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Histopathological Study of Gallbladder Associated with Gallstone Disease.

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Abstract

Gallstones are a medical condition that is found all over the world. However, the rates of occurrence vary significantly depending on the geographical location. The present study aims to examine the histopathological changes in the mucosa of the gall bladder in patients with cholelithiasis. The study involved 81 patients who were treated with a gallbladder removal operation due to cholecystitis. The patients were divided into groups according to their symptoms. The histological study involved fixing gallbladder samples in 10% formalin, dehydrating them in ethanol, clearing them in xylol, and embedding them with paraffin wax. Sections were cut, stained, and viewed under a light microscope. The result of this study reveals variations in gallstone disease distribution concerning symptoms. patients without symptoms represent (23.45%), Patients with mild symptoms and without complications represent (35.80%), patients with severe symptoms without complications represent (12.35%), and patients with severe symptoms and complications represent (28.40%). The following abnormalities in gallbladder tissue are presented: Some pouching of mucosal epithelium were found, which extended from the surface epithelium into the deep muscle layer of the gallbladder. Disruption of the epithelium, irregular surfaces, and vacuolated cytoplasm were found in the gallbladder epithelium. The tissue section showed infiltration of some inflammatory cells, lymphocytes, and plasma cells, and some types of macrophages of the gallbladder mucosa with chronic inflammatory cells associated with epithelial hyperplasia, bleeding in blood vessels was frequently occurring. The study concluded that pathological alterations in the gallbladder epithelium may contribute to gallstone development.

1. Introduction:

Gallstone disease is a significant medical issue globally, with varying incidence rates across different regions. Modern civilization has linked dietary and lifestyle factors to cholelithiasis, or the formation of gallstones [1]. According to reports, gallbladder cholelithiasis has been associated with specific risk factors, and gallstones are solid masses of varying sizes that form within the biliary canal [2]. In many instances, gallstones remain asymptomatic. Only around 10% of people diagnosed with gallstones symptoms will appear in approximately 5 years, with about 20% of individuals suffering symptoms within 20 years of their diagnosis [3].

In cholelithiasis, the gallbladder mucus regulates the condition by promoting the formation of stones. Gallstones are created by a combination of mucus, calcium, and lipids [4]. The gallbladder has three layers according to histology: the mucosa, the muscularis externa, and the adventitia or serosa [5]. Gallstones typically develop inside the biliary tract or gallbladder and can be symptomatic or asymptomatic. Gallstone

disease is regarded as a gallstone with symptoms or problems [6]. Around 80% of people with gallstones are asymptomatic; they do not know about the condition. Abdominal pain, fever, and jaundice, as well as aberrant leukocytosis, are common clinical symptoms and indicators [7]. There is no submucosa or muscular mucosa in it. Due to the presence of microvilli, the mucosa consists of a single layer of columnar epithelial cells featuring a brush border. The lamina propria, which is located beneath the epithelium, contains numerous elastic fibers that settle down. A mucous membrane's epithelium can absorb water and other materials from bile, forming a bubble pouch. Loose connective tissue, blood vessels, and some scattered lymphatic tissue are present in the underlying lamina propria [8].

The second layer is the smooth muscle layer, arranged in a circular, longitudinal, and oblique manner. The layer contains numerous elastic and collagen fibers, so-called fibromuscular layer. The function of this layer is the contraction and expulsion of bile during stimulation. The third layer is the serosa, or adventitial, and comprises thick, elastic fibers in the shape of networks in dense, fibrous connective tissue and adipose tissue Elastic fibers, massive blood and lymphatic arteries, and autonomic nerves are all present [8]. Most gallstones have rough surfaces that lead to ongoing irritation of the gallbladder mucosa. The prolonged presence of gallstones finally produces a series of histopathological changes in the gallbladder [9]. The aim was to find tissue changes in gallbladder of cholelithiasis patients.

2. Subjects and Methods:

The study involved 81 female patients who underwent cholecystectomy for symptomatic cholecystitis caused by cholelithiasis, their ages were between 20 to 60 years. According to the protocol for histological study 10% formalin were used for fixing some of the gallbladder samples; favorite parts were obtained by histopathologist; and ascending concentrations of ethanol (70%, 80%, 90% and 100%) were used for dehydration, cleared in xylol, followed by infiltration and then paraffin wax used for embedding. 4 micron thick paraffin sections were cut using rotary microtome tool and stained by hematoxylin and eosin stains, light microscope was used to show the sections under the power of (X 10 and X 40) [9]. The study was carried out in GMC Hospital in kalar-Sulaymaniyah - Kurdistan region -Iraq. The research was conducted between January 2020 and June 2021.

3. Results:

The result of this study in Table 1 revealed variations of gallstone disease distribution in relationship to symptoms. (23.45%) without symptoms while the rest are symptomatic which is categorized into: With mild symptoms and without complications represent (35.80%), patients with severe symptoms without complications represent (12.35%) and patients with severe symptoms and complications represent (28.40%).

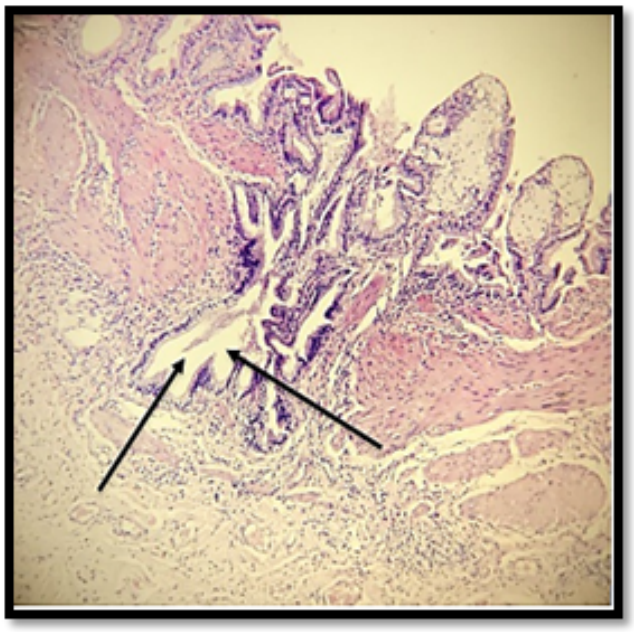

Figure 1. Image of gallbladder epithelium showing Rokitansky-Aschoff sinuses (X10).

Ten percent of gallbladder samples were obtained and underwent histological study, the following abnormalities are presented. Some outpouching of mucosal epithelium was found which extended from the surface epithelium into the deep muscle layer creating (Rokitansky- Aschoff sinuses) of the gallbladder Figure 1. Gallbladder epithelium showed disruption of epithelium, irregular surface and vacuolated cytoplasm Figure 2.

The tissue section showed infiltration of some inflammatory cells, lymphocytes, plasma cells, and some types of macrophage of the gallbladder mucosa with chronic inflammatory cells Figure 3. In the majority of gallbladders, bleeding and mucosal blood vessel congestion were frequently occurring. Congested blood vessels could be seen entering the lamina propria and reaching the surface epithelium of the mucosa Figure 4. In the muscular area, there is smooth muscle fibrosis and an inflammatory cell infiltration Figure 5 and Figure 6.

4. Discussion:

The histopathological characteristics and occurrence of gallbladder lesions differ based on race, country, and medical institutions. It is widely recognized that gallbladder diseases are more prevalent in women than in men. Cholelithiasis leads to

Table 1. The classification of patients based on their symptoms.

Gallstone Symptom	without symptoms	With mild symptoms and without complications	with severe symptoms without complications	with severe symptoms and complications	Total
No.	19	29	10	23	81
%	23.45	35.80	12.35	28.40	100

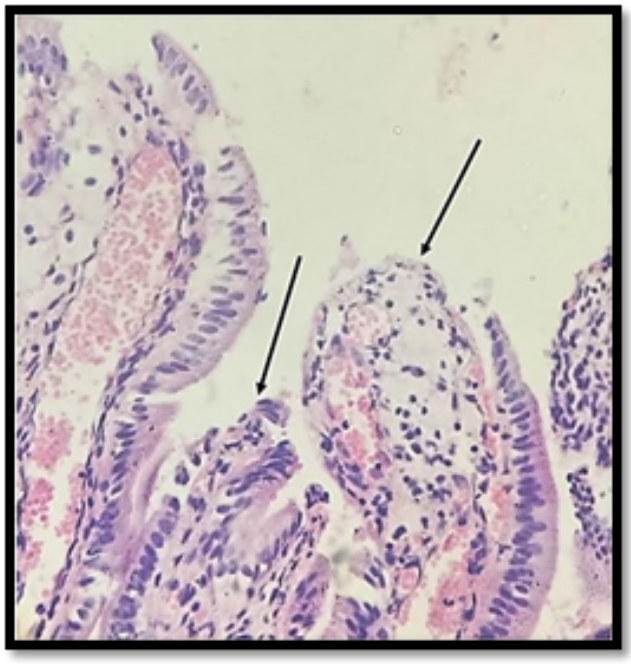

Figure 2. Gallbladder epithelium showed disruption of epithelium, irregular surface and vacuolated cytoplasm (X40).

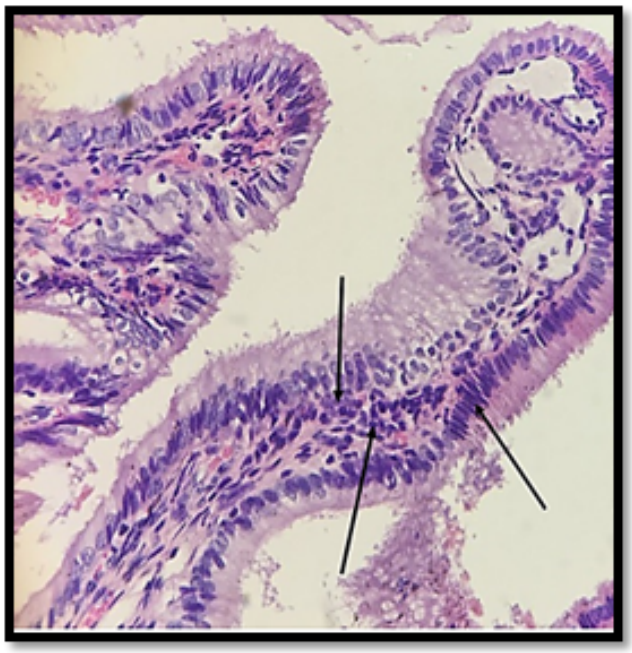

Figure 3. The tissue section shows infiltration of the gall bladder mucosa with chronic inflammatory cells, (X40).

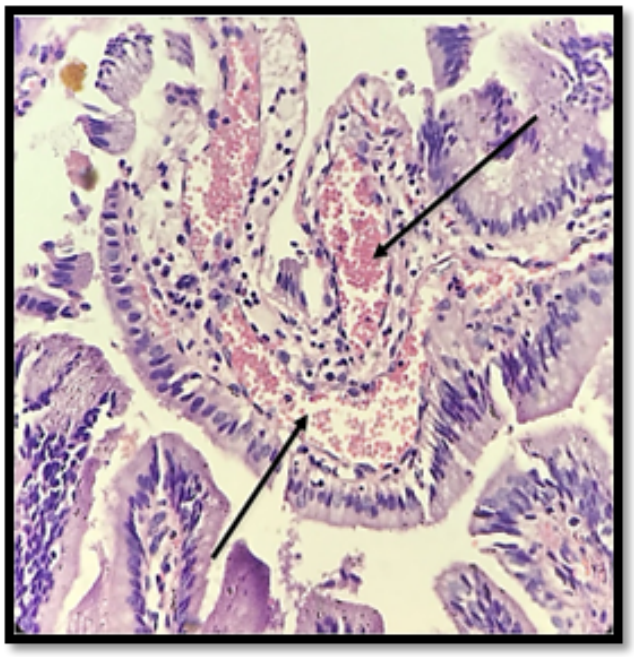

Figure 4. Image of hemorrhage of the mucosal blood vessels of gallbladder (X40).

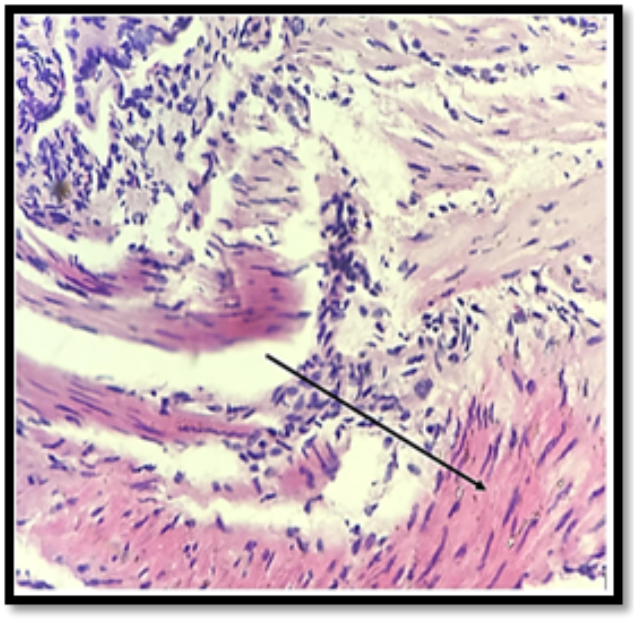

Figure 5. Image of fibrosis in gallbladder muscle (X40).

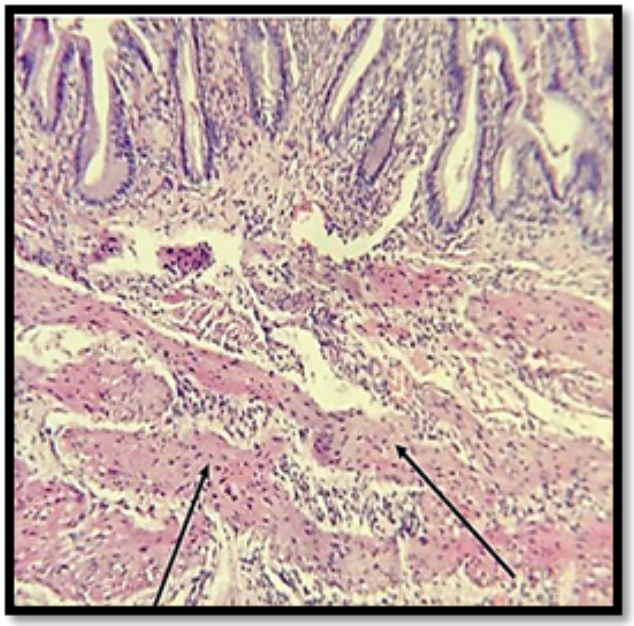

Figure 6. Image of inflammatory infiltrate cell in gallbladder muscle (X10).

diverse histopathological changes in the gallbladder mucosa, primarily including acute inflammation, hyperplasia, granulomatous inflammation, cholesterosis, dysplastic alterations, and carcinoma [10].

When gallstones are first forming, they are typically asymptomatic; however, when the size of the boulders exceeds 8 mm, symptoms start to show. By obstructing the flow of bile or digestive enzymes, gallstones can result in consequences that include inflammation, nausea, vomiting, and sharp, sudden pain in the top right abdomen [7]. Acute cholecystitis, acute cholangitis, and biliary pancreatitis are just a few of the dangerous and even fatal consequences that can result from symptomatic gallstone disease [11], [12]. Asymptomatic gallstone disease also called ”silent stones” are those gallstones that can remain without management. Most frequently, patients with symptomatic stones complain of right upper quadrant or epigastric pain regularly. This is likely due to a stone being stuck in the cystic duct [1] and they suffer from disturbance on the upper right side, mostly associated with nausea and vomiting, which may last and become continuous from thirty minutes to same hours [13].

In the current study, the important alteration in the gallbladder mucosa was shown as prominent Rokitansky-Achoff sinuses with stones. Similar findings have been reported by some studies [14], [15]. Rokitansky-Aschoff sinuses, also known as crypts, are diverticula of the gallbladder wall formed by the hyperplasia and herniation of epithelial cells through the fibromuscular layer. These invaginations can vary in size,

being either microscopic or macroscopic. They penetrate the smooth muscle gaps of the gallbladder [16].

In the current study examination of gallbladder sections was done using a light microscope, presenting epithelium that has been disturbed, displaying an uneven, discontinuous surface and vacuolated cytoplasm. The findings align with another study [17]. This could be attributed to the larger stones causing increased irritation to the mucosal lining of the gallbladder. Concentrated biliary substances, including cholesterol and potentially detrimental hydrophobic bile salts that may influence muscle contraction, coming into contact with the gallbladder epithelium and smooth muscle layer may lead to disruption of the gall bladder [18].

Another finding in this study is lymphatic infiltration into the gallbladder tissue. Similar studies found that the most frequently observed histopathological alteration in the gallbladder mucosa linked to cholelithiasis is chronic cholecystitis [19], [20].

In the current study, other investigations have noted the involvement of alterations in the mucosal blood vessel and hemorrhage, which documented previously [21]. This may be due to irritation of the stone with the tissue.

Fibrosis of muscle in this study was another feature and the results were in line with other studies that have also reported fibrosis of the gallbladder muscle [22], [23]. The gall bladder mucosa alters as the stone's weight, volume, and size rise.

Gallstones were associated with significant alterations in the epithelium of the gallbladder. These alterations are more pronounced in the mucosa of gallbladders containing cholesterol stones, likely because the larger stones cause increased irritation to the mucosal lining. Additionally, chronic inflammatory changes in the gallbladder mucosa can develop even before visible stones become apparent. Early identification of these microscopic changes can enhance clinical insights and the management of related health issues [24].

5. Conclusion:

All things considered, gallstone production may be significantly influenced by the pathological alterations of the gallbladder epithelium.

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Data Availability Statement: All of the data supporting the findings of the presented study are available from corresponding author on request.

Declarations:

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: The manuscript has not been published or submitted to another journal, nor is it under review.

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الدراسة النسيجة المرضية للمرارة المصاحبة لحصاة المرارة

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الخلاصة

حصى المرارة هي حالة مرضية موجودة في جميع أنحاء العالم. ومع ذلك ، فإن معدلات الاصابة تختلف اختلافا كبيرا بحسب الموقع الجغرافي. الهدف من هذه الدراسة هو معرفة التغيرات النسيجية المرضية في الغشاء المخاطي للمرارة للمرضى الذين يعانون من حصى المرارة. شملت الدراسة 81 مريضا اجريت لهم عملية إزالة المرارة بسبب التهابها. تم تقسيم المرضى على مجموعات وفقا لأعراضهم. تضمنت الدراسة النسيجية تثبيت عينات المرارة بنسبة (10%) ، وتجفيفها بالإيثانول، وتنظيفها بالزيلول، وتضمينها بشمع البارافين. تم تحضير المقاطع وصبغها وعرضها تحت المجهر الضوئي. كشفت نتائج هذه الدراسة عن اختلافات في انتشار مرض الحصوة فيما يتعلق بالأعراض. يمثل المرضى الذين لا يعانون من أعراض ومضاعفات (23.45%) ، والمرضى الذين يعانون من أعراض خفيفة وبدون مضاعفات (35.80%) ، والمرضى الذين يعانون من أعراض شديدة دون مضاعفات (12.35%) ، والمرضى الذين يعانون من أعراض ومضاعفات حادة (28.40%) ، بعض من الاضطرابات الأتية تم مشاهدتها ثم العثور على بعض الأكياس من ظهارة الغشاء المخاطي ، والتي امتدت من ظهارة السطح إلى طبقة العضلات العميقة في المرارة. العثور على اضطراب في الظهارة ، وعدم انتظام في الأسطح ، والسيتوبلازم فجوي في ظهارة المرارة. أظهرت نتائج فحص الأنسجة وجود بعض الخلايا الالتهابية والخلايا الليمفاوية وخلايا البلازما ، وبعض أنواع البلاعم في الغشاء المخاطي للمرارة مع الخلايا الالتهابية المزمنة ، المرتبطة بتضخم الظهارة. النزيف الأوعية الدموية المخاطية قد يحدث بشكل متكرر. وفي الخلاصة أن التغيرات المرضية في ظهارة المرارة قد تسهم في تطور حصوات المرارة.

الكلمات الدالة : حصى المرارة، المرارة، الخلايا الالتهابية.

التمويل: لايوجد.

بيان توفر البيانات: جميع البيانات الداعمة لنتائج الدراسة المقدمة يمكن طلبها من المؤلف المسؤول.

اقرارات:

تضارب المصالح: يقر المؤلفون أنه ليس لديهم تضارب في المصالح.

الموافقة الأخلاقية: لم يتم نشر المخطوطة أو تقديمها لمجلة أخرى، كما أنها ليست قيد المراجعة.