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IMMUNOHISTOCHEMICAL PROPERTIES OF THE LIVER OF INFANTS WHO DIED IN THE NEONATAL PERIOD BORN WITH PREECLAMATION

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Abstract. *In order to examine the changes in the liver of infants who died in the early neonatal period due to preeclampsia, immunohistochemistry was used to detect active changes in hepatocytes, to determine whether or not the characteristics of hepatocytes change, and to interpret the changes in the antigenic structure. It should be noted that through immunohistochemical examination, we were able to detect necrosis, pre-necrosis, proliferative indicators, and then to determine the activity of blood cells in the liver.*

Keywords. *Morphology, immunohistochemistry, liver, preeclampsia, infant.*

The urgency of the problem. It has been determined that the number of pregnant women with preeclampsia in the world has increased by 2.1 times in the last 10 years. At least 10-30% of them have signs of liver damage due to microangiopathy as part of a general disorder of blood vessels. This leads to a sharp deterioration in the metabolism between the mother and the fetus, the elimination of toxic substances. In the USA and European countries, this type of pathology accounts for an average of 2.1-5.7% of all pregnancies, while in the CIS countries this figure is 10.5-18.2%. In Central Asia, the number of deaths from preeclampsia and eclampsia in 2020 was about 8.9-12.7% of all pregnant women.

Signs of liver damage appear at the end of the II-III trimesters of pregnancy, usually against the background of a detailed clinical picture of hypertensive disorders. In the absence of arterial hypertension or proteinuria, an atypical course of preeclampsia can be observed. Liver damage can manifest itself only with laboratory changes (increased AST/ALT, mild thrombocytopenia), without jaundice and other complications, including the development of HELLP syndrome. It is precisely in these changes in the third trimester of pregnancy that sharp changes in fetal development occur, the inability to fully utilize harmful substances secreted by the fetus through the placenta, and lead to morphofunctional stress of the fetal liver.

Objective. To study the morphological and immunohistochemical changes that occur in the pathomorphological characteristics of the liver of infants whose mothers have preeclampsia.

Materials and methods. Liver tissue was obtained from 66 infants born to mothers with preeclampsia who were brought to the Republican Center for Pathological Anatomy from the RIPIATM for autopsy. Morphological, immunohistochemical and statistical research methods were used to improve the assessment of morphological changes characteristic of the liver of infants who died in the neonatal period due to preeclampsia.

Results and discussion. In order to examine the changes in the liver of infants who died in the early neonatal period due to preeclampsia, immunohistochemical studies were performed to identify active changes in hepatocytes, to determine whether there were any changes in the characteristics characteristic of hepatocytes, and to interpret changes in the antigenic structure. Let's remind, through

immunohistochemical examination, we examined the liver necrosis, pre-necrosis process, proliferative indicators, and then the activity of blood vessels in the liver.

Immunohistochemical studies were performed using monoclonal antibodies and a systemic imaging system (Ventana Ultra USA):

1. CD 31 (PECAM-1)
2. CK 34 (vascular growth factor, indicating neoangiogenesis)
3. P 53 is a factor indicating increased apoptosis of hepatocytes.
4. Ki 67 is a factor determining the proliferative index.

The Ki-67 marker is expressed mainly in the perinuclear region of any cells and is a marker determining proliferative activity. This plays an important role in assessing the proliferative index of fibroblasts. The significance of the markers used in immunohistochemical examination is as follows: the Ki-67 marker is a marker that determines cell proliferation and is expressed in different levels (light, medium and strong liver color) in all active phases of the cell: G1, S, G2, M. This marker is highly expressed from the initial phase of cell activation, from the G1 to the M phase, and is clearly visible in the metaphase of mitosis. In the initial phase of G1, the Ki-67 marker is located at the centromere of satellite DNA and at the telomere of the chromosome.

In the intermediate phases of cell activation, the Ki-67 marker is detected intranuclearly in the nucleolus, but by the G2 phase, it is expressed in the nucleolus and karyoplasm. When the cell enters the post-mitotic G0 phase, the Ki-67 marker is degraded by proteosomes and undergoes complete catabolism and is not expressed in cells in interphase. This is important in the evaluation of the proliferative index of fibroblasts, which are proliferatively active in hypotocytes.

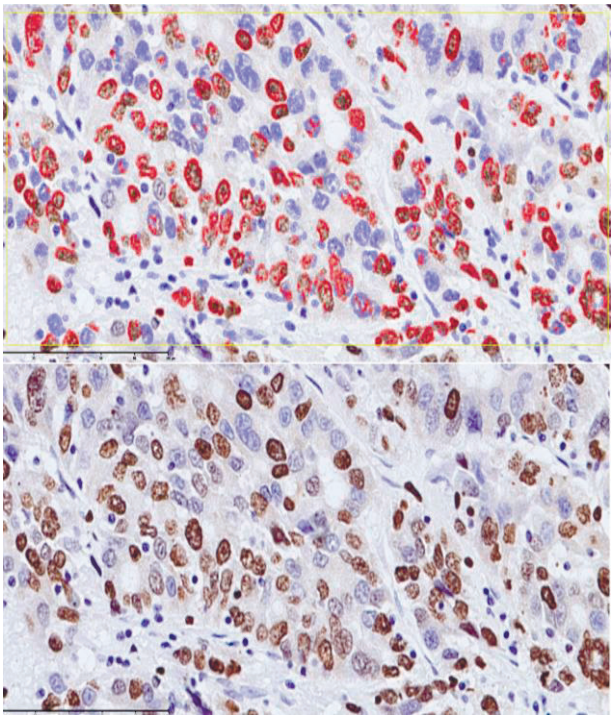

Fig. 1. An infant born with preeclampsia and dying in the early neonatal period. Protocol 46-D. High positive expression of the Ki-67 marker. Scanned and the level of expression was determined using the QuPath-0.4.0.ink. program. Expressed cells are dark red. Stain Dab chromogen. Size 10X10.

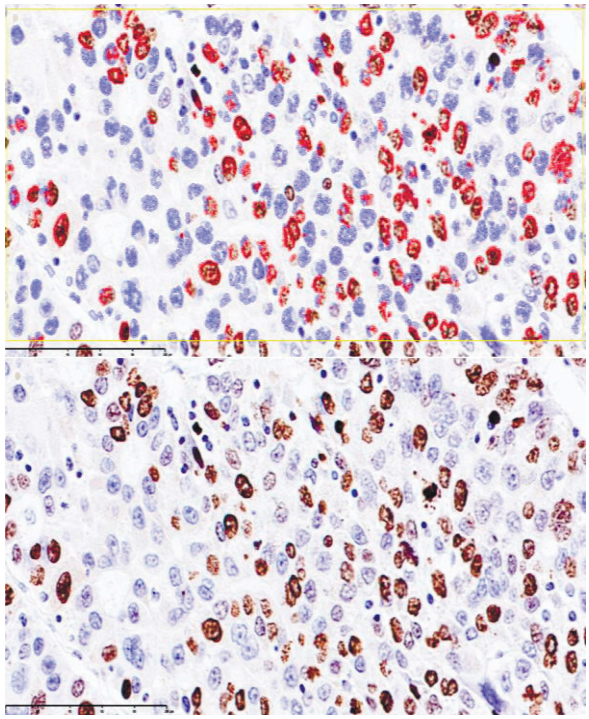

Fig. 2. An infant born with preeclampsia and dying in the late neonatal period. Protocol 17-DI. High positive expression of the Ki-67 marker. Scanned and the level of expression was determined using the QuPath-0.4.0.ink. program. Expressed cells are dark red. Stain Dab chromogen. Size 10X10.

In our study, high positive expression of the Ki-67 marker was detected in 63.1% of the subjects, mainly in hepatocytes, with very low expression in mesenchymal cells. This indicates that compensatory regenerative indicators are high in the liver parenchyma in infants, which indicates a high level and diversity of factors affecting the proliferative type of liver in preeclampsia. The high number of hepatocytes still undergoing 2 and 3 nuclear mitosis phases in the liver, the high number of hepatocytes that completely occupy the liver in terms of volume, the adaptation mechanism aimed at eliminating tissues and cells specific to the fetus in the early neonatal period, is directly related to the detoxification activity of the liver, and it is precisely in preeclampsia that the immunohistochemical aspects of these morphofunctional indicators in hepatocytes lag behind the norm.

In our study, moderate positive expression was detected in 29.9% of the subjects. The remaining 6.8% had a negative reaction, mainly expressed. This, in turn, led to the fact that in the early neonatal period, the positive expression of the Ki-67 marker in the liver of infants was high, and according to the proliferative index, by index, it was found that 38.23±1.42% The proliferative index in mesenchymal cells was 11.01±1.02%, and this indicator was not statistically different from the indicators in the control group, so it was not included in our study.

In the late neonatal period, the expression of the Ki-67 marker was mainly expressed in hepatocytes, with nuclear expression, heterogeneously expressed in the nuclear, subnuclear and perinuclear areas, and was lower than in the early neonatal period, indicating a decrease in the proliferative resources of the liver and a decrease in the self-repair function of the liver, as well as an increase in the process of fibrosis in the liver. This was revealed by the fact that the Ki-67 marker reacted mainly in hepatocytes, with relatively low values of about 10% in the late neonatal period. While in the early neonatal period, the highest rate of proliferation in the liver of infants was 48.9% (see Figure 4.1), in the late neonatal period this rate was 34.23% (see Figure 4.2). This also confirms the direct relationship between the duration of the influencing factors, the decrease in the liver's intracellular and extracellular matrix resources, and the increase in mesenchymal tissue.

The proliferative index of the Ki-67 marker in the late neonatal period of infants born against the background of preeclampsia was 25.38±1.05%, while the proliferative index in mesenchymal cells was mainly 14.78±1.66%, confirming the increase in the stromal components of the liver in this process.

In the next immunohistochemical study, the mutant protein transcription factor is studied by studying the reaction of the P-53 marker.

The P-53 protein is a proapoptotic factor, which, as a result of the accumulation of abnormal proteins, onoproteins, and various foreign mutant proteins in the cytoplasm of cells, triggers the apoptosis mechanism in the cell, which leads to organ failure, depending on the duration of this process and the level of influencing factors. This may lead to misunderstandings in our studies, since high positive expression of the P-53 marker indicates a high probability of tumor progression. It should be noted that this marker is characterized by high reactivity in stress, strong ionizing radiation, infectious diseases, infectious toxic shock, chemical toxic poisoning, chromosome and gene mutations. However, in our study, it was found that the ontogenesis of the liver is lagging behind in the background of preeclampsia, as well as the presence of secondary infectious factors, which is manifested by a high positive reaction of the P-53 marker. If the genetic apparatus of the cell is not damaged, then P-53 also occurs with a low positive reaction, and if DNA is damaged, P-53 is also activated. Thus, P-53 is activated when damaging factors accumulate in DNA. As a result of P-53 activation, the cell cycle stops and apoptosis occurs. The significance of the increased concentration of P-53 is that it rapidly replicates with DNA and damages the genetic apparatus, and this condition is considered to be the readiness of the cell to DNA damage. In well-differentiated cells with intact maturation, the latent P-53 protein is located in the cytoplasm of the cell. When the cell's proliferative activity increases, this P-53 protein translocates to the nucleus, and in the absence of stress on the cells, this protein is degraded within 5-20 minutes.

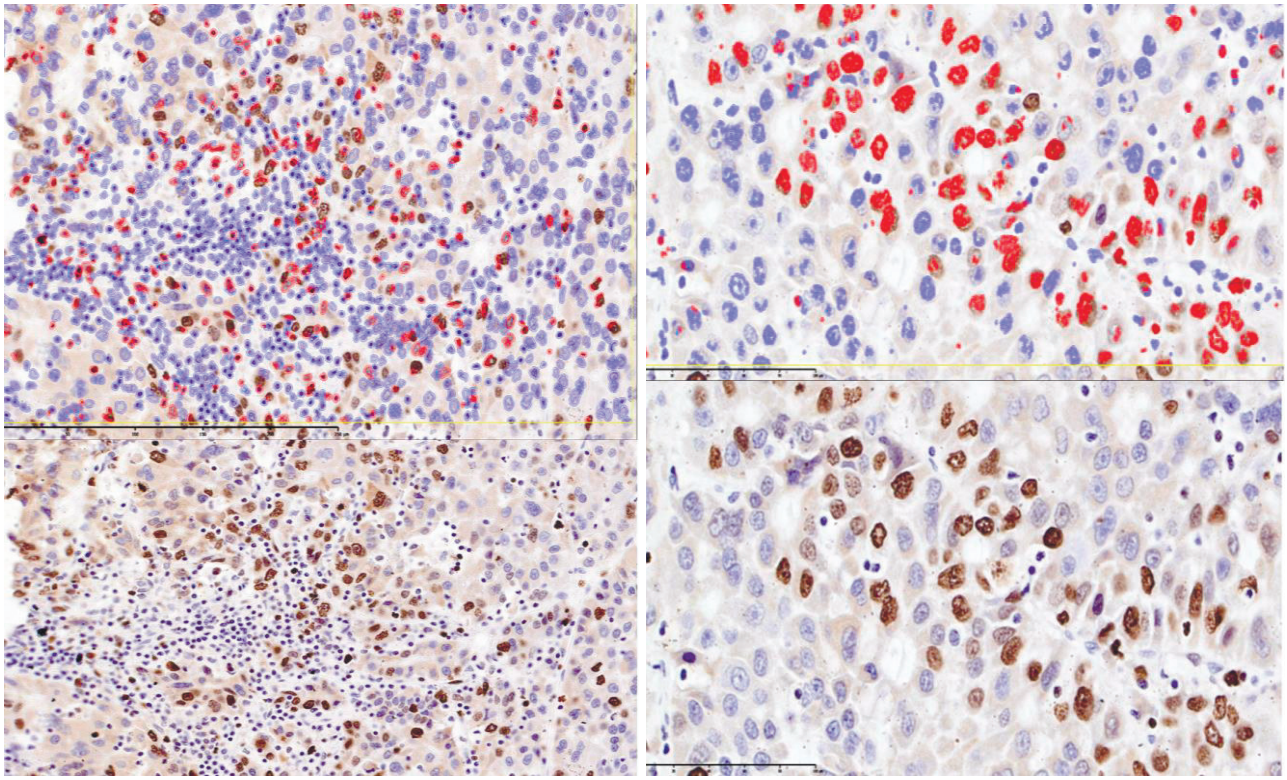

Fig. 3. A baby born with preeclampsia and died in the late neonatal period. Protocol 17-DI. High positive expression of R-53 marker. QuPath-0.4.0.ink. was scanned in the program and the level of expression was determined. Expressed cells are dark red. Paint Dab chromogenic.
The size is 10X10.

In our study, 71.1% of the subjects had high positive expression of the P-53 marker, indicating that the liver injury process is ongoing, and that the liver of infants born against the background of preeclampsia, as a result of toxic effects, causes damage to the nuclear structures of hepatocytes, which leads to the activation of this gene. In the early neonatal period, the highest level of positive expression of the P-53 marker was 26.8%, the lowest was 16.5%, and the average positive reaction was 19.3%, confirming the high level of liver injury. In the liver of infants born in the late neonatal period against the background of preeclampsia and living up to 8-28 days, the lack of tolerance to toxic substances, excessive accumulation of metabolites, manifested in the form of fatty and protein inclusions in the cytoplasm of hepatocytes, was manifested by the occurrence of cell decomposition, necrobiosis and induced apoptosis. Recall that in previous morphological studies, we have shown that in hematoxylin and eosin staining, apoptosis and necrosis processes occurred in parallel in hepatocytes. This, in turn, confirmed the fact that in IGH studies, in the late neonatal period, the P-53 marker was significantly lower than in the early neonatal period, confirming the fact that the damage process was proceeding rapidly.

Thus, in the late neonatal period, the positive rate of the P-53 marker is on average 22.9%, which leads to the clinical morphological manifestation of liver failure and hepatic coma.

CD-34 is a membrane protein expressed in cells of many tissues and is an intercellular adhesion molecule (adhesion between cells) involved in the early stages of hematopoiesis.

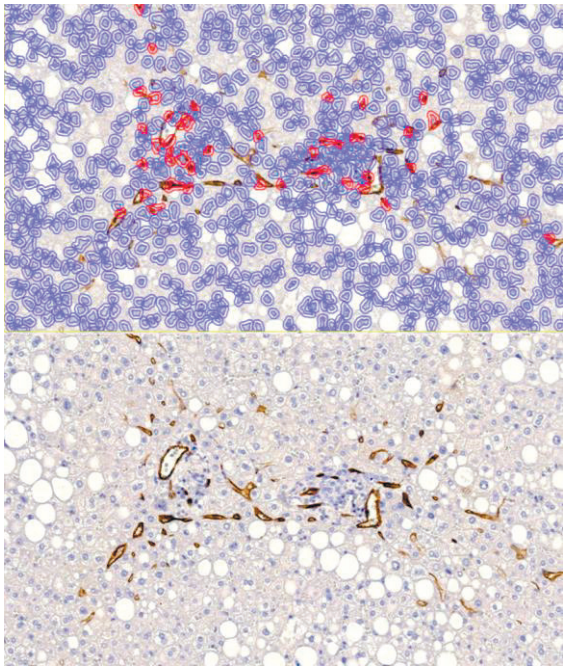

Fig. 4. Infant born with preeclampsia and dying in the early neonatal period. Protocol 17-DI. Low positive expression of the CD-34 marker. Scanned and the level of expression was determined using the QuPath-0.4.0.ink. program. Expressed cells are dark red. Staining Dab chromogen. Size 10X10.

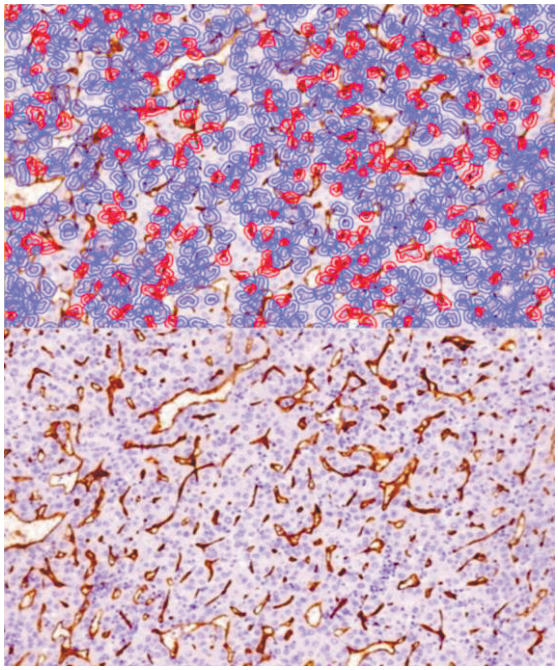

Fig. 5. Infant born with preeclampsia and dying in the late neonatal period. Protocol 17-DI. Low positive expression of the CD-34 marker. Scanned and the level of expression was determined using the QuPath-0.4.0.ink. program. Expressed cells are dark red. Staining Dab chromogen. Size 10X10.

It is a marker of vascular endothelium and is tested to assess the level of neoangiogenesis. Studying the processes of neoangiogenesis in benign and malignant tumors is an important promising direction in assessing tumor progression and developing antiangiogenic therapies.

The CD-34 marker was examined to assess the level of angiogenesis in reparative regeneration of liver damage in preeclampsia.

Low positive expression of the CD-34 marker in hemocapillaries in the liver confirms that in most cases, in areas of damage and secondary inflammation, fibroblasts, mainly from mesenchymal cells, are proliferating, and sparse fibrous connective tissue is formed in place of the lost vascular components.

Thus, in the early neonatal period, in most infants born against the background of preeclampsia, the number of cells undergoing necrosis and apoptosis in the liver under the influence of secondary infectious factors, and in areas where the stroma of the damaged segments is exposed, the transformation of endothelial cells into fibroblasts or the process of mesenchymal metaplasia is confirmed, confirming the proliferation of connective tissue in the liver.

In the late neonatal period, the intensification of this process and the parallel increase in the CD-34 marker by 5-6%, morphologically, in the damaged segments of the liver, small-caliber blood vessels are redeveloped mainly in the perilobular and periportal areas, which, as a result, confirms the slowing of blood circulation in the microcirculatory system in the liver.

CD-31 (PECAM-1) - PECAM-1 is involved in transendothelial migration of leukocytes, angiogenesis and integrin activation. In addition to the functions listed above, PECAM-1 serves as a mechanosensor of the cell. The purpose of the study is to determine that the protein molecules in the PECAM-1 marker are in a homophilic dimer state, and the molecule of one cell binds to the molecule of a neighboring cell, ensuring its stability and forming intercellular contacts. If mechanical stress is

observed between the cells and their separation from each other, this protein is synthesized in large quantities to bind them to each other. As a result, it ensures the stability of the state. In this case, the cytosolic state of the protein is associated with the actin filaments of the cell. Mechanical dilation of a vessel, for example due to increased blood flow, leads to the elongation of two interacting proteins relative to the actin filament within the cell.

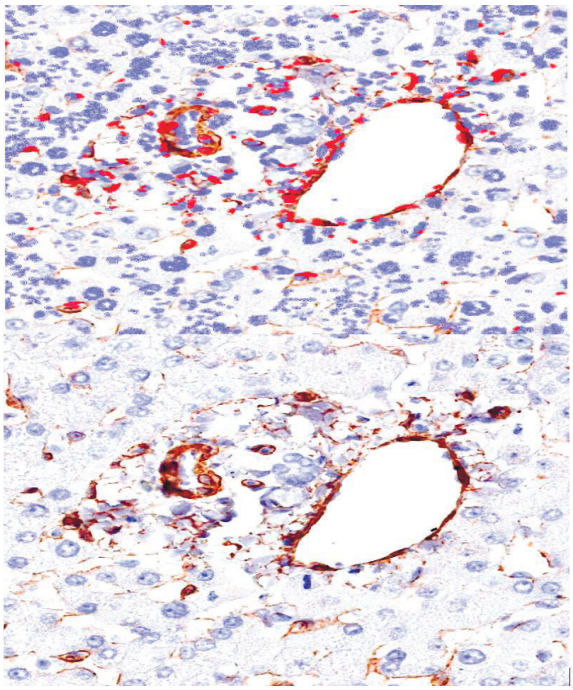

Fig. 6. Infant born with preeclampsia and dying in the early neonatal period. Protocol 17-DI. type. Low positive expression of the CD-31 PECAM marker. Scanned and the level of expression was determined using the QuPath-0.4.0.ink. program. Expressed cells are dark red. Staining Dab chromogen. Size 10X10.

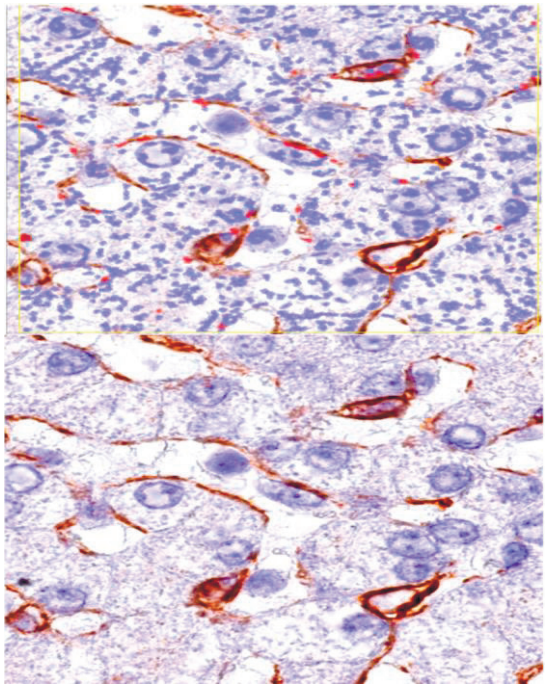

Fig. 7. Infant born with preeclampsia and dying in the late neonatal period. Protocol 17-DI. Low positive expression of the CD-31 PECAM marker. Scanned and the level of expression was determined using the QuPath-0.4.0.ink. program. Expressed cells are dark red. Staining Dab chromogen. Size 10X10.

This stretching leads to the action of tyrosines and their phosphorylation by tyrosine kinase, which activates the corresponding signaling pathway. Thus, the cellular response to changes in blood flow is morphologically manifested. In our study, these changes include a decrease in the number of hemocapillaries, compression of small-caliber vessels (recall that in preeclampsia, venous congestion and massive dystrophic processes in hepatocytes, varying degrees of expansion of sinusoids, parallel expansion of the spaces of Disse, leading to a pronounced violation of microcirculation, increased necrosis and apoptosis in hepatocytes, and the formation of sparse and coarse fibrous connective tissue). As a result, a sharp decrease in the positive reaction of this marker, clinically morphologically, leads to liver failure, and those born against the background of preeclampsia and die in the neonatal period mainly from liver failure. This is important in revealing the essence of our study and in interpreting the morphological basis of the resulting immunohistochemical changes.

In immunohistochemical studies, the CD-31 marker is a morphological marker of damage mainly to endothelial cells.

Specifically, it is confirmed that in the liver, it causes vasodilation of small vessels and simultaneously leads to functional tension of the active filament, a glycoprotein transmembrane protein, both extracellular and intracellular. It should be noted that the high positive expression of the

CD-31 (PECAM-1) marker by external factors is characterized by the binding of the membrane glycoprotein involved in the role of the active filament on the endothelial surface and the functional tension of endothelial cells, and in our study, the lack of a factor stimulating this process led to low positive expression.

In our study, in 76.8% of infants who died in the early neonatal period, low positive expression of the CD-31 (PECAM-1) marker was found, with a negative reaction in 23.2%.

In the late neonatal period, this indicator was expressed in 82.6% with low positive expression, and in 17.3 with a negative reaction. This, in turn, allows us to predict that in severe cases of hepatic preeclampsia, the process of damage begins during fetal development, mainly in dystrophic, necrobiotic forms, with a sharp narrowing of the sinusoids.

Thus, the lack of significant statistical differences in the low positive expression of the CD-31 (PECAM-1) marker in the early neonatal and late neonatal periods, characterized by a low positive reaction to this marker, and in clinically morphologically, in infants born against the background of preeclampsia, in assessing the morphofunctional indicators of the liver, of course, in the liver tissue, vascular endothelial growth factor in advance, and the use of angioprotective drugs, serves as one of the main criteria for diagnosing, saving the lives of infants and determining the economic and social effectiveness of treatment.

Conclusions. In infants born against the background of preeclampsia and dying in the early neonatal period, it was found that the predominance of the Ki-67 marker reaction of proliferative indicators occurred mainly in hepatocytes, while in the late neonatal period, the predominance of the P-53 marker was observed, which increased the apoptosis process and reduced the proliferative properties of hepatocytes, and the proliferation of fibrous connective tissue instead of vascular tissue was detected by the CD-31 and CD-34 markers.

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