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Morphological criteria of gallbladder destruction in patients with acute cholecystitis, which occurred against the background of COVID-2019

The aim of the work: to study the morphological features of the gallbladder of acute cholecystitis, which occurred against the background of COVID-2019.

Materials and Methods . Material for histological studies was collected from the proximal and middle third of the gallbladder, as well as from the fundus. The obtained fragments of the gallbladder wall after fixation in a 10 % solution of neutral buffered formalin were processed in the LogosOne histoprocessor and embedded in paraffin blocks. Tissue sections with a thickness of 5 μm obtained on an AMR400 rotary microtome were stained with hematoxylin and eosin. The obtained histological preparations were examined using a Nikon Eclipse Ci-E light microscope. A Sigeta M3CMOS14000 digital video camera with Toup View software was used for photo documentation. With the help of this program, morphometry of tissue structures was also carried out. All histological preparations were divided into 2 groups – patients with acute cholecystitis and those with acute cholecystitis against the background of COVID-19.

Results and Discussion. During the morphological examination of the gallbladder of patients in the cholecystitis + COVID-19 group, it was established that the thickness of the mucous membrane exceeded the similar indicator of the first group by 19.4 %, the differences regarding the muscular and serous membranes were insignificant – an increase of 2.2 % and 1.7 %. The height of covering epitheliocytes regressed by 21.8 %, and the relative volume of affected cells also increased significantly – by 26.0 %. The share of blood vessels increased by 14.4 % in the mucosa and by 9.8 % in the muscle. The described dynamics of morphometric parameters are relevant to the changes detected by microscopy. Thus, the thickening of the mucous membrane under the conditions of a combination of cholecystitis and COVID-2019 can be explained by more pronounced phenomena of edema and hyperemia of the mucous membrane. A decrease in the height of epitheliocytes and an increase in the proportion of damaged cells indicate more intense destructive changes in the epithelium. An increase in the proportion of blood vessels in different parts of the gallbladder wall does not indicate an improvement in its vascularization, but is instead a morphometric marker of the severity of hemodynamic disorders (dilatation and engorgement of vessels, thrombosis). In older people, there was a thinning of the muscle membrane by 11.6 %. Such a structural rearrangement, one should think, has a negative effect on the contractile function of the gallbladder, reduces the force of its tension and contraction. Based on the study of histological preparations, it can be stated that hemodynamic disorders and, in particular, thrombosis play an important role in the pathogenesis of acute cholecystitis, which occurs against the background of COVID-2019.

Key words: COVID-2019; gallbladder; acute cholecystitis.

Background. In developed countries, 10–15 % of the adult population is affected by gallstones. According to the third National Health and Nutrition Examination Survey, 6.3 million men and 14.2 million women aged 20 to 74 in the United States had gallbladder disease [1–6]. In Europe, the Multicenter Italian Study on Cholelithiasis (MICOL) examined nearly 33.000 subjects aged 30 to 69 years in 18 cohorts of 10 Italian regions. The overall incidence of gallstone disease was 18.8 % in women and 9.5 % in men. However, the prevalence of gallstone disease varies significantly between ethnicities. Biliary colic occurs in 1 to 4 % annually [1]. Acute calculus cholecystitis occurs in 10 to 20 % of untreated patients.

The aim of the work. To study the morphological features of the gallbladder of acute cholecystitis, which occurs against the background of COVID-2019.

Materials and Methods. Material for histological studies was collected from the proximal and middle third of the gallbladder, as well as from the fundus. The obtained fragments of the gallbladder wall after fixation in a 10 % solution of neutral buffered formalin were processed in the LogosOne histoprocessor and embedded in paraffin blocks. Tissue sections with a thickness of 5 μm obtained on an AMR400 rotary microtome were stained with hematoxylin and eosin [1]. The obtained histological preparations were examined using a Nikon Eclipse Ci-E light microscope. A Sigeta M3CMOS14000 digital video camera with Toup View software was used for photo documentation. With the help of this program, morphometry of tissue structures was also carried out. All histological preparations were divided into 2 groups – patients with acute cholecystitis and those with acute cholecystitis against the background of COVID-19.

Results and Discussion. During the histological examination of the wall of the gallbladder in both studied groups, the picture of acute phlegmonous cholecystitis prevailed. At the same time, a pronounced leukocyte infiltration of a diffuse nature was observed, which covered the mucous membrane with spread to the submucosa and muscle membrane (Fig. 1).

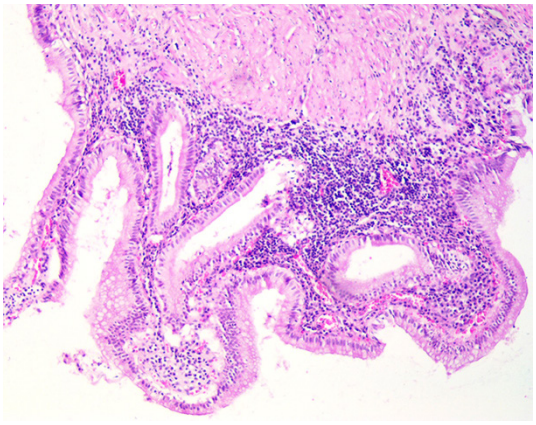

Fig. 1. Diffuse leukocyte infiltration of the mucous membrane of the gallbladder, inflammatory hyperemia. Study group 1. Staining with hematoxylin and eosin, x200.

Destructive changes were also observed in the form of desquamation of the mucous membrane epithelium with the formation of erosions, the formation of microabscesses in the submucosal layer (Fig. 2).

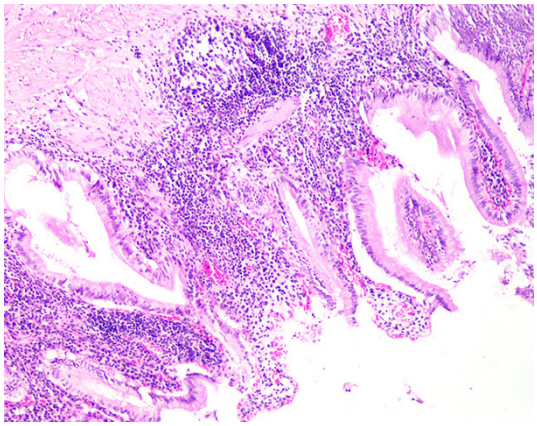

Fig. 2. Diffuse leukocyte infiltration of the mucous membrane of the gallbladder, desquamation of the epithelium, microabscesses. Study group 1. Staining with hematoxylin and eosin, x200.

In the second studied group, against the background of the acute inflammation described above, pronounced hemodynamic disorders occurred: hemorrhaging of vessels, hemorrhages, stasis phenomena in the vessels of the microcirculatory channel (Fig. 3). These dyscirculatory disorders had a diffuse nature and were visualized in all areas of the mucous membrane, as well as in deeper structures of the gallbladder wall. At the same time, in some places, the hemorrhages acquired a torrential character, forming extensive centers of hemorrhagic infiltration (Fig. 4).

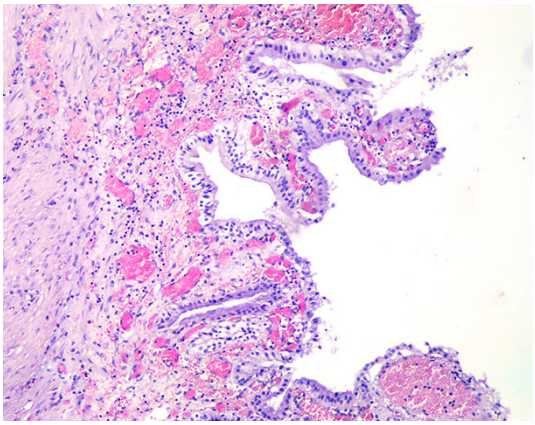

Fig. 3. Pronounced hyperemia, hemorrhages, phenomena of stasis in the vessels of the microcirculatory channel of the mucous and muscular membrane of the gallbladder, signs of acute inflammation. Study group 2. Staining with hematoxylin and eosin, x200.

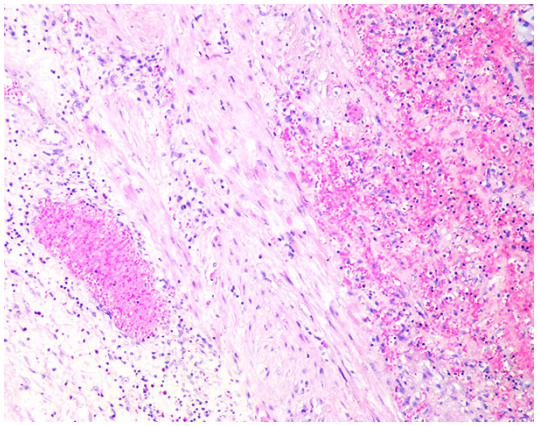

Fig. 4. A center of hemorrhagic infiltration of the muscular membrane of the gallbladder, vessel thrombosis, signs of acute inflammation. Study group 2. Staining with hematoxylin and eosin, x200.

Another element of the complex of pathological changes in patients with cholecystitis against the background of COVID-2019 was vascular thrombosis, which occurred in most observations. This process had a disseminated nature and was fixed both in the mucous membrane and in the muscle membrane (Fig. 5).

Also, in both studied groups, the manifestations of adenomyomatosis were observed in some places – focal proliferation of the mucous membrane epithelium with intussusception into the hypertrophied muscle membrane and the formation of pseudoglandular structures – Rokitansky-Ashoff sinuses (Fig. 6). Inflammatory infiltration was also noted perifocally around them.

According to the results of morphometry, the thickness of the mucous, muscular and serous mem-

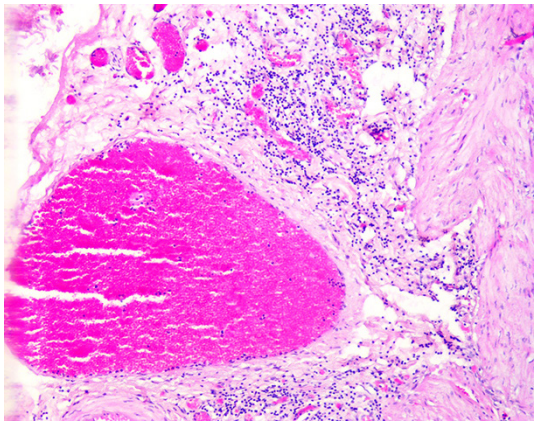

Fig. 5. Thrombosis of a vein of medium caliber and small vessels, sharply expressed perivasal leukocyte infiltration. Study group 2. Staining with hematoxylin and eosin, x200.

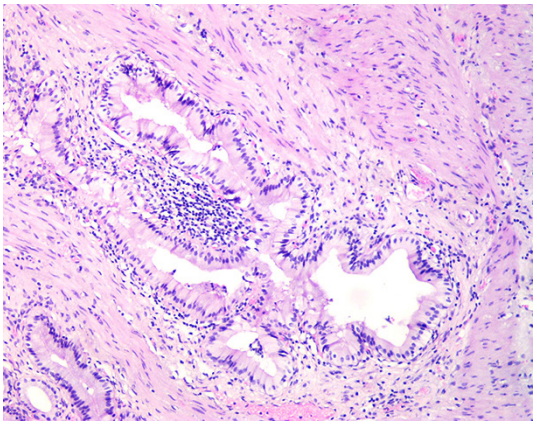

Fig. 6. Sinus of Rokitansky-Ashoff in the muscular membrane of the gallbladder. Study group 2. Staining with hematoxylin and eosin, x200.

branes of the gallbladder, the height of the covering epithelial cells of the mucous membrane, the relative volume of epithelial cells with signs of alteration, and the relative volume of blood vessels per unit area of the mucous and muscular membranes were evaluated.

Comparing the obtained data, it was determined that the thickness of the mucous membrane in the second studied group exceeded the similar indicator of the first group by 19.4 %, the differences in the muscular and serous membranes were insignificant – an increase of 2.2 % and 1.7 %. The height of covering epitheliocytes in the second group regressed by 21.8 %, and the relative volume of affected cells also increased significantly – by 26.0 %. The share of blood vessels increased by 14.4 % in the mucosa and by 9.8 % in the muscle. The described dynamics of morphometric parameters are relevant to the changes detected by microscopy. Thus, the thickening of the mucous membrane under the conditions of a combination of cholecystitis and COVID-2019 can be explained by more pronounced phenomena of edema and hyperemia of the mucous membrane.

A decrease in the height of epitheliocytes and an increase in the proportion of damaged cells indicates more intense destructive changes in the epithelium in the second group. The increase in the proportion of blood vessels in different parts of the gallbladder wall in the second studied group does not indicate an improvement in its vascularization, but is instead a morphometric marker of the severity of hemodynamic disorders (dilatation and engorgement of vessels, thrombosis), which is fully consistent with the histological picture depicted above. Within the second studied group (combination of cholecystitis and COVID-2019), a comparison of morphometric indicators of patients of young (up to 59 years, 4 cases) and older (more than 60 years, 8 cases) age groups was also performed. At the same time, the following differences were revealed. In older people, there was a thinning of the muscle membrane by 11.6 %. Such a structural rearrangement, one should think, has a negative effect on the contractile function of the gallbladder, reduces the force of its tension and contraction. Similar data on age-related aspects of gallbladder wall remodeling have been published by other researchers. The height of covering epitheliocytes in the older age subgroup regressed by 10.2 % relative to the indicators of younger age, in parallel, the relative volume of altered epitheliocytes increased by 17.2 %.

Conclusion. Based on the study of histological preparations, it can be stated that hemodynamic disorders and, in particular, thrombosis play an important role in the pathogenesis of acute cholecystitis, which occurs against the background of COVID-2019. The latter is probably a local reflection of the systemic imbalance of hemostasis inherent in the coronavirus disease. Thrombosis should be considered as a significant factor in the disruption of the blood supply of the mucous membrane of the gastrointestinal tract, which in turn leads to a greater severity of its alterative changes under the conditions of the association of cholecystitis and COVID-2019. The latter is also confirmed by the results of a morphometric study, in particular, a 26.0 % increase in the share of damaged epitheliocytes. In addition, morphometric data indicate more pronounced alterative changes in the epithelium of the mucous membrane of the gallbladder in patients after 60 years, as well as about the age-related remodeling of the structural components of the organ wall.

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МОРФОЛОГІЧНІ КРИТЕРІЇ ДЕСТРУКЦІЇ ЖОВЧНОГО МІХУРА У ХВОРИХ НА ГОСТРИЙ ХОЛЕЦИСТИТ, ЩО ВИНИК НА ТЛІ COVID-2019

Мета роботи: вивчити морфологічні особливості жовчного міхура при гострому холециститі, що виник на тлі COVID-2019.
Матеріали і методи. Забір матеріалу для гістологічних досліджень здійснювали із проксимальної та середньої третини жовчного міхура, а також із ділянки дна. Отримані фрагменти стінки міхура після фіксації в 10 % розчині нейтрального забуференого формаліну обробляли в гістопроцесорі LogosOne та заливали в парафінові блоки. Тканинні зрізи завтовшки 5 мкм, отримані на роторному мікротомі AMR400, забарвлювали гематоксиліном і еозином. Отримані гістологічні препарати досліджували за допомогою світлового мікроскопа Nikon Eclipse Ci-E. Для фотодокументування використовували цифрову відеокамеру Sigeta M3CMOS14000 з програмним забезпеченням Toup View. За допомогою цієї програми також здійснювали морфометрію тканинних структур. Усі гістологічні препарати були поділені на 2 групи – пацієнти, що перехворіли на гострий холецистит, та ті, що хворіли на гострий холецистит на тлі COVID-19.
Результати досліджень та їх обговорення. При морфологічному дослідженні жовчного міхура хворих у групі холецистит + COVID-19 встановлено, що товщина слизової оболонки перевищувала аналогічний показник першої групи на 19,4 %, відмінності щодо м'язової та серозної оболонок були несуттєвими – збільшення на 2,2 % та 1,7 %. Висота покривних епітеліоцитів регресувала на 21,8 %, також істотно – на 26,0 % – зростав відносний об'єм уражених клітин. Частка кровоносних судин зростала на 14,4 % у слизовій оболонці та на 9,8 % у м'язовій. Описана динаміка морфометричних параметрів релевантна змінам, виявленим при мікроскопії. Так, потовщення слизової оболонки за умов поєднання холециститу та COVID-2019 можна пояснити більш вираженими явищами набряку та гіперемії слизової оболонки. Зменшення висоти епітеліоцитів та зростання частки пошкоджених клітин вказує на інтенсивніші деструктивні зміни епітелію. Зростання частки кровоносних судин у різних відділах стінки жовчного міхура не свідчить про покращення її васкуляризації, натомість є морфометричним маркером виразності гемодинамічних розладів (дилатація та повнокрів'я судин, тромбоз). У осіб старшого віку мало місце стоншення на 11,6 % м'язової оболонки. Така структурна перебудова, слід думати, негативно впливає на скоротливу функцію жовчного міхура, зменшує силу його напруження та скорочення.
На підставі дослідження гістологічних препаратів можна стверджувати, що в патогенезі гострого холециститу, який перебігає на тлі COVID-2019, важливу роль відіграють гемодинамічні розлади і, зокрема, тромбоз.

Ключові слова: COVID-2019; жовчний міхур; гострий холецистит.