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**EPITHELIAL-MESENCHYMAL TRANSFORMATION AS A
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NEWBORNS FROM MOTHERS, WHOSE PREGNANCY WAS
COMPLICATED BY IRON DEFICIENCY ANEMIA AND
PREECLAMPSIA**

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Introduction. Numerous diseases of the urinary system organs in children occur in the antenatal, intranatal and postnatal periods of the development. Children's health is determined by many factors, primarily the state of the health parents, the course of pregnancy and delivery. Preeclampsia and iron deficiency anemia are frequent pregnancy complications in Ukraine. Our earlier researches revealed morphofunctional changes in the urinary system organs of fetuses and newborns that developed under conditions of maternal preeclampsia and iron deficiency anemia, in particular sclerotic changes, the morphogenesis of which remains unknown today.

The purpose of the study was to search the signs of epithelial-mesenchymal transformation in the kidneys, ureters and bladder of fetuses and newborns that developed under conditions of maternal preeclampsia and iron deficiency anemia, and to assess its role in the sclerotic changes development in these organs.

Materials and methods. The study material was the tissue of the kidneys, ureters and bladder of full-term fetuses and newborns. Three groups were formed: I – fetuses and newborns from mothers with physiological pregnancy; II – fetuses and

newborns from mothers whose pregnancy was complicated by iron deficiency anemia; III – fetuses and newborns from mothers, whose pregnancy was complicated by preeclampsia. Immunohistochemical staining methods with monoclonal antibodies to vimentin, smooth muscle actin, desmin, cytokeratins 18 and 19 were used.

Results. The analysis of immunohistochemical reactions with monoclonal antibodies to epithelial (cytokeratins 18 and 19) and mesenchymal (vimentin, desmin, smooth muscle actin) markers revealed the signs of epithelial-mesenchymal transformation in the kidneys, ureters and bladder of fetuses and newborns, which were more pronounced in the cases of their development in conditions of maternal preeclampsia compared with iron deficiency anemia, increasing from the fetus to the newborn and with the enlargement of maternal pathology degree of severity, explaining the development of sclerotic changes in these organs.

Conclusions. Epithelial-mesenchymal transformation, which takes an active part in the development of such irreversible process as sclerosis, in the urinary system organs of fetuses and newborns, which developed in conditions of maternal preeclampsia and iron deficiency anemia, must be given due attention that in future will help to find new methods of prevention and treatment.

Key words: epithelial-mesenchymal transformation, preeclampsia, iron deficiency anemia, urinary system organs, sclerosis, fetus, newborn.

Introduction. In Ukraine the urinary system organs diseases in children remains an urgent problem because of the high prevalence, the risk of complications and disability [1]. In recent years in the Kharkiv region, which is one of the largest regions in Ukraine, a significant number of cases in the total pathology of the urinary system organs in the child population accounted for infections of the urinary system organs (interstitial nephritis, acute and chronic pyelonephritis, infection of the

urinary system organs without identification of its localization, acute cystitis), dysmetabolic nephropathy, congenital malformations of the urinary system organs, glomerulonephritis, vesicoureteral reflux and neurogenic dysfunction of the bladder [2].

Many diseases of the kidneys, ureters and bladder in children end with the sclerotic changes development in these organs [3]. Among the processes leading to the development of sclerosis in the urinary system organs, the role of epithelial-mesenchymal transformation has been proven. This is a complex multistep process characterized by the loss of epithelium of its phenotypic features (E-cadherin, which provides cell-cell adhesion and cell polarity, cytokeratins expression) and its acquisition of mesenchymal cell properties (fibroblast-like form, vimentin and smooth muscle actin expression, ability to invasion and extracellular matrix production) [4].

Numerous diseases of the urinary system organs in children occur in the antenatal, intranatal and postnatal periods of the development [5]. Children's health is determined by many factors, primarily the state of the health parents, the course of pregnancy and delivery [1].

Preeclampsia and iron deficiency anemia are frequent pregnancy complications in Ukraine [6, 7]. Our earlier researches revealed morphofunctional changes in the urinary system organs of fetuses and newborns that developed under conditions of maternal preeclampsia and iron deficiency anemia, in particular sclerotic changes [8, 9], the morphogenesis of which remains unknown today.

The purpose of the study was to search the signs of epithelial-mesenchymal transformation in the kidneys, ureters and bladder of fetuses and newborns that developed under conditions of maternal preeclampsia and iron deficiency anemia, and to assess its role in the sclerotic changes development in these organs.

Material and methods. The study material was the tissue of the kidneys, ureters and bladder of full-term fetuses and newborns. All cases were divided into three groups: I – fetuses (n=13) and newborns (n=15) from mothers with physiological pregnancy; II – fetuses (n=41) and newborns (n=44) from mothers whose pregnancy was complicated by iron deficiency anemia of varying degrees of severity; III – fetuses (n=38) and newborns (n=40) from mothers, whose pregnancy was complicated by preeclampsia of varying degrees of severity.

The immunohistochemical study was conducted according to standardized protocols using monoclonal antibodies to vimentin (clone V9), smooth muscle actin (clone 1A4), desmin (clone D33), cytokeratins 18 (clone CY-90) and 19 (clone A53-B/A2.26). Microspecimens were studied using microscope «Olympus BX-41».

Results and discussion. Vimentin is a member of the type III intermediate filaments and is a marker of mesenchymal origin cells. Vimentin intermediate filaments are polymers of soluble tetrameric vimentin. Vimentin filaments are involved in motility, maintenance of cell shape, and endurance of mechanical stress of mesenchymal cells [10].

In group I in the kidneys vimentin-positive cells were identified in the capsule, where they were located evenly, the nephrogenic zone and the organ stroma. In the kidneys stroma the expressed vimentin fibroblastic series cells were characterized in some fields of view by diffuse location, and in other fields of view by focal location. Also vimentin was expressed by endothelial cells of renal stroma vessels, epithelial cells of the inner leaf of the Bowman capsule, some mesangial cells and endothelial cells of glomerular capillaries. In the ureters and urinary bladder of fetuses and newborns of group I vimentin was expressed by the vascular endothelial cells, fibroblastic series cells located in the mucous membrane lamina propria, submucosa, the stroma of muscle layer and adventitia. In group I in the kidneys, ureters and bladder it was detected an age-related

increase the number of vimentin-positive cells in newborns compared to fetuses.

In groups II and III the immunohistochemical reaction with vimentin was more pronounced compared to group I, and the expression of this monoclonal antibody increased with age. It was noted that vimentin expressed, firstly, such structures like in group I, and, secondly, some epithelial cells of the outer leaf of the Bowman capsule, tubular apparatus of nephrons, some epithelial cells of the mucous membrane epithelial layer of the ureters and bladder. Besides, the expression of vimentin in the urinary system organs of fetuses and newborns of groups II and III increased with the enlargement maternal pathology degree of severity and was more pronounced in group III compared to group II.

Numerous studies noted an association between vimentin overexpression and organ sclerosis development [11].

Smooth muscle actin and desmin are smooth muscle tissue markers. Desmin is a muscular differentiation marker, which appears in early embryogenesis, whereas smooth muscle actin appears only after childbirth, therefore desmin is an evolutionarily earlier smooth muscle marker [4, 12].

In group I in the kidneys smooth muscle actin was expressed by myofibroblasts arranged in the capsule and organ stroma, smooth muscle cells of the stromal vessels, mesangial cells of smooth muscle type. In this group in newborns compared to fetuses it was observed an increase the number of stromal vascular smooth muscle cells and smooth muscle mesangial cells, but the number of myofibroblasts in the stroma decreased.

In the ureters and bladder of fetuses and newborns of group I smooth muscle actin was expressed by myofibroblasts, located in the mucous membrane lamina propria, submucosa, smooth muscle cells of the vessels and muscle layer. In these organs an age-related increase the number of smooth muscle cells of the vessels and muscle layer and a decrease the number of myofibroblasts were determined.

In group II and, especially, in group III compared to group I it was detected an increase the expression of smooth muscle actin in the fetuses and newborns kidneys. Also the expression of smooth muscle actin in the kidneys of fetuses and newborns of groups II and III increased with the maternal pathology degree of severity enlargement. This monoclonal antibody expressed by the epithelial cells of the outer leaf of the Bowman capsule, mesangial cells of the smooth muscle type, smooth muscle cells of the stroma vessels, myofibroblasts located in the organ stroma, some epithelial cells of the tubular apparatus of nephrons. In these groups in comparison with group I age-related increase the degree of immunohistochemical reaction with smooth muscle actin in the kidneys was also noted, but the number of myofibroblasts in the stroma was significantly greater and increased with age.

In the ureters and bladder of fetuses and newborns of groups II and III smooth muscle actin was expressed by some epithelial cells of the mucous membrane epithelial layer, myofibroblasts located in the mucous membranes lamina propria, submucosa, smooth muscle cells of the vessels and muscle layer. In these groups in the ureters and bladder changes of the severity of immunohistochemical reaction with smooth muscle actin were similar with the changes that were described in the kidneys.

In the kidneys, ureters and bladder of fetuses and newborns of groups I-III during the analysis of immunohistochemical reaction with the monoclonal antibody to desmin it was noted that this monoclonal antibody was expressed by the same structures as smooth muscle actin; characterized by similar changes of the severity of immunohistochemical reaction with smooth muscle actin in groups II and III compared to group I; in groups II and III expressed in the structures where smooth muscle actin was not detected (in the kidneys in the epithelium of the tubules and the outer leaf of the Bowman capsule, in the ureters and bladder in the epithelial cells of the mucous membrane epithelial layer).

It is known that myofibroblasts are metabolically and morphologically distinctive cells expressing smooth muscle actin which are formed from resident fibroblasts or due to epithelial-mesenchymal and endothelial-mesenchymal transformation [13]. These cells play a key role in the fibrotic response development. In an activated state myofibroblasts cease to proliferate and start to synthesize large amounts of extracellular component proteins [14].

Under the action of damaging factors in the kidneys podocytes also possess the property of epithelial-mesenchymal transformation [13].

Cytokeratins are intermediate filaments proteins that form the cytoskeleton of the epithelial cells [15]. It is known that the epithelial cells of various parts of the nephron tubule system express different cytokeratin molecular forms [3, 16].

In groups I-III cytokeratin 19 in the kidneys was expressed by the epithelial cells of the distal tubules, Henle loops and collecting tubules, in the ureters and bladder by the transitional epithelium of the mucous membrane; cytokeratin 18 in the kidneys was expressed by the epithelial cells of the proximal and distal tubules, descending and ascending thin tubules, collecting tubules, in the ureters and bladder by the transitional epithelium of the mucous membrane.

In all groups an age-related increase of the severity of the above-described immune histochemical reactions with cytokeratins was detected, but in group II and especially in group III this change was less pronounced. In group II and especially in group III compared to group I it was noted a decrease of the severity of reactions with cytokeratins, and this feature became more pronounced with the enlargement maternal pathology degree of severity.

Tubular epithelial cells as a result of the damaging factors action in the process of epithelial-mesenchymal transformation are transformed into the myofibroblasts which migrate into the

interstitium, participate in the production of the extracellular matrix components and sclerosis development [13].

In experimental studies it has been established that the contact of the tubular epithelium with the basement membrane stabilizes the epithelial phenotype, and the contact disturbance stimulates epithelial-mesenchymal transformation [17].

Thus, the analysis of immunohistochemical reactions with monoclonal antibodies to epithelial (cytokeratins 18 and 19) and mesenchymal (vimentin, desmin, smooth muscle actin) markers revealed the signs of epithelial-mesenchymal transformation in the kidneys, ureters and bladder of fetuses and newborns, which were more pronounced in the cases of their development in conditions of maternal preeclampsia compared with iron deficiency anemia, increasing from the fetus to the newborn and with the enlargement of maternal pathology degree of severity, explaining the development of sclerotic changes in these organs that we identified in our earlier researches [8, 9].

It is known that the processes of epithelial-mesenchymal transformation may occur for a long time and increase in the case of the gain the inducing factor action [17]. This fact helps to explain revealed by us the increase of this process signs in the kidneys, ureters and bladder of fetuses and newborns in the cases of maternal pathology degree of severity enlargement.

Conclusions

1. A comprehensive analysis of the immunohistochemical reactions revealed the sign of epithelial-mesenchymal transformation in the kidneys, ureters and bladder of fetuses and newborns, characterized by loss of the cells epithelial phenotype and acquisition of mesenchymal phenotype, which were more pronounced in the cases of their development in conditions of maternal preeclampsia and less pronounced in maternal iron deficiency anemia, increased from the fetus to the newborn and with the maternal pathology degree of severity enlargement.

2. Epithelial-mesenchymal transformation, which takes an active part in the development of such irreversible process as

sclerosis, in the urinary system organs of fetuses and newborns, which developed in conditions of maternal preeclampsia and iron deficiency anemia, must be given due attention that in future will help to find new methods of prevention and treatment.

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