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SEXUAL DIMORPHISM OF THE STRUCTURAL ORGANIZATION OF THE LIVER OF THE INSULIN-RESISTANT RATS

Abstract. *In animals, under the conditions of insulin resistance (IR), a violation of lamellar organization of the liver due to dystrophic changes in hepatocytes in all its lobes, but mainly in their peripheral departments, has been revealed. Liver cells are enlarged in size. In females there is a more pronounced zonal character of degenerative processes of hepatocytes compared with males, whose dystrophy is predominantly diffuse in nature. A distinctive peculiarity in females with IR is also the presence of foci of fatty degeneration of hepatocytes.*

Key words: *insulin resistance, structural organization of the liver, sexual dimorphism.*

Topicality of the study. Diabetes mellitus (DM) is a relatively common disease of the endocrine system in the world, that has a tendency to increase. The total number of patients with DM can reach 440 million up to 2030 [1]. The risk of DM is in its multiple-organ damage, as well as in the high risk of development of comorbidity. In recent years, attention has been drawn to the diseases of the liver, which from year to year remain the leaders of the digestive tract pathologies. The liver plays a leading role in the metabolic processes of the organism, and the violation of its functional state is associated with the changes in the metabolic processes of various genesis [2, 3]. Therefore, it is relevant to study the structural organization of an organ under the conditions of DM.

The objective of the research. To study the structural peculiarities of the liver in rats with insulin resistance taking into account sexual dimorphism, in the experiment. **Materials and methods.** The study was performed using 40 nonlinear, mature rats (males and females) weighing 150-180 g. Rats of the experimental group (20 males and 20 females) were modeled IR by adding the 10% fructose solution to drinking water during 8 weeks [4]. Control group animals (20 males and 20 females) followed the standard diet. Care, feeding and euthanasia (decapitation using ketamine anesthesia, 100 mg/kg of the body weight) were in line with current international requirements regarding the humane treatment of animals. Histological preparations were stained with hematoxylin and eosin according to Shabadash for evaluation of structural changes in the liver, and PAS-reaction was performed [5]. When analyzing indices, gender peculiarities were taken into account. Histological studies were performed on the Leica DME light-optical microscope. In order to objectivize quantitative studies, computer morphometry of objects in histological preparations was performed. During the first stage, the digital copies of the optical image of microscopic preparations were obtained using a Nikon Coolpix 4500 digital camera, followed by an analysis of digital copies of the images using the computer software Image Tool 3.0 for Windows. The morphometric analysis of the liver was performed taking into account the following parameters: the average perimeter and area of the hepatocyte, the average perimeter and nucleus area of the hepatocyte, the average perimeter and nucleolus area of the hepatocyte, and the average thickness of the vascular wall. The nuclear-cytoplasmic index and the index of the ratio of the nucleolus area to the area of the nucleus of the liver cell were also calculated. Statistical analysis of the results was performed using Microsoft Exel and Statistica 5.5 (Multiple Regression) computer software.

Results of the research and their discussion. The structural construction of the liver in animals of the control group, both males and females, is characterized

by a trabecular organization (Fig. 1). Hepatocytes of rectangular shape, with a granular eosinophilic cytoplasm, contain small granules of glycogen with an average area of 3.23±0.65 μm^2 (Fig. 2).

Granules are round-shaped, rich in purple tint, are diffusely located in the cytoplasm of cells. The liver cells have a centrally located round nucleus with heterogeneous chromatin. The nucleus is characterized by a central and paracentral rounded form of the nucleoli. Between the trabeculae of hepatocytes there are sinusoidal hemocapillaries that contain erythrocytes. In peri-sinusoidal spaces, solitary macrophages are visualized. In the peripheral parts of the lobes, the portal tracts are visualized, in the central departments – there are central veins, which are mainly filled with blood. The examined metric indices of the hepatocytes are represented in Table 1, and indicate a lack of a significant difference between animals of different genders. In males with IR, the lamellar organization of the liver is violated due to the dystrophic changes in hepatocytes in all the lobes, but mainly in their peripheral areas. Hepatocytes are of polygonal form, the borders of cells are slightly veiled. Liver cells have light cytoplasm, in which eosinophilic granularity is noted. The analysis of the staining according to Shabadash indicates that the cytoplasmic lucent areas are due to the loss of liver cells of glycogen (Fig. 3).

The liver cells are enlarged in size (average perimeter and area of hepatocytes at 29% and 65% exceed the control data, $p<0.05$). An increase in metric parameters of hepatocytes is accompanied by their compact location. The nuclei of the liver cells are mostly round regular-shaped, with a fine-particle uniformly dispersed chromatin in the karyoplasm, with a central and paracentral rigorous visualization of the rounded form of the nucleoli. The morphometric parameters of liver hepatocytes in rats with IR are represented in Table 1. In some cases, hepatocytes with more than one nucleolus in the nucleus, accompanied by an increase of the metric parameters of the nuclei, are observed. Somewhere there are marked single binuclear hepatocytes. A significant increase of the nuclear-cytoplasmic index (at 19% in males and at 25% in females, $p<0.05$), and the presence of binuclear hepatocytes suggest the development of regenerative processes in the cell, in particular, in the nucleus, but the tension of the regenerative process is low, an index of which is the reduction of the ratio of the nucleolus area to the area of the hepatocyte nucleus. In some hepatic cells, the nuclei are compact, somewhere fragmented, deformed,

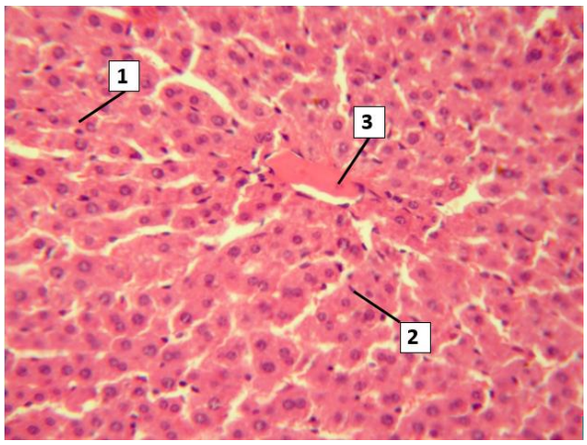

Fig. 1. Structural organization of the liver in the control group animals. Staining: hematoxylin and eosin. Magnification: ×400. 1 – trabeculae, 2 – sinusoidal hemocapillaries, 3 – central vein.

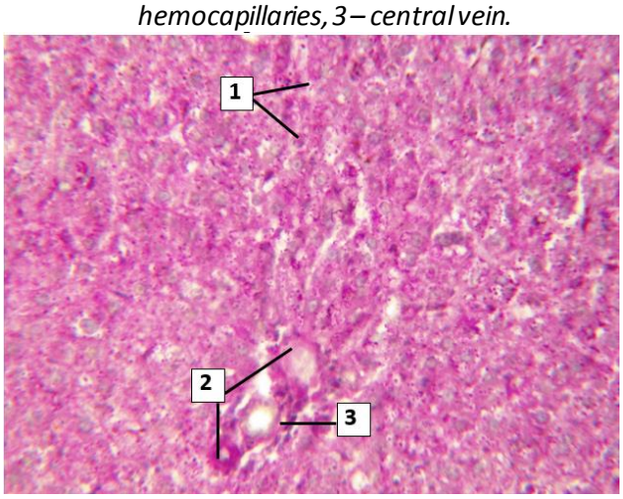

Fig. 2. Glycogen granules in the liver in the control group animals. Staining: according to Shabadash. Magnification: ×400. 1 – granules of glycogen, 2 – vessels of the portal tract, 3 – interlobular bile duct.

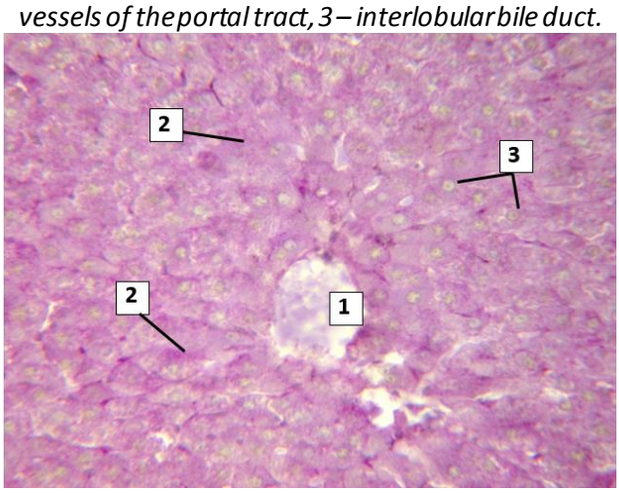

Fig. 3. Liver of rat-male with insulin resistance Staining: according to Shabadash. Magnification: ×400. 1 – central vein, 2 - solitary glycogen granules in the cytoplasm of the liver cells, 3 – nuclei of hepatocytes.

Table 1

Morphometric parameters of liver hepatocytes of control (intact animals) and experimental (animals with insulin resistance) groups of animales (M±m)

Parameter	Control group		Experimental group	
	Male	Female	Male	Female
Average perimeter of the hepatocyte, mkm	71.82±5.81	73.45±3.15	92.89±8.26*	82.64±8.75
Average area of the hepatocyte, mkm^2	348.12±2.59	362.04±10.40	574.63±37.61*	446.99±12.45*#
Average perimeter of the hepatocyte nucleus, mkm	30.64±2.92	31.97±1.15	40.18±3.49*	35.86±2.16
Average area of the hepatocyte nucleus, mkm^2	56.41±3.77	57.16±3.01	111.75±13.58*	87.63±11.37*#
Nuclear-cytoplasmic index	0.16±0.02	0.16±0.02	0.19±0.01	0.20±0.02
Average perimeter of the hepatocyte nucleolus, mkm	9.45±1.05	9.81±1.23	12.22±2.24	10.58±1.13
Average area of the hepatocyte nucleolus, mkm^2	7.87±0.93	7.55±1.04	9.74±1.52	6.98±1.45
Ratio of the nucleolus area to the area of the nucleus of the hepatocyte	0.14±0.01	0.13±0.1	0.09±0.01*	0.08±0.01*

*Note: * - reliable difference to control (p<0.05); # - reliable difference to data in males within the group (p<0.05)*

and sometimes they have the form of bent sticks. A cellular stromal reaction is not observed around such degenerative altered hepatocytes (Fig. 4). The compact location of the hepatocytes enlarged in size, is accompanied by the compression of sinusoidal hemocapillaries, their deformation

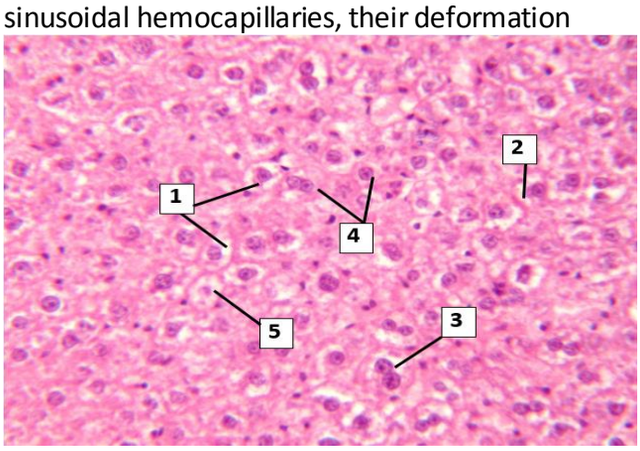

Fig. 4. Liver of rat-male with insulin resistance Staining: hematoxylin and eosin. Magnification: ×400. 1 – increased in size hepatocytes with light granular cytoplasm, 2 – compressed sinusoidal hemocapillaries, 3 – binuclear hepatocytes, 4 – centrally located nucleoli of rounded shape, 5 – apoptotic altered hepatocytes.

(see Fig. 4). In the lumen of the capillaries there are compactly located erythrocytes, in the single fields of view – with solitary leukocytes. The central veins and vessels of the portal tract are filled with compactly located erythrocytes with preserved tinctorial properties. In portal tracts there are solitary macrophages and lymphocytes, in some cases – insignificantly pronounced infiltration with non-parenchymal cellular elements with the presence of lymphocytes and macrophages, which do not extend beyond the boundary plate and are not accompanied by the degenerative changes of surrounding liver cells. The wall of the part of the vessels is insignificantly and segmentary thickened and substantially (2.4-fold, p<0.05) exceeds the control group data. In these zones, the wall is represented in the form of a homogeneous saturated eosinophilic nuclear-free mass, on the inner side of which there are localized elongated endothelial cells along the perimeter, outwards – solitary lymphocytes, macrophages. Staining using the PAS method indicates the accumulation of glycoproteins in the form of saturated pink nuclear-free masses in the wall. The wall of individual vessels is segmentary unevenly thickened; in the PAS reaction the accumulation of glycoproteins of light-purple color with focus foam is observed, which is a reflection of plasma oozing (Fig. 5).

Co-directed structural changes are also determined in females with IR. At the same time, in females under the conditions of IR, there is a more pronounced zonal character of hepatocyte degenerative processes compared to males, whose dystrophy has a more diffuse nature. A distinctive peculiarity is the presence of cells of fatty dystrophy of hepatocytes. As in males, an increase of the nuclear-cytoplasmic index indicates the intensity of the regenerative process in the cell (Fig. 6).

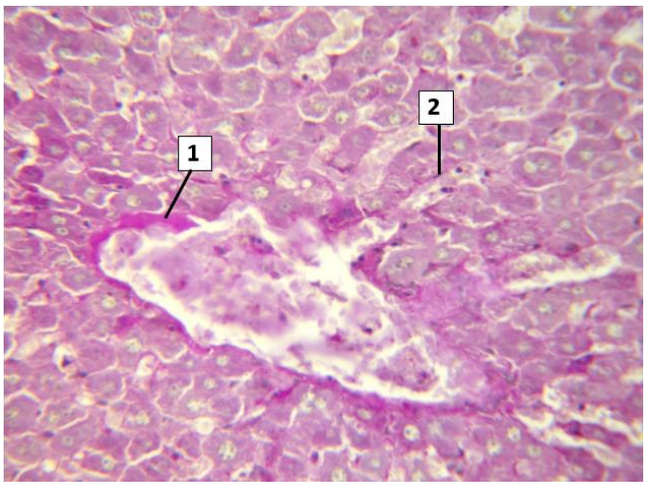

Fig. 5. Liver of rat-male with insulin resistance Staining: PAS. Magnification: ×400. 1 – plasma oozing of the central vein wall, 2 – basal membrane of sinusoidal hemocapillaries.

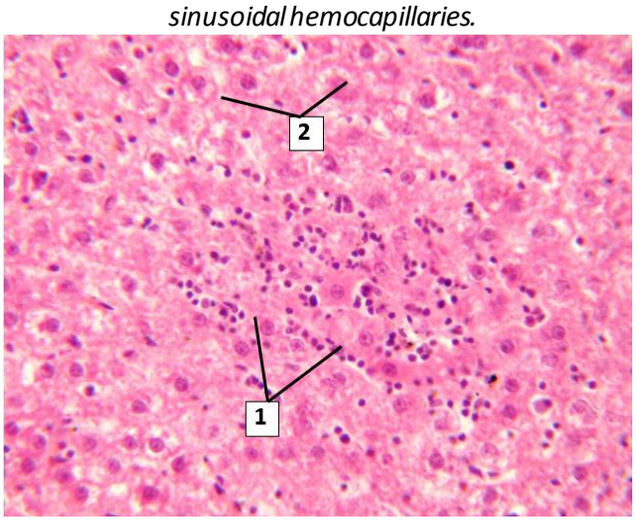

Fig. 6. Liver of rat-female with insulin resistance Staining: hematoxylin and eosin. Magnification: ×400. 1 – leukocytes in the lumen of sinusoidal hemocapillaries, 2 – dystrophically altered hepatocytes with enlightened cytoplasm.

Conclusion. In rats with IR, there was revealed the development of regenerative processes in the cell, in particular, in the nucleus, which is indicated by an increase of the nuclear-cytoplasmic index and the presence of binuclear hepatocytes, but the intensity of the regenerative process is low (decrease of the ratio of the nucleolus area to the area of the hepatocyte nucleus). Sexual dimorphism is in the presence of foci of the fatty dystrophy of hepatocytes and the pronounced zonal character of hepatocyte dystrophic processes in females compared with males, whose dystrophy is more diffuse in nature.

Prospects for further research. Study of the dynamics of the structural changes in hepatocytes as the IR formation and their reversibility.

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