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Destructive Cholecystitis: Peculiarities of Gallbladder Structure on the Light and Scanning Electron Microscopy

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Abstract The investigation results of gallbladder on the light and scanning microscope at calculous cholecystitis were presented in the article. The light microscopy revealed that the rate of changes in the gallbladder wall varied not only subject to the inflammation type but subject to anatomic part of organ (bottom, body, neck). Investigation by scanning microscope helped to specify and to demonstrate the results of light optic research visually.

Keywords Cholelithiasis, Calculous cholecystitis, Electron microscopy

1. Introduction

The issue of modern diagnostics and treatment of cholelithiasis, its numerous and severe complications still is not finally solved and attracts attention of the wide circle of the researchers and practitioners [1-16].

A large quantity of researches is devoted to the issues of pathogenesis, diagnostics and treatments of the biliary tracts diseases. Classical investigations were finished at the beginning of the 20th century. But this issue still remains actual as well.

The conducted analysis of available literature has shown that numerous works of both domestic [1-4, 6, 10, 14, 16] and foreign [5, 7, 9, 11, 13, 15] authors are devoted to the study of cholelithiasis. Those investigations were mainly devoted to the study of pathogenesis and classification of cholelithiasis, to working-out of new methods for conservative and surgical treatment of this pathology.

Pathologic anatomy of the cholelithiasis is closely connected with formation and growth of gallstone, its migration, obstruction of bile ducts lumen and the development of these phenomena outcome: atrophy and flatness of mucous membrane, sclerosis of subjacent layers, certain atrophy of gallbladder. Such changes can be coursed without inflammation, but in majority of cases the cholelithiasis is developed and it is connected with bile properties abnormality, edema of mucous membrane, occlusion of lymphatic and venous vessels promoting the development of inflammatory process. Pathomorphology at acute lesion of the gallbladder and bile ducts, and it has wide range of changes. They can be destructive and nondestructive [21-22].

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Subject to clinical manifestations and pathomorphologic changes in the gallbladder, independently from the etiology, they differentiate acute and chronic cholecystitis. This division is conditional, because there are interjacent forms of the disease – chronic recurrent cholecystitis. Severity rate of acute cholecystitis can be different – from catarrhal inflammation to the gallbladder gangrene [23].

The issues of morphologic changes estimation in gallbladder at different rate of bile-excreting tracts obstruction still remain unsolved. While studying recent decades literature we did not find fundamental works devoted to the morphologic and morphometric features of gallbladder at cholelithiasis. There are also no studies devoted to the comparison of the results of light optic and electron scanning investigations of the gallbladder.

2. Materials and Methods

The research was carried out on bioptic material of the Republican Research Centre of Emergency Medicine. We investigated gallbladders of 357 patients undergone surgeries subject to destructive cholecystitis (DCh). The gallbladders taken at autopsy of the patients died from the pathology which was not related to hepatobiliary system (n=45) were investigated as a control group (CG).

The shards of gallbladder tissue from bottom, body and neck, 1x1 cm in size were cut for microscopic investigation. Material was fixed in 10% neutral formaldehyde solution and conducted through alcohol of increasing concentration and was poured into paraffin by the method of Lloyd Z. et al. [17]. Sections (4-5 μm thick) were stained by hematoxylin and eosin. At performing light optic microscopy there was detected a heterogeneity of the gallbladder wall changes and 2 groups of observations were separated: acute phlegmonous cholecystitis (APCh) – (n=237) and acute gangrenous

cholecystitis (AGCh)) – (n=120).

For study the biological objects on the scanning electron microscope, tissue samples after fixation in 1% glutaraldehyde and additional fixation in 1% solution of OsO4 were conducted through aqueous solution of acetone of increasing concentration (up to 100%). Then the samples were conducted through the series of amyl acetate-ethanol mixtures of increasing concentration (up to 100%) [18-20]. The dehydrated samples were dried by the transition of the critical point and were covered by platinum in the "Joel" chamber.

Axioskop 40 plus light optic microscope by "Carl Zeiss" and JSM-6010LV/6010LA scanning microscope by "Jeol" were used for microscopy.

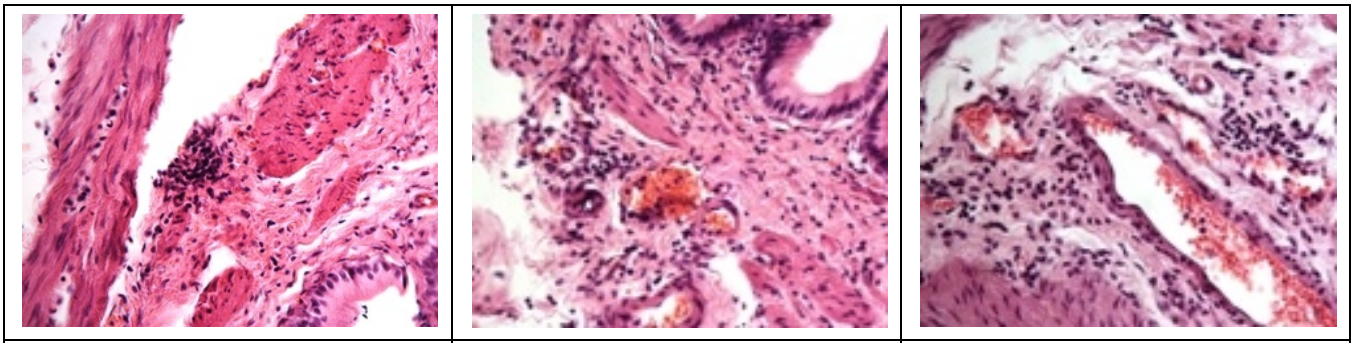
3. Results

Numerous microscopic investigations showed that gallbladder wall had specific morphologic picture subject to not only the inflammation form, but also subject to the zone of investigation. It was revealed in the control group that in the area of the bottom a mucous membrane formed an abundance of high and narrow folds which were adjoined closely to each other (Fig.1 a). The height of the folds was preserved in the zones of the body and the neck, but their quantity was decreased per unit area (Fif.1 b,c). A muscular tunic of gallbladder was formed by longitudinal and transversal muscle fibers. Maximal thickness of the muscular tunic was defined in the body area, but at the neck and the bottom area that tunic was manifested insignificantly (Fig. 1). Inflammatory infiltration in gallbladder wall of that group was insignificant, with a predominance of mononuclear cells in all layers of the wall: by their maximal quantity in the bottom area and a sharp decrease in the areas of gallbladder body and neck.

It was detected at SEM that the layers of gallbladder wall were clearly perceivable between each other with insignificant enlargement of friable fibrous connective tissue in interstitia (Fig. 2 a). The folds of mucous membrane were high and were situated irregularly (Fig. 2 b). Longitudinal and transversal directivity of muscular fibers with enlargement of friable fibrous tissue among them were clearly defined in muscular tunic (Fig. 2 a,c). External layer was presented by friable, tender fibers of connective tissue (Fig. 2 d).

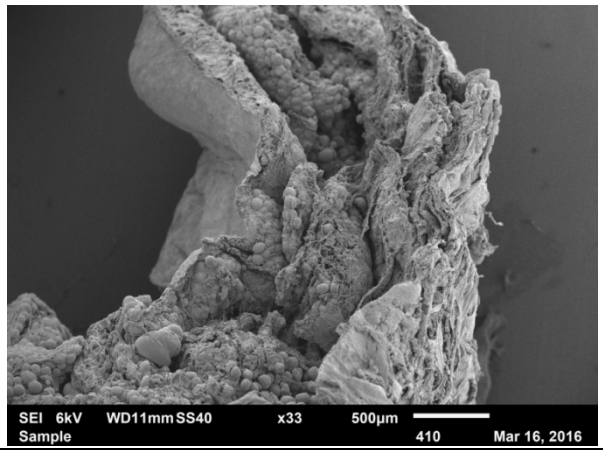
The folding of mucous membrane was preserved in all areas of investigation at destructive forms of cholecystitis. But the height and the folds quantity per unit area were changed. Mucous membrane at APCh had low and wide folds in the bottom and neck areas. The folds were mainly high in the neck area, but their height was decreased at some parts. The inflammatory infiltration of that layer in the bottom and neck areas was diffuse and polymorphous, but with the prevalence of segmentonuclear basophiles and neutrophilic leukocytes (Fig. 3). Mononuclear cells (monocytes and lymphocytes) were the prevailing ones in the neck area, with a preservation of a diffusive infiltration in the upper third of mucous and submucous layers. Muscular tunic at phlegmonous inflammation of gallbladder was thickened at all investigation areas subject to the hypertrophy of muscle fibers, edema and a massive inflammatory infiltration (Fig. 3a,b). There was a dystrophy of myocytes with occurrence of myolysis in the gallbladder bottom and neck at more preserved structure of muscle fibers in gallbladder neck (Fig. 3c,d). Inflammatory infiltration of a muscular tunic as well as mucous layer had diffuse and polymorphous origin. Segmentonuclear and neutrophilic leukocytes dominated in the bottom and neck of infiltration, and mononuclear cells dominated in the neck. Maximal quantity of inflammatory cells was defined in the bottom area of gallbladder (Fig. 3b).

SEM investigation revealed hypertrophy of gallbladder wall (Fig. 4 a). The folds in the mucous membrane were low and wide subject to enlargement of the connective tissue in the submucous layer (Fig. 4 a,b). A sharp hypertrophy of myocytes with myolysis foci and enlargement of rough connective-tissue into the interstitial tissue was visualized in the muscular tunic (Fig. 4e). Adventitia was also thickened subject to both connective and adipose tissues. Massive polymorphous inflammatory infiltration was visualized in all layers of the wall (Fig. 4 c,d,f).

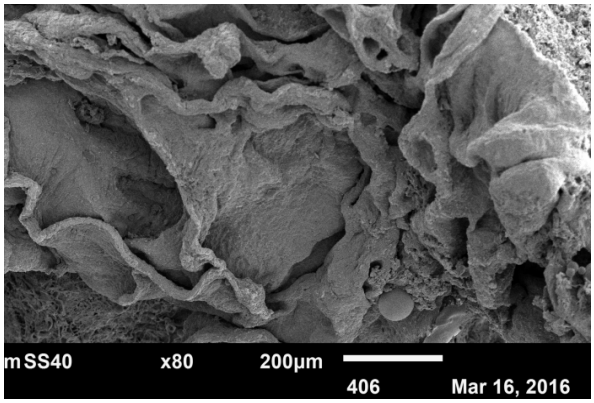


A. Gallbladder bottom	B. Gallbladder body	C. Gallbladder neck

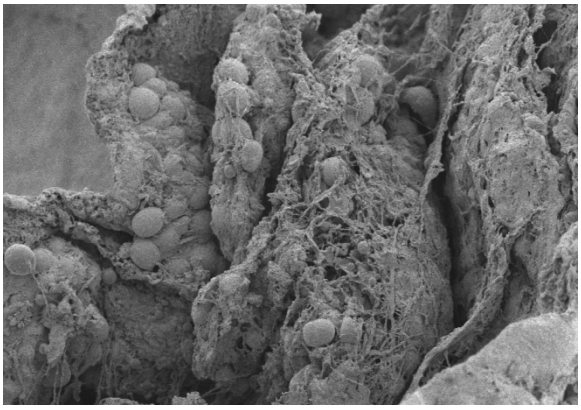
Figure 1. Gallbladder of the control group at the light microscopy. Stained by hematoxylin and eosin, x40



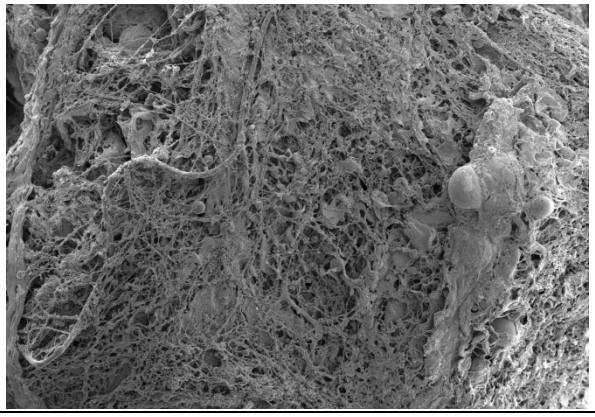
A. Gallbladder wall with clear visualization of the layers in the body area, x33



B. Gallbladder mucous membrane with high folds in the bottom area, x80

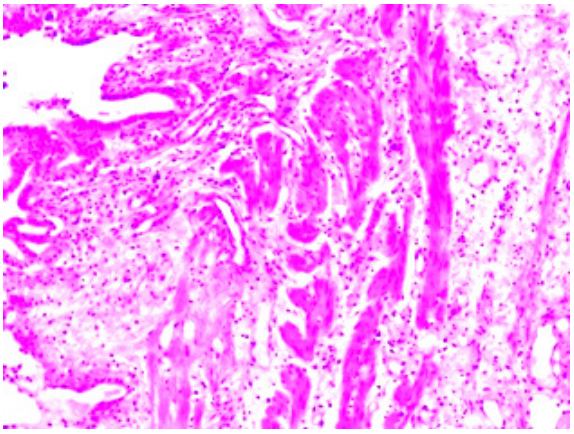


C. Muscular tunic with friable fibrous tissue in the body area, fragment of Fig.1 a, x33

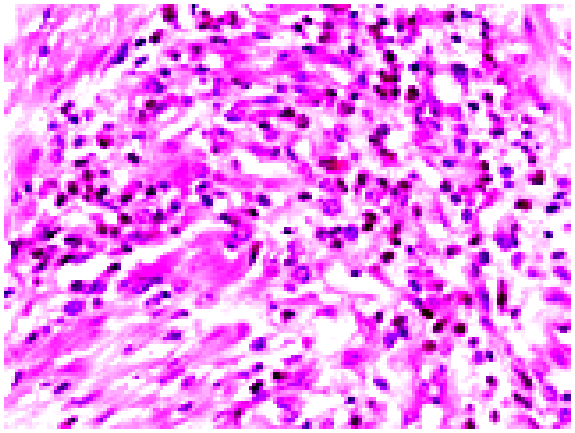


D. External layer formed by friable fibrous tissue in the bottom area, x90

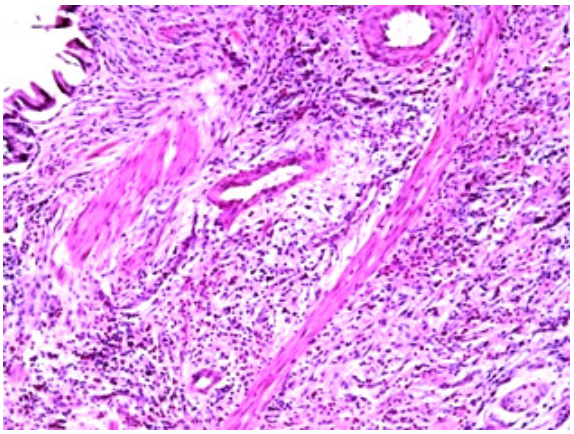
Figure 2. Gallbladder of the control group at SEM



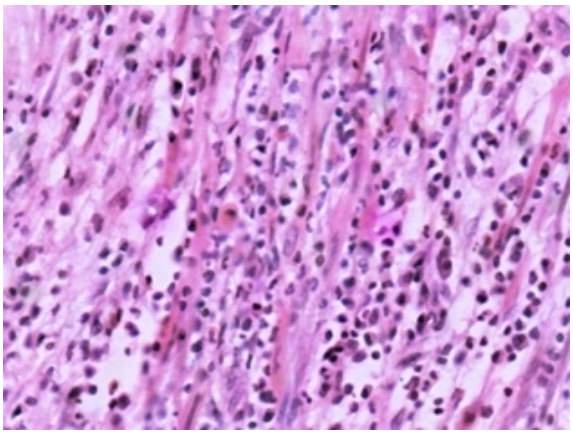
A. Gallbladder bottom with wide and low folds of mucous membrane, hypertrophy of muscle fibers, edema and inflammatory infiltration of the wall layers, x10



B. The same specimen with diffuse infiltration by segmentonuclear basophilic and neutrophilic leukocytes, x40

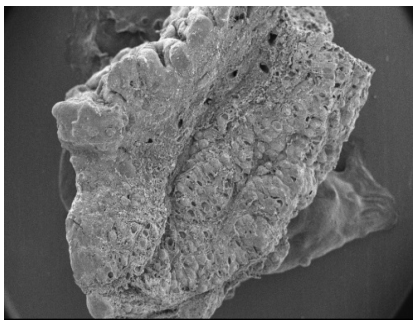


C. Gallbladder body with flattened folds, necrosis of muscular tunic with evident inflammatory infiltration, x10

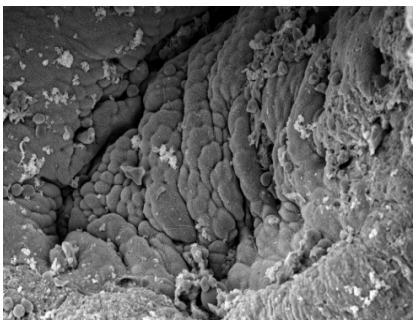


D. The same specimen with muscular fibers necrosis and polymorphic inflammatory infiltration, x40

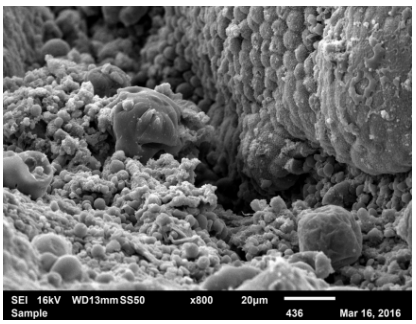
Figure 3. Gallbladder at acute phlegmonous inflammation. The light microscopy. Stained by hematoxylin and eosin



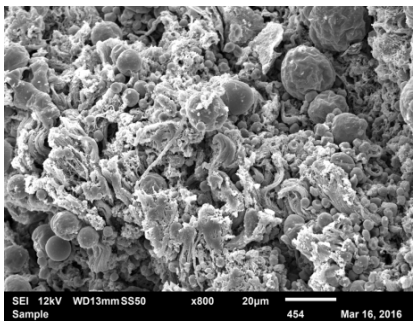
A. Gallbladder wall with a clear visualization of layers in the bottom area, x30



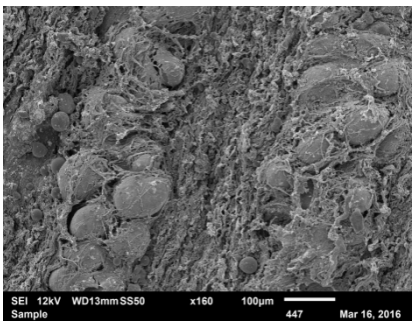
B. Gallbladder mucous membrane with low and wide folds in the body area, x230



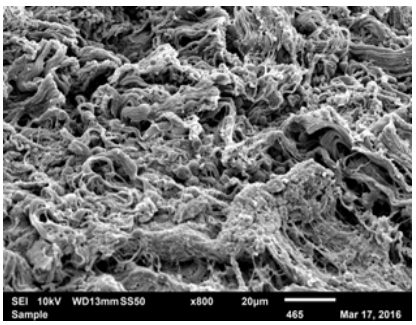
C. Mucous membrane with polymorphous inflammatory infiltration, x800



D. Fibrous connective tissue of submucous layer with inflammatory infiltration in the bottom area, x800



E. Hypertrophy of the muscular tunic in the bottom area with an enlargement of connective tissue in the intermuscular space, x160



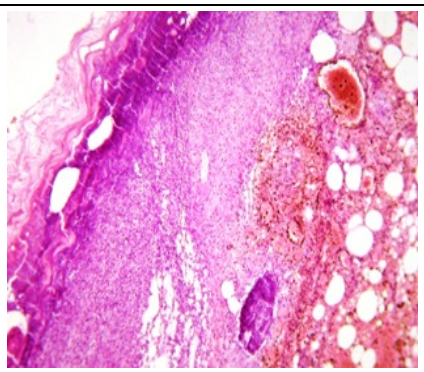
F. Evident inflammatory infiltration between thickened fibers of the connective tissue of the external layer in the neck area, x800.

Figure 4. Gallbladder at phlegmonous inflammation. SEM

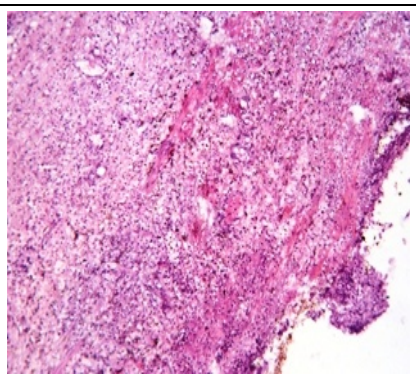
The sharp reduction of quantity and height of mucous membrane folds, their lysis was visualized at AGCh in all areas of investigation. A total absence of inflammatory infiltration was registered in mucous membrane of gallbladder body and bottom at the evident lytic process. There was a massive polymorphous inflammatory infiltration in the neck area. Muscular fibers in the bottom area were lysed and it was impossible to distinguish a position of fibers. In the body area at preservation of the fibers lysis pattern there were defined foci with preservation of the structure, but with evident dystrophic changes. In the neck area muscular fibers had unaffected structure, but with dystrophic changes. There was no inflammatory infiltration in the bottom area. There was an evident inflammatory infiltration with a prevalence of segmentonuclear and neutrophilic leukocytes. Diffuse polymorphous inflammatory infiltration was in the neck. The enlargement of the fibrous and fatty tissues was in all areas of

investigation at all types of acute inflammation.

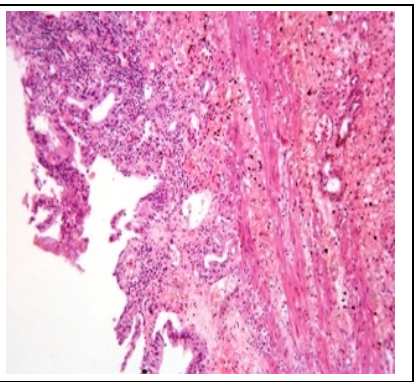
There were defined tissue edema and diffuse inflammatory infiltration with prevalence of segmentonuclear and neutrophilic leukocytes in all parts of external layer.



A. Gallbladder bottom with a gangrene of wall layers without infiltration but with evident inflammatory infiltration of external layer.

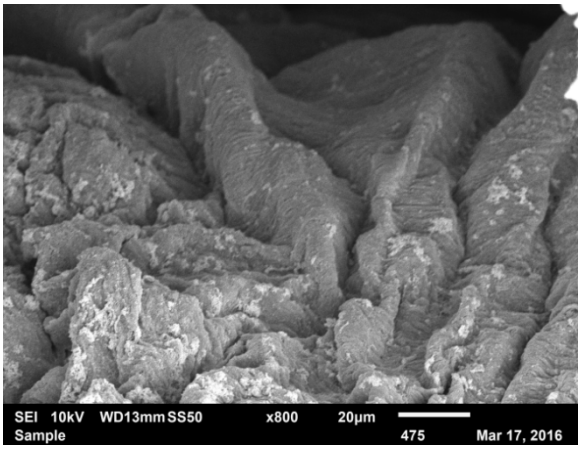


B. Gallbladder body with a total absence of the folds, myolysis and evident diffuse inflammatory infiltration.

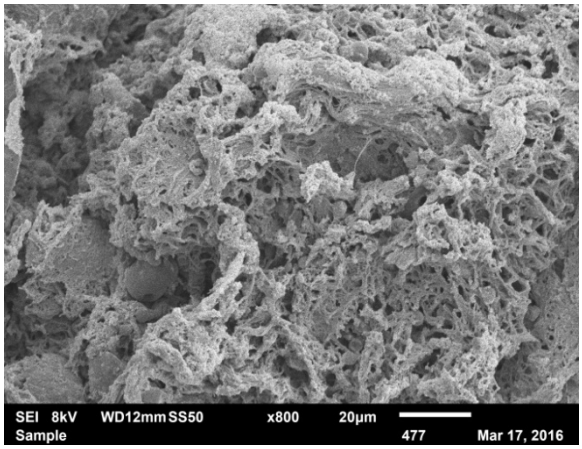


C. Gallbladder body with preservation of mucous layer folding, with distinguishable muscular fibers and diffuse inflammatory infiltration.

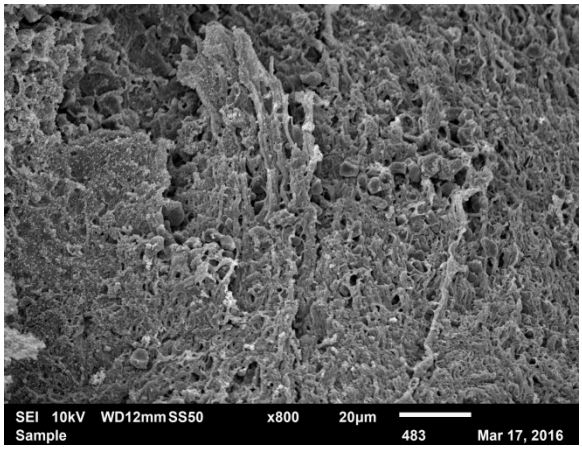
Figure 5. Gallbladder at gangrenous cholecystitis. The light microscopy. Stained by hematoxylin and eosin, x10



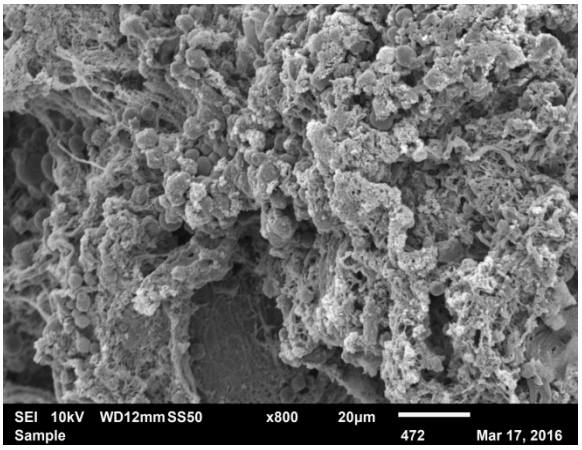
A. Mucous membrane with smoothed folds in the body area, x800



B. Necrosis of submucous layer without inflammatory infiltration in the body area, x800



C. Necrosis of muscular tunic in the bottom area, x800



D. Rough fibrous connective tissue of external layer with inflammatory infiltration in the neck area, x800

Figure 6. Gallbladder at gangrenous cholecystitis. SEM

SEM detected that a surface of mucous membrane at AGCh had a smoothed shape subject to irregular folding (Fig.6 a). There was a tissue necrosis without inflammatory infiltration in the submucous and muscular layers (Fig.6 b,c), an evident polymorphous inflammatory infiltration of necrotizing tissue was detected in the adventitia. It was an important fact, that connective tissue fibers in the external layer had a rough structure with increase of their quantity per unit area (Fig.6 d).

4. Conclusions

1. The light microscopy has been remaining a basic type of investigation for diagnostics of morphologic forms of gallbladder inflammation at calculous cholecystitis in operated patients.
2. The research revealed that changes were variable subject to the areas of investigation at destructive forms of calculous cholecystitis. APhCh is specified by manifested changes in the body area, at AGCh – in the bottom area and gallbladder neck remains safer at such types of cholecystitis
3. Scanning electron microscopy being the next level of investigation gives essential information for understanding and deep analysis of tissue structural changes in different areas of gallbladder.
4. At scanning microscopy of phlegmonous-changed gallbladder there was a hypertrophy of gallbladder wall, an excrescence of connective tissue to submucous layer, sharp hypertrophy of myocytes with myolysis nidus and excrescence of rough connective tissue fibers in interstitial tissue, massive polymorphous inflammatory infiltration. At gangrenous cholecystitis there were detected smoothed relief, tissue necrosis without inflammatory infiltration. Inflammation cells were detected only in adventitia.
5. Light electronic microscopy shows the changes of layers patterns at different forms of inflammation and it is the source of demonstrable material of various structural elements correlation between each other.

5. Discussions

Available researches devoted to the morphologic study of the gallbladder were carried out in the middle of the last century, without clear morphologic characteristics of the changes subject to anatomic part of the gallbladder. We have not also found studies which were devoted to the comparison of the gallbladder changes at the light and scanning electronic microscopy. Exactly these facts were a starting point for this research.

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