 and epidermal growth factor receptor in gallbladder epithelium according to gallstone composition. *Korean J Gastroenterology* 2003 42:330-336.
7. Yamagiwa H, Tomiyama H, Intestinal metaplasia-dysplasia-carcinoma sequence of the gallbladder. *Acta Pathol Jpn* 1986; 36:989-997.
8. Albores-saavedra J, Nadji M, Henson DE, Ziegels-weissman J, Mones JbM. Intestinal metaplasia of the gallbladder: a morphologic and immunohistochemical study. *Hum Pathol* 1996; 17:614-620.
9. Duarte I, Llanos O, Domke H, Harz C, Valdivieso V. Metaplasia and precursor lesions of gallbladder carcinoma. Frequency, distribution, and probability of detection in routine histological samples. *Cancer* 1993; 72:1878-1884.
10. Dowling GP, Kelly JK, The histogenesis of adenocarcinoma of the gallbladder. *Cancer* 1986; 58:1702-1708.
11. Tazuma S. and Kajiyama G.). Carcinogenesis of malignant lesions of the gallbladder. The impact of chronic inflammation and gallstones. *Langenbecks Arch Surg.* 2001; 386(3): 224-229.
12. Drury R.B. and Wallington E.A). Carleton's Histological Technique. 5th edition, Oxford University Press, UK. 1980; PP: 49,137,140.
13. Khanna R, Chansuria R, Kumar M, Shukla HS. Histological changes in gall bladder due to stone disease. *Indian Journal of Surgery* 2006;68(4):201-204.
14. Elfving E, Silvonen E, Teir H, Mucosal hyperplasia of the gallbladder in cases of cholelithiasis. *Acta Chir Scan* 1999; 135:519-522.
15. Rubin E. and Farber J. The gallbladder and extra hepatic bile ducts: In Pathology. 3rd edition. Lippincott-Williams & Wilkins. Philadelphia 1999; 830-838.
16. Chang HJ, Suh JI, Kwon SY. Gallstone formation and gallbladder mucosal changes in mice fed a lithogenic diet. *J Korean Med Sci* 1999; 14:286-292.
17. Zaki M, Al-Refeidi A. Histological changes in the human gall bladder epithelium associated with gall stone. *OMJ* 2009 ;24:269-273.
18. Geetha A. Evidence foe oxidative stress in gallbladder mucosa of gallstone patients. *J Biochem Mol Biol Biophys.* 2002 Dec; 6(6):427-432.
19. Pingitore R, Bonucci M, Bigini D, Ciancia EM, Vignati S. Chronic calculus cholecystitis: elementary and dysplastic lesions, echographic

- correlations and immunohistochemical approach. *Pathologica* 1992; 84:171-185.
20. Portincasa P, Di Ciaula A, VanBerge-Henegouwen GP. Smooth muscle function and dysfunction in gallbladder disease. *Curr Gastroenterol Rep* 2004; 6:151-62.
 21. Putz P and Willems G. Cell proliferation in the human gallbladder epithelium: effect of distension. *Gut* 2002; 20:246-248.
 22. Baig SJ, Biswas S, Das S, Basu K, Chattopadhyay G. Histopathological changes in gallbladder mucosa in cholelithiasis: correlation with chemical composition of gallstones. *Trop Gastroenterol* 2002;23(1):25-27.
 23. Vilkin A, Nudelman I, Morgenstern S, Geller A, Bar Dayan Y, Levi Z, Rodionov G, et al. Gallbladder inflammation is associated with increase in mucin expression and pigmented stone formation. *Dig Dis Sci* 2007; 52:1613-20.
 24. Lee SP. Hypersecretion of mucous glycoprotein by the gallbladder epithelium in experimental cholelithiasis. *J Pathology* 2008;134:199-207.
 25. Albores-saavedra J, Henson DE. Pyloric gland metaplasia with perineural invasion of the gallbladder: A lesion that can be confused with adenocarcinoma. *Cancer* 1999;1586(12):2625-2631.
 26. Fernan des JEF, France MIF, Suzuki RK, Tavares NM, Bromberg SH Intestinal metaplasia in gall bladders: Prevalance study. *Sao Paulo Medical Journal* 2008; 126(4):131-151.
 27. Zaki M, Al-Refeidi A. Histological changes in the human gall bladder epithelium associated with gall stone. *OMJ* 2009; 24:269-273.
 28. Hakkinen I. and Laitio M.. Epithelial glycoprotein of human gallbladder. *Ach Path.* 1970;37-142.

* Department of Anatomy, College of Medicine, University of Mosul, Mosul.

** Department of Anatomy, College of Medicine, University of Mosul, Mosul.

*** Department of Anatomy, College of Medicine, Al-Mustansiriya University, Baghdad.