THE NEW ARMENIAN MEDICAL JOURNAL
Vol.11 (2017), No 2, p.33-39

THE FEATURES OF SMOOTH MUSCLE ACTIN EXPRESSION IN THE KIDNEYS, URETERS AND BLADDER OF THE NEWBORNS EXPOSED TO CHRONIC INTRAUTERINE, ACUTE POSTNATAL AND MIXED HYPOXIA

Sorokina I.V.*, Myroshnychenko M.S., Korneyko I.V.

Department of Pathological Anatomy, Kharkiv National Medical University, Kharkiv, Ukraine

Received 5/04/2016;accepted for printing 18/06/2017

Abstract

The study aimed to determine the characteristics of smooth muscle actin expression in the kidneys, ureters and bladder of the newborns exposed to experimental chronic intrauterine, acute postnatal and mixed hypoxia.

In this study an experiment was carried out on WAG rats on modeling chronic intrauterine, acute postnatal and mixed hypoxia. The material of the study was the tissue of the kidney of newborn rats. Immunohistochemical study was performed by the standard method using monoclonal antibodies against smooth muscle actin. Morphometrical investigation was conducted while studying the slides.

In newborns, chronic intrauterine and mixed hypoxia result in significant reduction of mean value of the muscular fiber thickness in the muscular layer of the ureter and bladder, as well as in non-uniform expression of smooth muscle actin by the muscular layer cells of these organs. Acute postnatal hypoxia does not produce a damaging effect on the qualitative and quantitative characteristics of the muscular layer of the ureter and bladder of the newborns. In infants, acute postnatal hypoxia does not affect the mean value of the muscular fiber thickness in the arterioles and venules of the kidneys, ureters and bladder, while chronic intrauterine and mixed hypoxia result in a significant reduction of these indicators. Chronic intrauterine hypoxia and mixed hypoxia amplify severity of expression of smooth muscle actin by myofibroblasts in the kidneys, ureters and bladder, as well as by mesangiocytes, glomerular epithelial cells, and epithelial cells of renal tubules, which can lead to the further development of sclerotic changes in these organs in children at different stages of ontogenesis.

The analysis of the features of smooth muscle actin expression in the urinary system organs of newborns found that chronic intrauterine and mixed hypoxia have a damaging effect on the urinary system organs of newborns while acute postnatal hypoxia has no damaging effect.

Keywords: *kidney, ureter, bladder, newborn, hypoxia, smooth muscle actin, experiment.*

Introduction

At present, pathology of the urinary system in children not only loses its relevance, but also remains a serious and significant problem in medicine. Epidemiological studies in Ukraine and in the countries around the world testify to the widespread disease of the urinary system among children [*Makovetskaia G, Kozlova T, 2000; Harambat J et al., 2012; Kolibaeva T et al., 2013*]. Nowadays, the researchers note an atypical clinical picture of the urinary system diseases in children, prevalence of both chronic, latent forms and manifest, aggressive, severe forms of the disease that are resistant to conventional therapies, combination of different pathologies of this system in one patient against a background of anomalies, dismetabolic disorders and microbial-inflammatory pro-

Address for Correspondence:

Irina V. Sorokina
4 Nauky Avenue, Kharkiv 61022, Ukraine
Tel.: (+38 050) 1699763
E-mail: msmyroshnychenko@ukr.net

cesses [*Chugunova O, Panova L, 2010; Kolibaeva T et al., 2013*]. Pathology of the urinary system in children dramatically reduces their quality of life, in some cases leads to development of chronic renal failure, requiring the use of expensive methods of replacement therapy [*Chugunova O, Panova L, 2010; Erman M, Pervunina T, 2012*].

Currently, the concept of a close relationship between the development of the urinary system pathology in children and the effect of different unfavorable factors in the antenatal, intranatal or postnatal periods of the organism development is intensively developing [*Erman M, Pervunina T, 2012; Koleganova N et al., 2012; Gafarova F, 2015*]. The most frequent cause of problems in an embryo, fetus and newborn is oxygen deprivation or hypoxia [*Shevchenko L et al., 2011*], which can be acute, chronic, or mixed. Acute hypoxia occurs at premature detachment of the placenta, development of multiple infarctions in it, loss of the umbilical cord, umbilical cord knots or its loops around the neck or limbs of the fetus, leading to acute disorders of uteroplacental or fetoplacental circulation. Chronic fetal hypoxia is a manifestation of chronic placental insufficiency, which develops due to the presence of various maternal genital or extragenital pathologies [*Gilany K, Vafakhah M, 2010*].

According to different authors, under the influence of hypoxia on the organism of the infant, the signs of the urinary system involvement in the pathological process are diagnosed in 80% of cases [*Pogodaeva T, Luchaninova V, 2012; Dorey E et al., 2014*]. As a result of hypoxia the child can develop respiratory distress syndrome, central nervous and cardiovascular systems, disorders, which further increase the chances of the damage to the urinary tract organs in these children [*Galyant O et al., 2013*]. The reason is simple: the kidneys are integrating bodies; therefore, whatever disease develops in a child, the organs of urine production and excretion suffer in varying degrees [*Pogodaeva T, Luchaninova V, 2012; Alaro D et al., 2014*].

It should be noted that the literature data on the effect of hypoxia on the urinary system organs in fetuses and newborns are unstructured, in the majority of cases they have clinical orientation and are not morphologically confirmed [*Gupta B et al., 2005; Makovetskaia G, Kozlova T, 2000; Shunkina G, 2010; Erman M, Pervunina T, 2012; Koleganova N et al., 2012*]. In addition, the data on the effect of acute postnatal and mixed hypoxia on the structural and functional features of the urinary system in newborns are absent. All this testifies to the urgency and necessity of present research.

The study aimed to determine the characteristics of smooth muscle actin expression in the kidneys, ureters and bladder of the newborns exposed to experimental chronic intrauterine, acute postnatal and mixed hypoxia.

Material and methods

This study involved an experiment simulation of high-altitude hypoxia in Wistar Albino Glaxo rats at the experimental biological hospital of Kharkiv National Medical University. The study was approved by the Commission on Ethics and Bioethics of Kharkiv National Medical University and conforms to the principles of the Guide for the Care and Use of Laboratory Animals published by US NIH (No 85-23, revised in 1985) [*International Ethical Guidelines for Biomedical Research Involving Human Subjects, 1993*].

High-altitude hypoxia was simulated using a sealed pressure chamber, from which the air was pumped out creating the conditions of sharp reduction in the atmospheric pressure. The rats were placed daily for 20 minutes at the same time in the conditions corresponding to the altitude of 7500 *m* with the respective pressure of 287 *mm Hg*.

The animals were divided into four groups: I (control) – pregnant female rats (n=3) were not subjected to high-altitude hypoxia and the resulting offspring from them (n=11) in the first day after birth were taken out of the experiment, II (simulation of chronic intrauterine hypoxia) – pregnant female rats (n=4) were exposed to high altitude hypoxia and the resulting offspring from them (n=10) in the first day of life were taken from the experiment, III (modeling of acute postnatal hypoxia) – pregnant female rats (n=2) were not subjected to high-altitude hypoxia, however, the resulting offspring from them (n=8) in the first day of life were exposed to single high-altitude hypoxia and then taken out of the experiment, IV (modeling mixed hypoxia) – pregnant female rats (n=3) during pregnancy were exposed to high-altitude hypoxia, and then their offspring (n=8) in the

first day of life were exposed to single high-altitude hypoxia and taken out of the experiment.

The materials of the study were the kidneys of the newborns. Immunohistochemical investigation was performed by standard methods using monoclonal antibodies against smooth muscle actin (DAKO, Denmark). These slides were examined under "Olympus BX-41" microscope (Japan) with processing using "Olympus DP-soft version 3.1" software (Japan), which determined the thickness of the muscular fibers, the cells of which expressed smooth muscle actin in the muscular layer of the ureter and bladder, as well as arterioles and venules of the kidneys, ureter, and bladder of the rats. In other cases, to assess the grade of immunohistochemical reaction, a semiquantitative scale, i.e. "+" – poor, "++" – fair, "+++" – pronounced reaction, was used.

Non-parametric U-Mann-Whitney criterion was used for statistical evaluation of the values obtained in the groups. Significance of differences between the values was taken at significance level of $p < 0.05$. Statistical calculations were performed using Statistic Soft 6.0 and Microsoft Excel 2007 software.

Results and discussion

All four groups demonstrated smooth muscle actin expression on immunohistochemical investigation in the form of a clear brown cytoplasmic staining of the smooth muscle cells that formed muscular fibers of the muscular layer of the bladder and ureter (Fig. 1). The analysis of muscle fiber thickness in the ureter and bladder (Table 1) revealed a significant decrease in this indicator in II and IV groups compared with I group, which suggests of the damaging effect of chronic intrauterine and mixed hypoxia on quantitative parameters of the muscular membrane of the ureter and bladder in the newborns that can manifest by certain functional disorders in these organs. While comparing to I group, in III group significant differences were absent in terms of the mean value of the thickness of the muscular fibers in the muscular layer of both ureter and bladder, which suggests that acute postnatal hypoxia does not have a damaging effect on the quantitative indicators of the muscular membrane of the ureter and bladder in newborns. In II and IV groups, no significant differences were observed in terms of mean thickness of the muscular fiber in the muscular layer of the ureter and bladder.

Table 1

Mean value of the muscular fiber thickness in the muscular layer of the ureter and bladder in newborn rats of four groups ($\times 10^{-6} m$)

Groups	Location	
	Ureter	Bladder
I	8.09±0.547	12.55±0.562
II	5.20±0.327 $p_1<0.05$	8.50±0.543 $p_1<0.05$
III	8.38±0.497 $p_1>0.05$ $p_2<0.05$	11.95±0.563 $p_1>0.05$ $p_2<0.05$
IV	4.75±0.250 $p_1<0.05$ $p_2>0.05$ $p_3<0.05$	8.13±0.295 $p_1<0.05$ $p_2>0.05$ $p_3<0.05$

Notes: *p_1 – significance of differences compared to I group; p_2 – significance of difference compared to II group; p_3 – significance of difference compared to III group.*

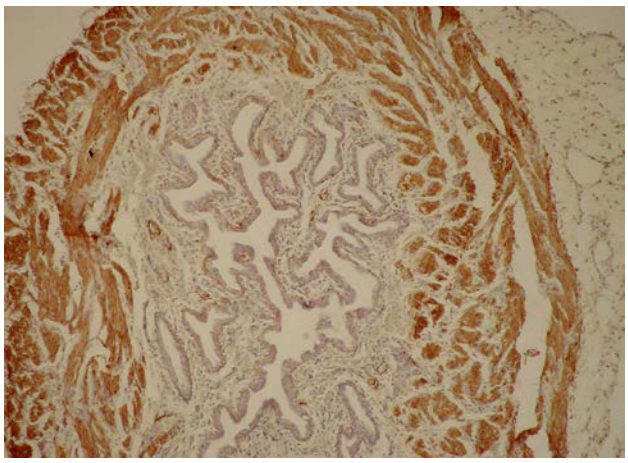

Figure 1. *Expression of smooth muscle actin in the ureter of the newborn exposed to chronic intrauterine hypoxia. Peroxidase reaction with monoclonal antibodies to smooth muscle actin, ×100*

In II and IV groups, the muscular fibers of the muscular layer of the ureter and bladder demonstrated heterogeneity in expression of smooth muscle actin. Thus, some muscular fibers expressed smooth muscle actin well, while the others expressed it slightly or moderately. Uneven expression by the muscular fibers of smooth muscle actin is possible due to the fact that some of the fibers develop dystrophic and atrophic changes induced by chronic intrauterine or mixed hypoxia, while the others develop hypertrophy of the muscular fi-

bers, which is a unique compensatory-adaptive response necessary to compensate for the quantitative deficiency of smooth muscle cells resulting from their destruction and elimination under the action of the above damaging factor.

It is known that smooth muscle tissue is an obligatory structural and functional component of the wall of the bladder and ureters; it plays an important role in their normal function. Smooth muscle cells are traditionally classified into two main types – visceral and vascular. Visceral smooth muscle cells are referred to the so-called "phase" smooth muscle cells, which are electromechanically paired and function as a single syncytium. The action potential in this syncytium is transferred from one smooth muscle cell to another through gap junctions. An important feature of these cells is the ability of a small number of cells spontaneously to generate action potential, i.e. to be pacemakers. The vascular smooth muscle cells are "tonic" and function as individual units [*Lushnikova E et al., 2012*].

Expression of smooth muscle actin by the smooth muscle cells that form muscular fibers of the tunica of the arterioles and kidney venules of the kidneys, ureters and bladder was identified. In the urinary organs in all groups, the muscle fibers were significantly thicker in the wall of the arterioles compared to the venules, which is a variant of the norm and is due to their functional characteristics (Table 2). The analysis also revealed a significant decline in the mean muscular fiber thickness

Table 2

Mean value of the muscular fiber thickness in the arterioles and venules of the kidney, ureter and bladder in newborn rats of four groups ($\times 10^{-6} m$)

Location	Vessel type	Groups			
		I	II	III	IV
Ureter	Arteriole	2.19±0.229	1.51±0.270 p_1<0.05	2.15±0.073 p_1>0.05 p_2<0.05	1.49±0.050 p_1<0.05 p_2>0.05 p_3<0.05
	Venule	1.38±0.189 p_4<0.05	0.88±0.107 p_1<0.05 p_4<0.05	1.35±0.056 p_1>0.05 p_2<0.05 p_4<0.05	0.83±0.117 p_1<0.05 p_2>0.05 p_3<0.05 p_4<0.05
Bladder	Arteriole	2.92±0.207	2.20±0.198 p_1<0.05	2.94±0.080 p_1>0.05 p_2<0.05	2.24±0.081 p_1<0.05 p_2>0.05 p_3<0.05
	Venule	2.11±0.160 p_4<0.05	1.49±0.239 p_1<0.05 p_4<0.05	2.16±0.082 p_1>0.05 p_2<0.05 p_4<0.05	1.52±0.059 p_1<0.05 p_2>0.05 p_4<0.05
Kidney	Arteriole	1.79±0.048	1.19±0.059 p_1<0.05	1.70±0.046 p_1>0.05 p_2<0.05	1.10±0.073 p_1<0.05 p_2>0.05 p_3<0.05
	Venule	1.28±0.086 p_4<0.05	0.73±0.030 p_1<0.05 p_4<0.05	1.20±0.057 p_1>0.05 p_2<0.05 p_4<0.05	0.70±0.060 p_1<0.05 p_2>0.05 p_3<0.05 p_4<0.05

Notes: *p_1 – significance of differences compared to I group; p_2 – significance of difference compared to II group; p_3 – significance of difference compared to III group; p_4 – significance of difference compared to the arteriole in this group.*

in the arterioles and venules of the kidney, ureter and bladder in the newborns from II and IV groups, compared to I group, which indicates the damaging effect of chronic intrauterine and mixed hypoxia on the muscular component of the vascular walls of microcirculatory basin that can disrupt tissue nourishment in these organs and further result in sclerotic changes in them. Acute postnatal hypoxia does not produce a damaging effect on quantitative indicators of muscular fibers of the arterioles and venules of the urinary system in newborns. In the newborns from II and IV groups, no significant differences were observed in terms of the mean value of thickness of the muscular fibers of arterioles and venules.

The kidneys, ureters and bladder of the newborns from I group demonstrated moderate expression of smooth muscle actin by myofibroblasts, which were defined in the stroma of these organs (Fig. 2, 3). Myofibroblasts were identified in the process of development and maturation of the urinary system of humans and laboratory animals by many researchers [*Carey A et al., 1992; Naruse K et al., 2000*].

It is known that myofibroblasts are main profibrogenic cells characterized by unique functional capabilities. Thus, myofibroblasts are able to produce a number of key components of the extracellular matrix, including I, III, V, VII type collagens. Possibility of production of the basal membrane components, i.e. type IV collagen and laminin by myofibroblasts have been proven. In addition, this cell line produces a wide range of sulfated proteoglycans of the matrix and basement membrane (in particular, decorin, nidogen and perlecan) influencing the migratory and proliferative anility of the connective tissue cells and epithelium. Important products of myofibroblast secretion are fibronectin and tenascin-C. In addition to the components of the extracellular matrix, myofibroblasts produce a great variety of metalloproteinases and their tissue inhibitors, which play an important role in matrix remodeling, cellular migration activity regulation [*Barinov E, Sulayeva O, 2010; Lawson J et al., 2015*].

No specific features of smooth muscle actin expression by myofibroblasts were detected in III group 3 compared to I group. In II and IV groups, compared with I group, marked expression of smooth muscle actin by myofibroblasts was revealed, which may have occurred as a result of proliferation and activation of resident fibroblasts exposed to simulated chronic intrauterine and mixed hypoxia, which change their phenotype (begin to express smooth muscle actin) and secrete matrix proteins [*Strutz F, Zeisberg M, 2006; Lawson J et al., 2015*]. Additionally, the increase in the number of myofibroblasts may occur due to the changes in the phenotype of tubular epithelial cells during epithelial-mesenchymal transformation, which can be explained by the detected clear cytoplasmic staining of some tubular epithelial cells. Epithelial-mesenchymal transformation begins with destruction of the tubular basement membrane exposed to hypoxia and other damaging factors, as well as certain cytokines. The cells

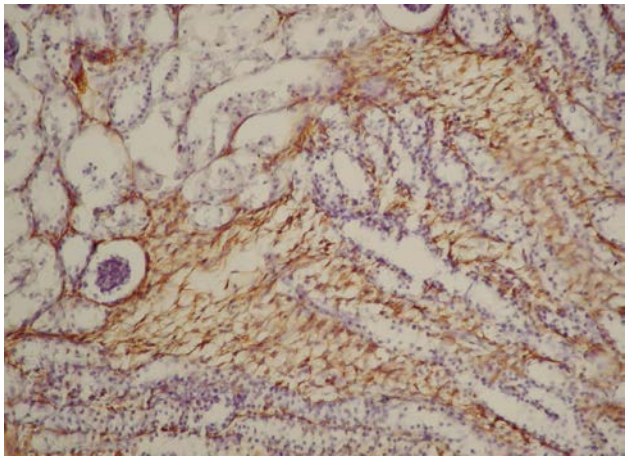

Figure 2. *The expression of smooth muscle actin in the kidney of the newborn exposed to mixed hypoxia. Peroxidase reaction with monoclonal antibodies to smooth muscle actin, ×200*

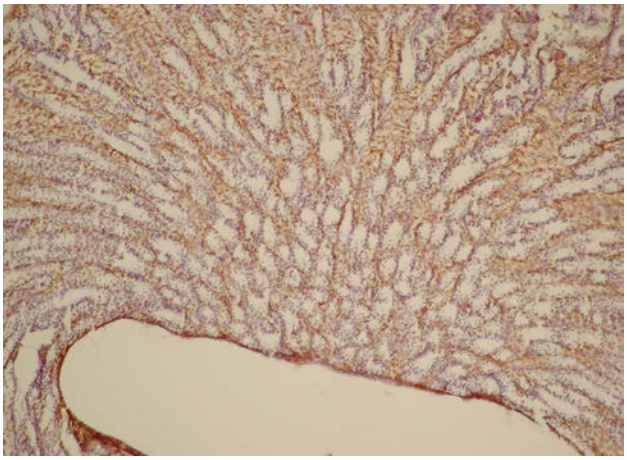

Figure 3. *The expression of smooth muscle actin in the kidney of the newborn exposed to chronic intrauterine hypoxia. Peroxidase reaction with monoclonal antibodies to smooth muscle actin, ×100*

lose apical-basal polarity, begin to express mesenchymal markers and reduce the expression of epithelial markers, then change their shape due to the changes in endogenous cytoskeleton, migrate into the inter-tubal space, turning into activated myofibroblasts [*Barinov E, Sulayeva O, 2010; Puchinskaya M, 2015*].

In I and III groups, some glomeruli demonstrated a small amount of mesangiocytes expressing smooth muscle actin, which has been described by many researchers studying the process of kidney development [*Carey A et al., 1992; Naruse K et al., 2000*]. In II and IV groups, the glomeruli demonstrated moderate to pronounced expression of smooth muscle actin by not only mesangiocytes but also the endothelial cells, possibly, due to the negative effect of chronic intrauterine and mixed hypoxia, which will lead to the future development of sclerotic changes in the urinary system of the child.

Conclusion

In newborns, chronic intrauterine and mixed hypoxia result in significant reduction of mean value of the muscular fiber thickness in the muscular layer of the ureter and bladder, as well as in non-uniform expression of smooth muscle actin by the muscular layer cells of these organs. Acute postnatal hypoxia does not produce a damaging effect on the qualitative and quantitative characteristics of the muscular layer of the ureter and bladder of the newborns.

In infants, acute postnatal hypoxia does not affect the mean value of the muscular fiber thickness in the arterioles and venules of the kidneys, ureters and bladder, while chronic intrauterine and mixed hypoxia result in a significant reduction of these indicators.

Chronic intrauterine hypoxia and mixed hypoxia amplify severity of expression of smooth muscle actin by myofibroblasts in the kidneys, ureters and bladder, as well as by mesangiocytes, glomerular epithelial cells, and epithelial cells of renal tubules, which can lead to the further development of sclerotic changes in these organs in the children at different stages of ontogenesis.

REFERENCES

1. *Alaro D, Bashir A, Musoke R, Wanaiana L*. Prevalence and outcomes of acute kidney injury in term neonates with perinatal asphyxia. Afr Health Sci. 2014; 14(3): 682-688.
2. *Barinov EF, Sulayeva ON*. [Gastrointestinal myofibroblasts: role in regulation of physiological activity and reparation of gastro-intestinal tract] [Published in Russian]. Russian journal of gastroenterology, hepatology and coloproctology. 2010; 20(3): 9-18.
3. *Carey AV, Carey RM, Gomez RA*. Expression of α-smooth muscle actin in the developing kidney vasculature. Hypertension. 1992; 19(2): II168-II175.
4. *Chugunova OL, Panova LD*. [Risk factors and diagnosis of the urinary system diseases in newborns] [Published in Russian]. Russian herald of perinatology and pediatrics. 2010; 1: 12-20.
5. *Dorey ES, Pantaleon M, Weir KA, Moritz KM*. Adverse prenatal environment and kidney development: implications for programing of adult disease. Reproduction. 2014; 147(6): R189-R198.
6. *Erman MV, Pervunina TM*. [Fetal programming of renal function] [Published in Russian]. Practical medicine. 2012; 9(65): 56-60.
7. *Gafarova FM*. [Correlation of extragenital pathology of parturient women and the state of newborns' health] [Published in Russian]. Practical medicine. 2015; 7(92): 45-48.
8. *Galyant OI, Senkevich OA, Satsko LV, Kirsheva TYu*. [Multi-organ damage in newborns with hypoxic-ischemic brain injuries] [Published in Russian]. Far East Medical Journal. 2013; 3: 58-60.
9. *Gilany K, Vafakhah M*. Hypoxia: a review. JPS. 2010; 1(2): 43-60.
10. *Guide for the care and use of laboratory animals*. Bethesda, Md.: U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health, 1985. NIH publication no. 85-23. Rev. 1985. 83p.
11. *Gupta BD, Sharma P, Bagla J, Parakh M, Soni JP*. Renal failure in asphyxiated neonates. Indian Pediatr. 2005; 42(9): 928-934.

12. *Harambat J, van Stralen KJ, Kim JJ, Tizard EJ*. Epidemiology of chronic kidney disease in children. Pediatr Nephrol. 2012; 27: 363-373.

13. *International Ethical Guidelines for Biomedical Research Involving Human Subjects*. Prepared by the Council for International Organizations of Medical Sciences (CIOMS) in collaboration with the World Health Organization (WHO). Geneva, Switzerland. 1993. 61p. ISBN 92 9036 056 9.

14. *Koleganova N, Benz K, Piecha G, Ritz E, Amann K*. Renal, cardiovascular and metabolic effects of fetal programming. Nephrol Dial Transplant. 2012; 27(8): 3003-3007.

15. *Kolibaeva TF, Markovsky VD, Sorokina IV, Myroshnychenko MS, Podvalnaya NA*. [Structure of urinary system pathology in children: regional aspect] [Published in Russian]. Modern pediatrics. 2013; 6(54): 153-157.

16. *Lawson J, Lawson J, Elliott J, Wheeler-Jones C, Syme H, Jepson R*. Renal fibrosis in feline chronic kidney disease: known mediators and mechanisms of injury. Vet J. 2015; 203(1): 18-26.

17. *Lushnikova EL, Nepomnyashchikh LM, Neimark AI, Isaenko VI, Molodykh OP, Koldysheva EV, Zhuk AG, Likhachev AG*. [Role of structural-functional reorganizations of detrusor and prostatic smooth muscle cells in development of overactive bladder] [Published in Russian]. Fundamental research. 2012; 5: 68-73.

18. *Makovetskaia GA, Kozlova TV*. [On kidney diseases in newborns and first months of life children] [Published in Russian]. Nephrology and Dialysis. 2000; 2(1-2): 51-54.

19. *Naruse K, Fujieda M, Miyazaki E, Hayashi Y, Toi M, Fukui T, Kuroda N, Hiroi M, Kurashige T, Enzan H*. An immunohistochemical study of developing glomeruli in human fetal kidneys. Kidney Int. 2000; 57(5): 1836-1846.

20. *Pogodaeva TV, Luchaninova VN*. [Prediction of the formation of kidney diseases in the fetus and newborn] [Published in Russian]. Russian herald of perinatology and pediatrics. 2012; 4(1): 75-80.

21. *Pogodaeva TV, Luchaninova VN*. [Study of the homeostatic functions kidney for prediction newborns nephropathies] [Published in Russian]. Fundamental research. 2012; 10: 290-295

22. *Puchinskaya MV*. [Epithelial-mesenchymal transition in health and disease] [Published in Russian]. Archives of Pathology. 2015; 1: 75-83.

23. *Shevchenko LI, Znamenskaya TK, Rozova EV*. [Influence of the hypoxic states of different genesis on the development of fetus and flow of early neonatal period at new-born] [Published in Russian]. Neonatology, surgery and perinatal medicine. 2011; 1(1): 113-118.

24. *Shunkina GL*. [Role of biochemical investigations in assessment of the newborn kidney function damage after a hypoxia] [Published in Russian]. MTM. 2010; 4: 104-106.

25. *Strutz F, Zeisberg M*. Renal fibroblasts and myofibroblasts in chronic kidney disease. J Am Soc Nephrol. 2006; 17(11): 2992-2998.