

Results of the study of the influence of viral liver damage in white rats under experimental conditions on liver tissue (trichrome Masson and immunohistochemistry marker cd68)

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Abstract. This article is supposed to determine the level of activity of viral (including SARS-CoV-2) damage to the liver tissue and the stage of liver fibrosis resulting from experimental viral damage to the liver. A viral lesion (including SARS-CoV-2) was also detected in the liver tissue, as a result of which the expression of the CD 68 marker in the liver tissue of normal and experimentally infected liver tissue was determined.

1 Introduction

Currently, the number of infections caused by viruses, and the atypical course of clinical symptoms of diseases caused by these viruses, cause more and more severe complications, which leads to an increase in the number of complications and an increase in disability and morbidity rates. a decrease in the quality of human life causes an increase in the amount of excess costs for the study of pathologies that can be observed in the liver as a result of exposure to various viruses and the development of measures to prevent these pathological processes is a requirement of time, despite the fact that many medical workers, scientists, research institutes conducted and are conducting scientific and practical work, the consequences for human health remain at risk. Therefore, the development of pathological conditions in the organs of the human body, especially in the liver, and measures to prevent them are not only the call of the times, but also require us to develop a new view of diseases, new modern measures.

The purpose of this study was to determine liver damage caused by viral infections and the reaction of Kupffer cells to this damage using the CD 68 marker, which was used as an immunohistochemical method, as well as to study the expression of this marker in normal and experimental viral liver damage, and at the same time to determine the reaction liver for viral infection (including CoV-2) is aimed at determining the level of fibrosis that occurs in the liver as a result of damage.

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2 Material and methods

In total, 30 white outbred rats were allocated for the study, divided into two groups. 10 outbred rats were selected for the control group and 20 outbred rats for experimental liver injury. For general morphology, 1.5x1.5 cm sections, i.e. large section, small section and medium section, were cut from each liver and frozen in 10% neutral formalin. After washing in running water for 2 hours, they were dehydrated in alcohol of increasing concentration, placed in xylene-paraffin for 4 hours, then embedded in paraffin, and paraffin blocks were prepared. Sections of 5-8 μm thick were prepared from paraffin blocks, stained with Masson's trichrome and examined immunohistochemically with the CD 68 marker. When examining morphological changes in liver cells and tissues, the necessary information was obtained, liver hepatocyte cells were photographed using a trinocular microscope, pathological processes observed in vessels.

3 Results and conclusions

CD 68 is a glycoprotein belonging to the LAMP family and expressed on the surface of macrophages and monocytes, and therefore it is used as a macrophage marker. Macrophages located in the liver tissue were identified by K. R. Kupffer in 1876, therefore macrophages in the liver are called Kupffer cells. Kupffer cells are located in the sinusoid space of the liver. Kupffer cells are located in the endothelial cells of the sinusoids and are involved in the formation of the wall of the sinusoidal vessel. Kupffer cells are amoeba-like in shape, have microvilli and pseudopodia on their surface, with which they swallow and phagocytose iodine bodies. Kupffer cells are located in the centrilobular and periportal regions of the liver lobe. Kupffer cells in the periportal region are larger and contain more lysosomes and are characterized by high phagocytosis. The cells of the centrilobular region are adapted to the generation of superoxide radicals. In the liver, two groups of macrophages are distinguished: lipid metabolism, cleaves protein complexes, and small particles phagocytize apoptotic cells. The cells of this group differ from monocytic macrophages in that they have proliferative properties and restore their numbers. The second group is cells that are formed in the bone marrow and transform into macrophages after moving to the liver. The function of Kupffer cells is to break down foreign bodies that have entered the liver, phagocytosis, and produce cytokines that increase inflammation and stop inflammation. At the same time, biologically active substances produced by Kupffer cells activate Ito cells, resulting in fibrosis. Based on the CD68 marker, the expression level of macrophage cells can be calculated. A total of 500 cells were counted in the count, and it was determined how many of them expressed this marker at a positive level, and what percentage of all cells were positive. 1) 10% - low level, 2) 10-20% - medium level, 3) more than 20% is considered a high level of expression.

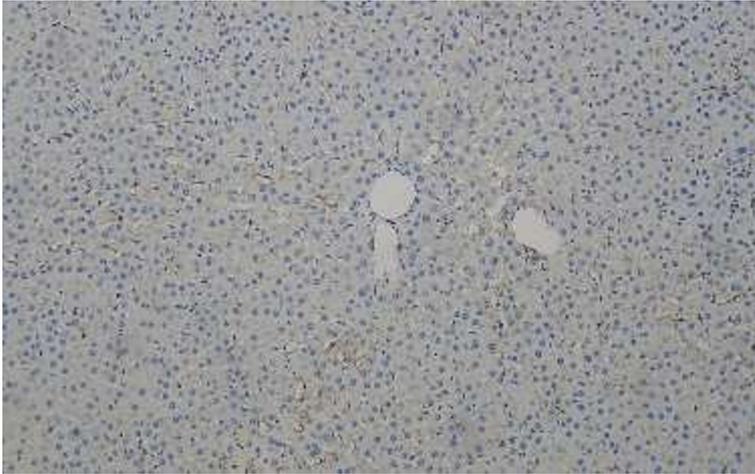


Fig. 1. Low expression of the CD68 marker in the sinusoidal space, periportal area, and central vein area in the liver of a white outbred rat of the control group. Immunohistochemical staining. Size 10x4.

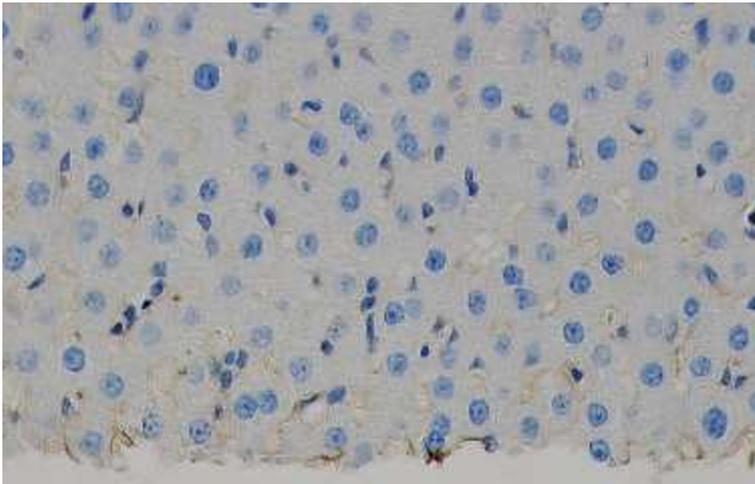


Fig. 2. Liver of a white outbred rat of the control group. Low expression of the CD68 marker in the sinusoidal cavity. Immunohistochemical staining. Size 10x4.

Taking into account the above, the aim of this study was to determine the level of expression of the immunohistochemical marker CD 68 in normal and experimental viral liver lesions and the level of liver damage. In the study of micropreparations prepared from the liver of white rats of the control group, the expression of the CD 68 marker was less than 10%. It was found that expression occurs in the periportal region and centrilobular region of the sinusoids of the liver (Fig. 1, 2). Micropreparations prepared from the liver of 20 outbred rats were studied in experimental viral liver damage. In 45% of cases, the CD 68 marker was expressed at a low level, in 35% of cases it was expressed at an average level, and in 20% of cases it was expressed at a high level. The severity was high in the area of the portal vein, in the perinatal area, in the bile ducts. Circles and in the region of the central vein of the liver (Fig. 3, 4).

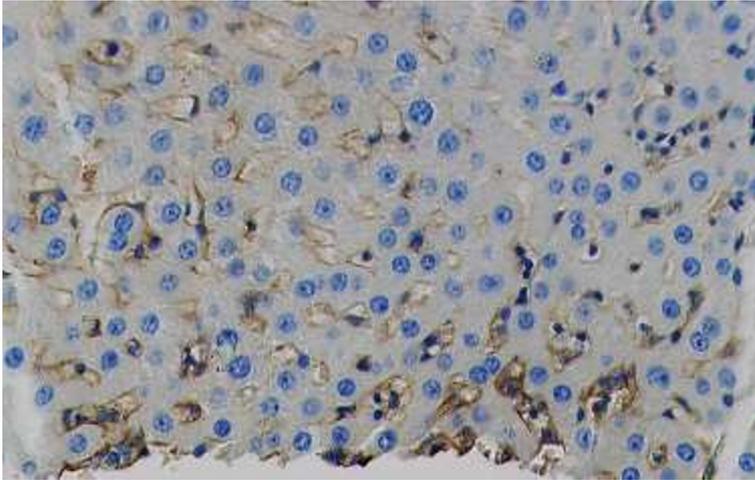


Fig. 3. High expression of the CD 68 marker in the sinusoidal spaces of the liver in experimental viral liver injury. Immunohistochemical staining. Size 10 x 40.

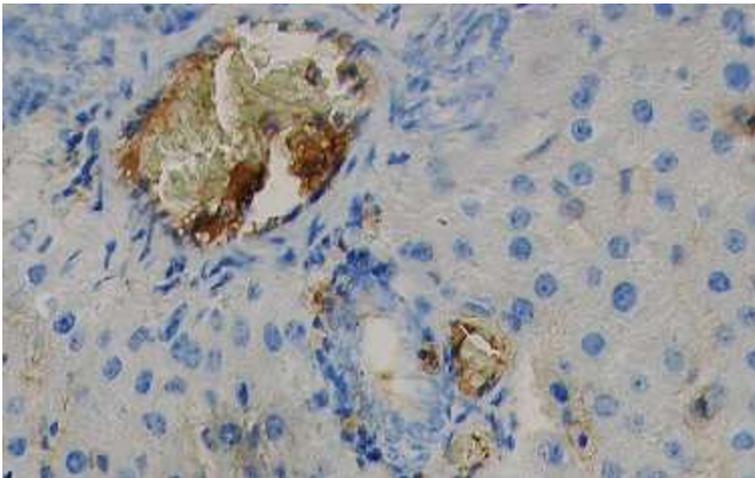


Fig. 4. High expression of the CD 68 marker in the region of the hilum, around the central vein and around the bile ducts in experimental viral liver damage. Immunohistochemical staining. Size 10 x 40.

Another of our goals was to determine the level of fibrosis that occurs in the liver with control and experimental viral liver damage. Masson's trichrome stain is a widely used method in histology and pathology. With its help, connective tissue, muscle fibers and cells in biological tissues are detected. Using this method, you can determine the level of fibrosis in tissues. Collagen is stained blue, cytoplasm, keratin, muscle fibers are red, erythrocytes are yellow, stained in color, fibrosis in the liver was assessed by metavir. (IASL, Butts-Ludwig and Metavir). Butts-Ludwig is a widely used system for determining the degree of fibrosis, and the results obtained from this system were evaluated. According to Butts-Ludwig metavir, the degree of fibrosis is assessed from 0 to 4 stages. 0 or F0 - no fibrosis, 1 or F1 - fibrous dilatation of the saphenous vein, 2 or F2 - fibrosis with few obstructions emerging from the portal tract, 3 or F3 fibrosis with multiple obstructions protruding from the portal tract. 4 or F4 stage of cirrhosis, in which the regenerative nodes are surrounded by fibrous tissue. The results of viral liver damage in the control and experimental groups were obtained and evaluated by metavir. Micropreparations taken from the liver of white rats of the control group were stained with Masson's trichrome, a normal amount of collagen was

found around the portal tract, it can be seen that the central vein of the liver section was stained to a minimal extent (Figure No. 5, 6).

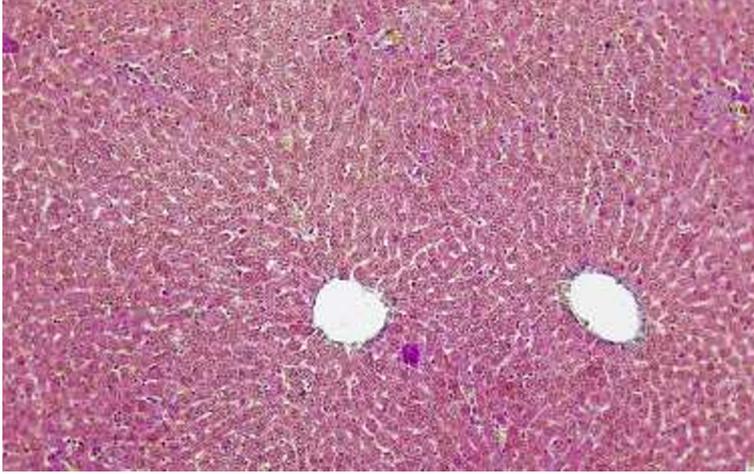


Fig. 5. Liver of outbred rats of the control group, stained with Masson's trichrome, with a normal amount of blue collagen around the portal tract and central vein. Size 10x4.

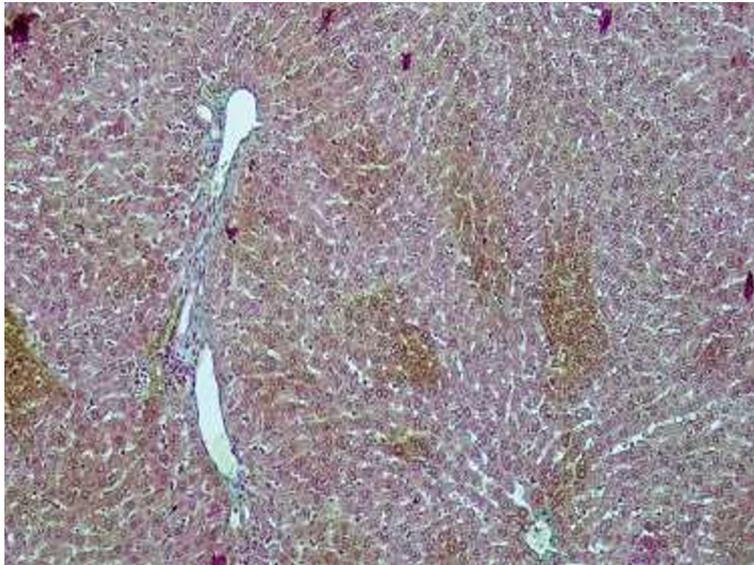


Fig. 6. Masson's trichrome-stained liver of control white outbred rats with normal amounts of blue collagen around the portal tract and central vein. Size 10x4.

Micropreparations obtained from the group of experimental viral liver injury were evaluated by Metavir and the results obtained in percentage were determined as follows: 30% F0 was detected, of which 50% F 1, i.e. expansion of portal vein fibrosis. 17% of them had F2, i.e. a small number of short obstructions around the portal fibrosis tract, and in 3% of cases there was a large number of obstructions from the F3 portal fibrosis tract (photo # 7, 8).

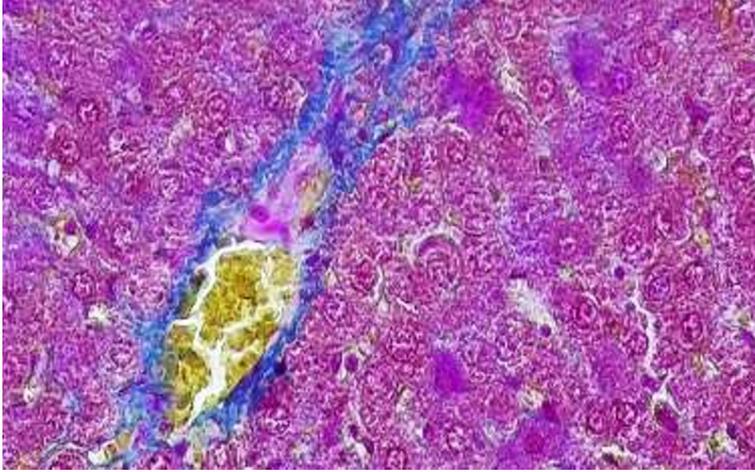


Fig. 7. Liver of a outbred rat of the experimental viral liver injury group B, stained with Trichrome Masson, increased levels of fibrosis around the portal tract and central vein, blue collagen. Size 10x40.

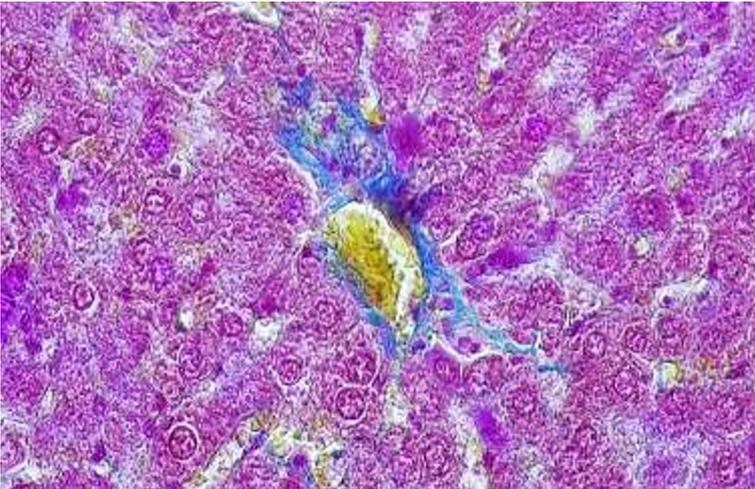


Fig. 8. Liver of a outbred rat of the experimental viral liver injury group stained with Trichrome Masson, increased levels of fibrosis around the portal tract and central vein, blue collagen. Size 10x40.

4 Conclusions

In our study, when studying micropreparations prepared from the liver of white rats of the control group, the expression of the CD 68 marker was lower by 10%, the expression was detected in the periportal and centrilobular sinusoidal areas of the liver.

When studying microsections prepared from the liver of outbred white rats with experimental liver damage, the expression of the CD 68 marker was found at a low level in 45% of cases, at an average level in 35% of cases and at a high level in 20% of cases. Expression was high in the portal vein, in the peripartum, around the bile ducts, and in the central vein.

When staining micropreparations obtained from the liver of a white outbred rat of the control group with Masson's trichrome, it can be seen that the normal amount of collagen is located around the portal tract, there is minimal staining around the central vein of the liver section.

Micropreparations obtained from the group of experimental viral liver damage were evaluated by Metavir and the results obtained in percentage were determined as follows: 30% F0 were detected, of which about 50% F 1, i.e. fibrosis of the portal vein. Of these, 17% had F2, i.e., a few short obstructions around the portal fibrosis tract, and 3% of cases had a large number of obstructions emerging from the F3 portal fibrosis tract.

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