

Ultrastructural Changes In Human Gall Bladder Epithelium In Cholelithiasis

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الخلاصة

تكوين الحصوات الصفراوية يعرف على انه وجود حصى ضمن تجويف اكياس المرارة او في الشجيرات الصفراوية الخارج كبدية. الهدف من هذه الدراسة هو التعرف على التغييرات النسيجية الدقيقة في النسيج الطلائي لاكياس المرارة في حالة تكوين الحصوات الصفراوية.

نماذج اكياس المرارة جمعت من مرضى اجريت لهم عملية استئصال اكياس المرارة. النماذج الدقيقة حضرت و عوملت لدراسة التركيب النسيجي الدقيق للنسيج الطلائي لاكياس المرارة.

التغييرات النسيجية الظاهرة في المقاطع الشبه رقيقة لصبغة المثيل الازرق اظهرت وجود تمزقات مع عدم تماسك في النسيج الطلائي للمرارة مترافقا مع ظهور تجايف روكتانسكي اشوف مع وجود تضخم وعدم انتظام التناسق في الخلايا الطلائية مع حؤول في الغدد المخاطية في الصفيحة الرقيقة لاكياس المرارة. فحص التركيب النسيجي الدقيق اظهر وجود سحق في الزغيبات الدقيقة مع تحطم لبيوت الطاقة و توسع في الفراغات بين الخلوية متزامنا مع ظهور الفجوات في داخل الخلايا الطلائية مع وجود عدم انتظام و انفقاق في محيط الخلايا. الخلايا الطلائية احتوت على قطيرات مخاطية، شوهد بعضها مفرزا الى خارج الخلية.

حصى المرارة يرافقها تغييرات نسيجية اساسية في الانسجة الطلائية كما ظهرت في المجهرين الضوئي و الالكتروني.

مفتاح الكلمات: اكياس المرارة، تكوين الحصوات الصفراوية، التغييرات النسيجية الدقيقة.

Abstract

Cholelithiasis is defined as the presence of stones within the lumen of the gall bladder or in the extrahepatic biliary tree. The goal of this study was to identify the ultrastructural ultrations of gall bladder epithelium in cholelithiasis.

Gallbladder specimens were collected from patients who underwent cholectstectomy. Minute specimens were also fixed and processed to evaluate the fine structures of the gall bladder epithelium.

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The histological changes in semithin sections of methylene blue stain showed disruption of gall bladder epithelium and discontinuity of this epithelium with appearance of Rokitansky-Aschoff sinuses, there is hyperplasia of epithelial cells, all these changes associated with mucous gland metaplasia in the lamina propria of cholecystitic gall bladder. At the ultrastructural level, abraded and altered microvilli accompanied by mitochondrial damages, dilatation of intercellular spaces was revealed by thin-section electron microscopy associated with intracellular vacuoles and irregularity and herniation of cell outlines. The epithelial cells contain mucous droplet, some of these droplets shedded to the exterior of the cells.

Gallstones are accompanied by major changes in the gallbladder epithelium, as shown by both light and electron microscopy.

Key words: gall bladder, cholelithiasis, ultrastructural changes.

Introduction

Gallstone disease is a common health problem worldwide.⁽¹⁾ It is commonly believed that bile stasis is the prime factor for gallstone formation. The function of the gallbladder is not only to store bile, but also to concentrate it during the interdigestive phase by means of salt-dependent water reabsorption.⁽²⁾ Gallstones are common, affecting about one fourth of women and 10% to 15% of men over the age of 50⁽³⁾.

Documented risk factors for gallstone disease include age, sex, obesity, rapid weight loss, high dietary intake of fat, multiple deliveries and congenital hemolytic anemias, and some medications^(4,5,6).

Epithelium of the gallbladder and biliary tract is exposed to high concentrations of potentially harmful exogenous and endogenous compounds excreted into primary bile.⁽⁷⁾ All columnar epithelial cells are lined by a blanket of mucus, a native physiological gel-like secretion which separates the host mucosal cells from the external milieu.⁽⁸⁾ The gallbladder mucus plays a regulatory role in cholelithiasis as it promotes the nucleation of stones.⁽⁹⁾ Mucus, calcium and lipids act in concert to form the gallstones.⁽¹⁰⁾

Gallbladder mucin is one of the key factors in gallstone formation. However, there is little information about the diversity of mucin secretion according to the stone composition.⁽¹¹⁾ A major causative agent for stasis is gallbladder dyskinesia which in turn may be a consequence of gallbladder wall pathology.⁽¹²⁾ However, it was observed that gallbladder tension increased, rather than decreased during the early stage of gallstone formation.⁽¹³⁾

Many studies tried to identify risk factors of biliary lithiasis, in the west have focused on hypersaturation of cholesterol in bile in the nucleation process, a critical step in the genesis of bile stones⁽¹⁴⁾.

Cholelithiasis produces diverse histopathological changes in gallbladder mucosa namely acute inflammation, chronic inflammation, glandular hyperplasia, granulomatous inflammation, cholesterosis, dysplasia, and carcinoma.⁽¹⁵⁾

Aims of the study

This study aims to identify the frequencies of different histological changes of the gallbladder mucosa in a group of individuals who underwent cholecystectomy and have gallstones, using electron microscope.

Methods

Gallbladders of six female patients aged between 27 - 35 years who underwent cholecystectomy for gallstone disease with chronic cholecystitis with multiple stones were obtained. Each gallbladder was sectioned serially from the neck to the fundus. The sections were opened and carefully washed with 0.15 N saline, cut into 2 mm sections and fixed with 2.5% gluteraldehyde diluted by 1% phosphate buffer at pH 7.4 for electron microscopy.

For transmission electron microscopy, one millimeter cube-thick gluteraldehyde-fixed tissue specimen were post fixed with 1% osmium tetroxide, dehydrated in ascending concentrations of ethanol, cleared in propylene oxide, and embedded in araldite. Semithin sections were prepared using ultramicrotome (Reichert, Jung) and stained with 1% methylene blue then examined with a

light microscope. Ultrathin sections were cut in an average thickness of 60 - 90 nanometer by ultra microtome (Reichert, Jung) also, handled on a grid were stained with uranyl acetate and lead citrate, and examined with (Philips CM 10) electron microscope.⁽¹⁶⁾

Results

On light microscopy, the epithelium appeared disrupted with discontinuous, irregular surface associated with Rokitansky-Aschoff sinuses (fig 1). Hyperplasia of epithelial cells also observed (fig. 2), and the changes in lamina propria accomplished by the appearance of mucous gland metaplasia (fig. 3).

On electron microscopy, columnar epithelial cells that contain apical mucous secretory granules and bulging apices were seen (fig. 4) and these were actively secreted into the lumen of the gallbladder. The contents of the mucous granules were released by exocytosis to the exterior of the cell (fig. 5). However, occasionally cells which had their intracellular mucous granules released extracellularly, could be seen with the contents expanded to acquire the form of a spherical mass. Vacuolation of the cytoplasm of these columnar epithelial cells also were observed (fig. 4,5,6,7). In some specimens, cholecystocyte changes were also seen. These are characterized by abraded, altered and spared microvilli with herniation of epithelial cells (fig. 5,6) accompanied by mitochondrial damages in its cristae (fig. 7). Dilatation of intercellular spaces was also observed in these specimens (fig. 5).

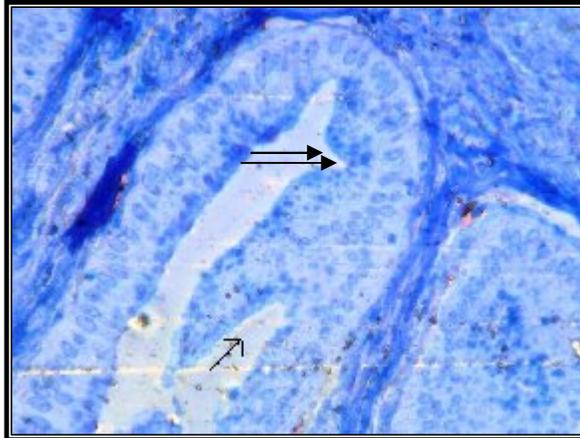


Figure 1: A photomicrograph of a section in a cholecystitic human gallbladder showing disrupted epithelium (double arrows) with Rokitansky-Aschoff sinuses (arrow) with discontinuous epithelium. (methylene blue X 400)

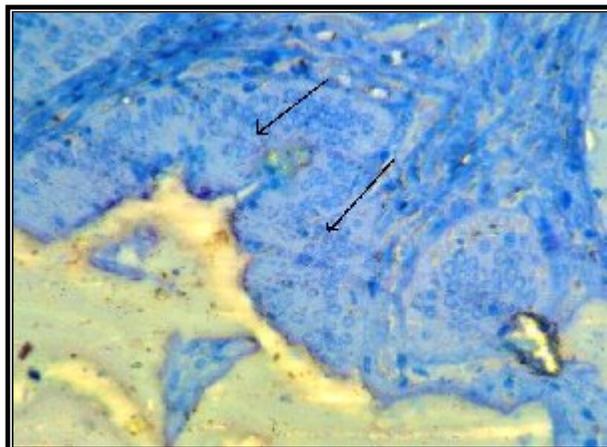


Figure 2: A photomicrograph of a section in the cholecystitic human gallbladder showing a disrupted epithelium and hyperplasia of the epithelial cells (arrows) with irregular surface of its epithelium. (methylene blue X400)

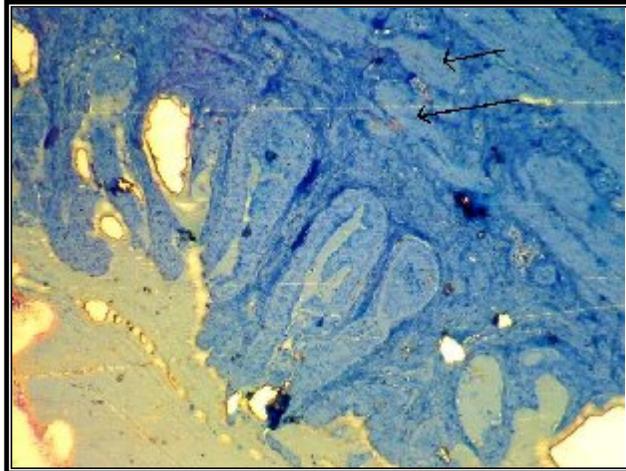


Figure 3: A photomicrograph of a section in the cholecystitic human gallbladder showing a disrupted epithelium and Rokitansky-Aschoff sinuses with mucous gland metaplasia in the lamina propria (arrows). (methylene blue X100)

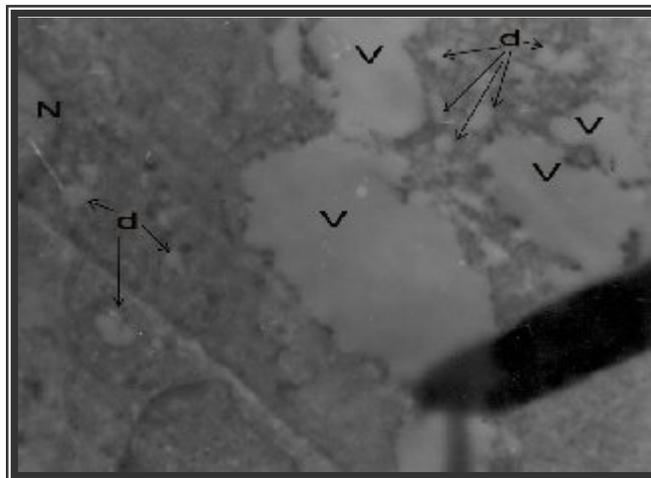


Figure 4: Electron micrograph of human gall bladder with cholangitis showing mucous droplets of epithelial cells (d), nucleus (N), intracellular vacuoles (V). X 8700.

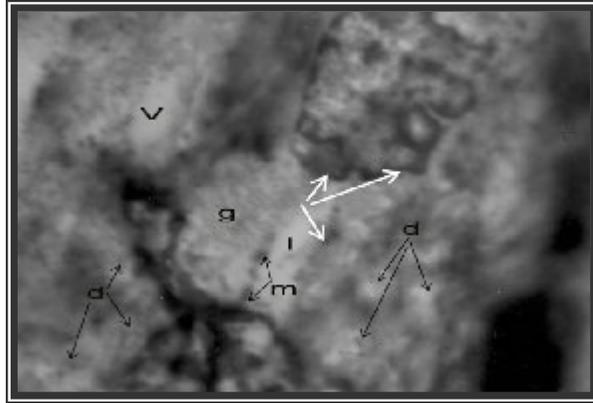


Figure 5: Electron micrograph of human gall bladder with cholysistitis showing the epithelial cells with dilated intercellular spaces (I), irregularity and herniation of cell outlines (white arrows), vacuoles (V), mucous granules shedding to the exterior (g), shedding of some microvilli (m), mucous droplets (d). X 8700.

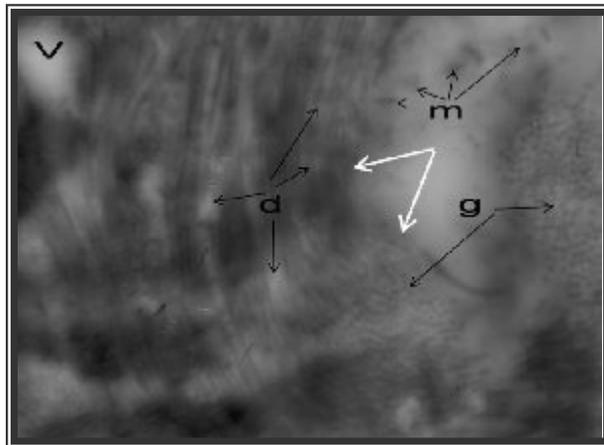


Figure 6: Electron micrograph of human gall bladder with cholysistitis showing mucous granules released to the exterior of the cell (g), shedding of some microvilli (m), vacuoles of cytoplasm (V), mucous droplets in the apex of cell (d), irregular cell outlines (white arrows). X 16000.



Figure 7: Electron micrograph of human gall bladder with cholecystitis showing huge and dilated mitochondria (M), intracellular vacuolation (V). X 8700.

Discussion

Gallstone formation results from many complex factors working together. Among them, the bile stasis caused by impaired gallbladder emptying is thought to be the fundamental kinetic factor.⁽¹⁷⁾

The pathologic factors related to gallstone formation are still the hot debate. Bile stasis secondary to gallbladder dyskinesia, is the most widely accepted theory. Gallbladder dyskinesia may be the result of gallbladder wall pathology.⁽¹²⁾ This view is supported by the finding that sphincterectomy of ampula of Vater may prevent the formation of gallbladder stone and partially improve the contractility of gallbladder.^(18,19)

In the present study, light microscopical examination of sections of the cholecystitic gallbladder, showed disrupted epithelium with discontinuous and irregular surface. The gallbladder epithelium and smooth muscle layer were exposed to concentrated biliary solutes, including cholesterol and potentially toxic hydrophobic bile salts, which are able to influence muscle contraction.⁽²⁰⁾ Also, Rokitansky-Aschoff sinuses that are the result of hyperplasia and herniation of epithelial lining of the inflamed gallbladders has been well documented.⁽²¹⁾

Formation of gall stones is usually associated with increase in the intraluminal pressure and weakening of the wall by distension which might cause inward proliferation of the mucosa to the wall of the gallbladder leading to the formation of Rokitansky-Achoff sinuses.^(1,22) Hyperplasia was a common finding in the gall bladder mucosa with stones. This hyperplastic activity of the mucosa is a reactive process which is related to mucosal irritation and regeneration. This change was also reported by many studies^(22,23). Putz and Willens (1978),⁽²⁴⁾ stated that cholelithiasis induces active proliferation of the epithelium in response to chronic irritation. So the surface epithelial cells looks more crowded, taller than normal and have a pseudostratified appearance^(22,23,24).

Other form of changes found in this study is the occurrence of mucous glands metaplasia within the lamina propria of the gallbladder which is known as pseudopyloric glands (because those glands are similar to the pyloric glands of the stomach) may be due to the toxic effect of lithogenic bile on the gall bladder mucosa. This change was also found by other workers^(22,25,26). Pyloric gland metaplasia of the gallbladder should be added to the long list of benign epithelial proliferation that is associated with gall stones⁽²²⁾, other type of metaplasia (intestinal metaplasia) could not be found in our study, this type of metaplasia is regarded by many workers as a precancerous lesion in the gallbladder mucosa^(22,25,27) in contrast to the pyloric gland metaplasia which is regarded as a benign condition.

The surface irregularity was due to the interruption of the brush border. Sulfomucins have a greater role in gallstone formation than the neutral mucins and also that the sialomucins and sulfomucins play an important role in cancer progression and metastasis. The results challenge the glycobiologists to delve deeper in elucidating the role of mucins in gastric malignancy and in gallstone formation.⁽²⁸⁾

Mucin is a high molecular weight glycoprotein that plays an important role in protecting the gallbladder epithelium from the detergent effect of bile.¹⁸ Human gallbladder mucin has been implicated to play a role in gallstone disease.^(29,30)

Moreover, it was observed that despite the diverse mechanisms of stone induction and the differences in stone composition, there is a quantitative increase in the epithelial mucus production in the period before stone formation.

Transmission electron microscopy studies of the cholecystitic gallbladder showed columnar epithelial cells that contain apical mucous secretory granules and bulging apices and these were actively secreted into the lumen of the gallbladder. The contents of the mucous granules were released by exocytosis to the exterior of the cell. However, occasionally cells which had their intracellular mucous granules released extracellularly could be seen with the contents expanded to acquire the form of a spherical mass. A mechanism of mucous granule exocytosis by columnar epithelial cells must take into account the unique physical-chemical properties of mucin glycoproteins and the resultant mucus.

Mucins are expressed in a cell and tissue-specific pattern in normal tissue.⁽³¹⁾ Alterations of the expression pattern of mucins have been described in the formation of gallstones and the expression of neutral mucins was predominant stone-containing gallbladder epithelium.⁽³²⁾ In addition, numerous mucous droplets in the apical portion of all the epithelial cells were apparent which confirm that the gallbladder epithelium may play an important role in regulating the stone formation.⁽³³⁾ Ultrastructural study of a group of selected specimen in the current study revealed cholecystocyte changes characterized by abraded, altered and spared microvilli and herniation of epithelial cells. Mitochondrial damage in the cristae, dilatation of intercellular spaces was also observed in these specimens. In one study, abraded and altered microvilli accompanied by mitochondrial damage in the apical regions of the inflamed gallbladders were seen. These principle ultrastructural changes in the human biliary epithelium is occurred due to the alteration of these epithelial cells from being primarily absorptive epithelium to a predominantly secretory epithelium.^(34,35)

Conclusion

The observations from this study indicate a relationship between pathologic changes of gallbladder epithelium and gallstone formation, and a possible pathway in the pathogenesis of gallstone formation. Overall, the pathological changes of the gallbladder epithelium may play an important role in the process of gallstone formation.

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