THE ROLE OF ENDOTHELIAL SYNTHASE (eNOS) IN THE PATHOGENESIS OF HYPOXIC-ISCHEMIC INJURIES BRAIN DAMAGE IN PREMATURE INFANTS

Asadova T. A.

Abstract. 102 premature infants with perinatal hypoxic-ischemic CNS lesions from mothers with a burdened obstetric history aged from 1 day to 1 month of life were examined. Most of the children were born to mothers under the age of 35-72 (70.6%), the rest were over 35 – 30 (29.4%). Premature children of the male sex were 53 (52%), female – 49 (48%); residents of the city – 70 (68.7%), the village – 32 (31.3%). The examination of premature infants was carried out on days 1-3, 5-7, and again on days 10-14. The following research methods were used: clinical, morphofunctional immaturity parameters, anthropometric indicators, functional state of organs and systems. The gestational age (g/v) of infants was determined based on the mothers ' anamnesis and ultrasound results. Endothelial nitric oxide synthase (eNOS or NOS-3) was determined by enzyme immunoassay (ELISA). Studies were carried out: RN-diagnostics of the chest organs, NSG; Doppler studies of the vascular system ; EchoCG, ECG.

Results. Cerebral ischemia (CI) of the first stage was detected in n=51; CI of the second stage in n=32; CI of the third stage in n=19 premature infants, respectively. To identify the role of vasoregulatory mechanisms in CNS damage, the nitric oxide synthase system was studied, in particular, the role of endothelial synthase (eNOS) in 71 premature infants, depending on g/v and the severity of CI.

eNOS has been shown to play a leading role in the development of cerebral ischemia. It was revealed that the increased expression and activation of eNOS directly depends on the gestational age of the infant. The more pronounced the effect of hypoxia and ischemia, the more pronounced the violation of the ability of adequate function and structure of the endothelial cells of the brain vessels, which contributes to the occurrence of cerebral ischemia of varying severity, leading to serious consequences.

Conclusions. In the pathogenesis of hypoxic ischemic encephalopathy, eNOS is the main indicator of the processes of endothelial dysfunction of the cerebral vessels, simultaneously reflecting both its cause and effect.

Key words: prematurity, pathology of the central nervous system, hypoxic lesion of the central nervous system, endothelial synthase eNOS.

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DETERMINATION OF THE DEGREE OF THE KIDNEY INJURY IN LOW BIRTH WEIGHT INFANTS DEPENDING ON THE CORRESPONDENCE OF THEIR ANTHROPOMETRIC PARAMETERS TO GESTATIONAL AGE

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The connection of the publication with planned research works. This work is fragment of the dissertation for the degree of Doctor of Philosophy in medicine "Early diagnosis and prognosis of ischemic nephropathy in low birth weight infants".

Introduction. Low birth weight newborns' (LBW) pathology accounts for a significant portion of the causes of perinatal morbidity and mortality. According to the American Academy of Pediatrics, Acute Kidney Injury affects 12.5-18% of low-birth-weight infants, with a mortality rate of 42% relative to low-birth-weight infants that do not have kidney damage (5%). SGA (Small for gestational age) newborns hold a unique position among LBW children, since this group of children suffers from chronic intrauterine hypoxia due to a variety of pathological causes. This suggests that the hypoxia factor affects them for a longer period of time, and they have less nephrons at birth, resulting in residual nephron hypertrophy and hyperfiltration. In the context of other pathological conditions, such as chronic hypoxia and asphyxia, a kidney functioning in the hyperfiltration mode is more susceptible to the effects of various pathological factors [1, 2].

There is a high incidence of ischemic nephropathy (IN), a pathology that is a manifestation of kidney damage, in newborns, especially premature SGA infants, who have experienced perinatal asphyxia [3, 4].

The early detection of this disorder in newborns is difficult as the changes in the kidneys are often "masked" as signs of other diseases [5]. The commonly accepted kidney injury criteria, based on the urinary syndrome markers, do not allow for an early diagnosis of the severity and the localization of glomerular or tubular lesions [6].

The discovery of biomarkers associated with the early stages of nephropathy that are independent of the kidneys' filtration capability, is linked to the pursuit for new and improved methods of diagnosing hypoxic kidney damage.

KIM-1 (Kidney Injury Molekule-1) and NGAL (Neutrophil Gelatinase-Associated Lipokalin), as well as Cystatin C, a marker of glomerular function disorder, are currently the most promising biomarkers of ischemic nephropathy.

KIM-1 (Kidney Injury Molecule) is a transmembrane glycoprotein that is not found in the urine in a healthy kidney but is synthesized in high concentration by epithelial cells of the proximal tubules after ischemic damage and persists until tubular function is completely restored [7].

The presence of KIM-1 enables epithelial cells to identify and engage in the utilization of postischemic kidney dead cells. KIM-1 is a phosphatidylserine receptor that detects apoptotic cells and converts proximal tubular epithelial cells into "semi-professional" phagocytes [8, 9].

KIM-1 has been added to a short selection of kidney injury markers used in drug investigation protocols by the FDA and EMEA (American and European Medicines Control Associations).

NGAL is a neutrophil gelatinase associated lipocalin. This protein has a molecular weight of 25 kDa and exists as a monomer, homodimer, or heterodimer in urine and neutrophils. It is linked to matrix metalloproteinase-9. Many organs, such as the lungs and liver, release NGAL into the blood plasma when they are affected. However, tubular injury associated with ischemia in acute renal failure (ARF) due to acute tubular necrosis or tubulointerstitial nephropathy causes an early and sharp increase in urine and serum NGAL levels. NGAL synthesis is induced by inflammation in epithelial cells, including proximal tubules [10].

The thick segment of the ascending limb of the loop of Henle and the collecting ducts secrete NGAL into the urine, where it provides antimicrobial and antioxidant defense by iron chelation.

Since glomeruli filter plasma NGAL freely, a decrease in glomerular filtration due to renal pathology causes NGAL to accumulate in the systemic circulation [11].

Serum Cystatin C is a cysteine proteinase inhibitor with a low molecular weight. In comparison to creatinine, the amount of this inhibitor is determined solely by the glomerular filtration rate, which is unaffected by gender, body weight, age, or nutrition. Since this protein does not participate in the metabolism of substances, its synthesis is independent of the body's metabolic processes. In comparison to serum creatinine, serum Cystatin C can be considered an almost perfect endogenous biomarker of renal function [12].

The purpose of the study. Using novel biomarkers of kidney damage, to compare the functional state of kidneys in SGA and AGA newborns with ischemic nephropathy (IN).

Object and methods of the research. A total of 72 newborns were included in the study. SGA (small for gestational age) newborns comprised 33, while AGA (appropriate for gestational age) newborns comprised 39 of the total number of infants. They were split into three groups based on the intensity of their IN (children with IN of I, II, or III degrees), with each group divided into two subgroups. Newborns with I degree IN (N=36) were divided into smaller subgroups of SGA (N=16) and AGA (N=20); newborns with II degree IN (N=20) were divided into SGA (N=9) and AGA (N=11); and newborns with III degree IN (N=16) were divided into SGA (N=8) and AGA (N=8) subgroups.

On days 1-3 and 7-10 of the patient's life, blood and urine samples were taken to determine the levels of KIM-1 and NGAL in the urine, as well as Cystatin C in the blood serum.

The levels of biomarkers were determined by the enzyme-linked immunosorbent assay (ELISA). NGAL using a kit of reagents from RayBiotech, İnc (USA), KİM-1 – using a kit of reagents from Arqutus Medical, BioAssayWorks, and Cystatine C in blood serum using a kit of reagents Human Cystatin C ELISA from BioVendor (Czech Republic). Additionally, we determined the levels of creatinine in blood serum, and performed renal artery Doppler Ultrasound for Resistance Index (RI) assessment. Statistical processing – accumulation, adjustment, systematization and visualization of the results obtained was carried out using standard programs for mathematical and statistical analysis Microsoft Excel 2013 and SPSS 20.0. (Armonk, NY: IBM Corp). To compare the data obtained, we used the methods of nonparametric analysis using the Mann-Whitney U-test. The obtained values of the U-Mann-Whitney test were estimated by the comparison with the tabular data. In that case, if the calculated value of the U-Mann-Whitney test was equal to or less than the critical value, the statistical significance of the differences was recognized. Outliers were eliminated using the Fischer test.

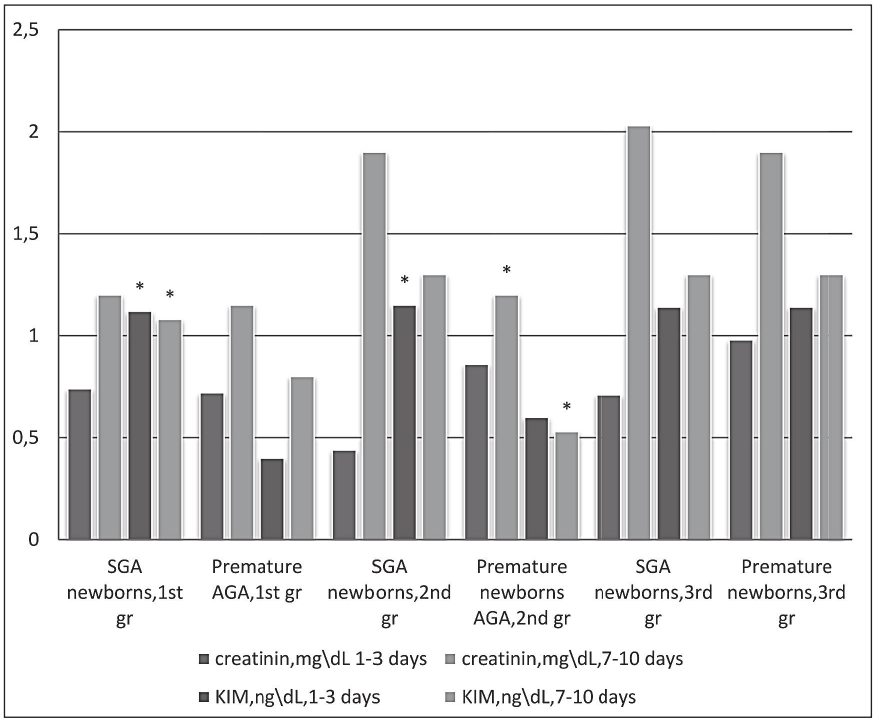

Figure 1 – The comparative analysis of KIM-1 and Creatinine levels in SGA and AGA newborns.
Note: *- difference within one subgroup, in relation to AGA newborns at p<0,05

The study was approved by the Ethics Committee of the Azerbaijan Medical University (protocol N 7, from 27.07.2019). In the research and the collection and processing of patient data were obtained informed consent of children parents.

The results of the study and their discussion. We discovered the following results while performing a comparative study of markers characterizing the functional status of the kidneys in low birth weight infants based on the correspondence of their

anthropometric parameters to gestational age and the degree of ischemic nephropathy.

The difference in creatinine levels between the subgroups on days 1-3 and 7-10 was significant only in the II degree of nephropathy, where it was higher in the SGA newborns' subgroup. While newborns with III degree nephropathy had the highest creatinine values in the SGA subgroup, there was no statistically significant difference among the subgroups.

In both the I and II degrees of nephropathy, the values of KIM-1 in SGA newborns substantially exceeded the values of this marker in the AGA subgroup. The highest concentrations of KIM-1 were found in the subgroups of III degree IN, with nearly equal values **(Figure 1).**

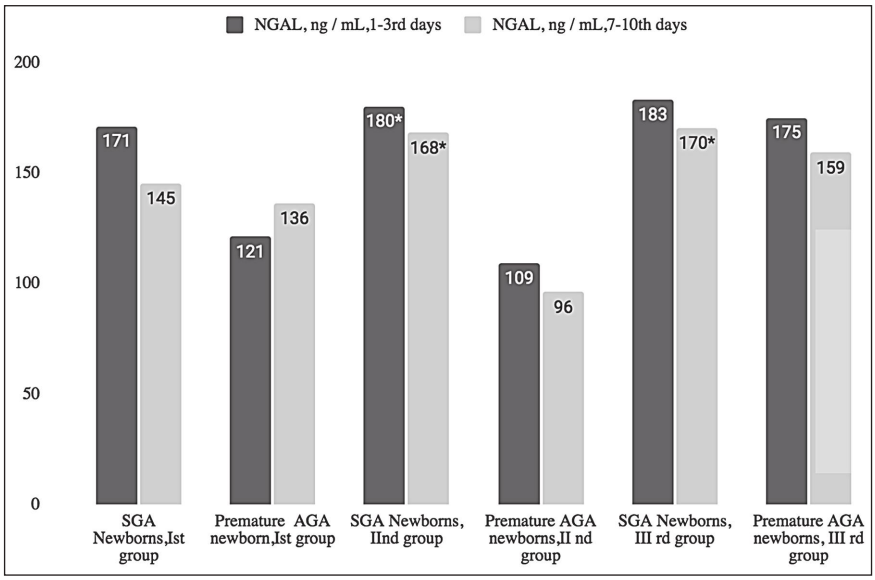

Figure 2 – The comparative analysis of NGAL levels in SGA and AGA newborns.
Note: *- difference within one subgroup, in relation to AGA newborns at p<0,05

The subgroup with SGA newborns had slightly higher levels of NGAL in the urine on days 1-3 with II degree IN and days 7-10 with III degree IN **(Figure 2).**

When evaluating glomerular renal function, it was discovered that the amount of Cystatin C in SGA newborns with I and II degrees of IN did not significantly surpass the markers of children with AGA, both in the first and second measurements. Similarly to low birth weight infants with III degree IN, statistically significant Cystatin C values were found in newborns with developmental delays on the 1st-3rd days of life and on the 7th-10th days of life **(Figure 3).**

There were no major variations in the subgroups of the main group with a high index of resistance in all three groups, suggesting an improvement in peripheral vascular resistance in the kidneys **(Figure 3).**

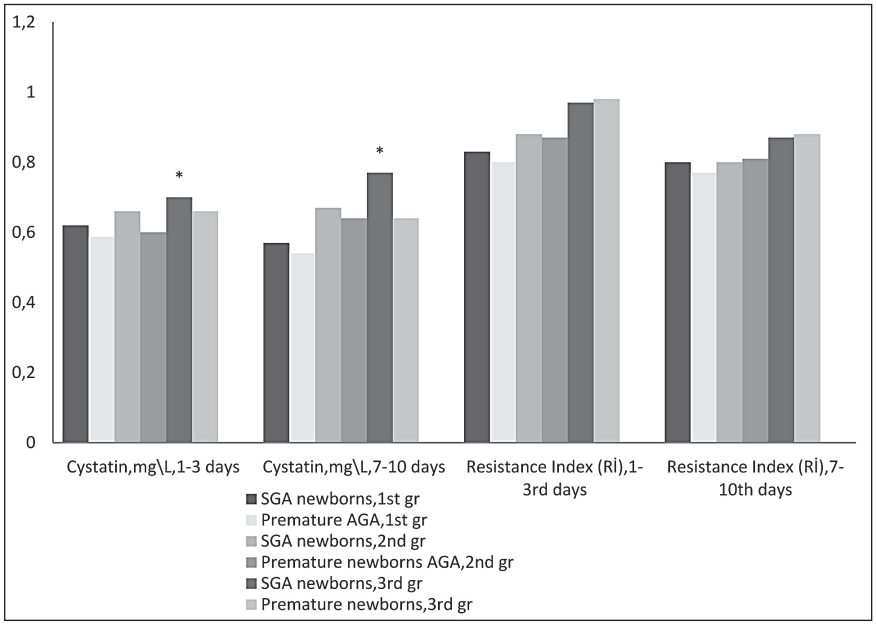

Figure 3 – The comparative analysis of Cystatin C level and RI in SGA and AGA newborns.
Note: *- difference within one subgroup, in relation to AGA newborns at p<0,05

Thus, relative to premature infants with a weight that corresponds to their gestational age, the tubular apparatus of the kidneys in newborns with intrauterine growth retardation is more vulnerable. This confirms the kidneys' high sensitivity to the unfavorable factors of the antenatal period that reduce the fetus' intrauterine development. High KIM-1 values, which represent histotoxic hypoxia of the kidney tissues from birth to the end of the early neonatal period, indicate the severity of tubular disorders in SGA newborns at the I degree of IN. Despite the lack of a statistically significant difference in creatinine levels between subgroups with III degrees of IN, the level of NGAL in infants who had a developmental delay was statistically significantly higher than in AGA newborns, indicating that this category of newborns may develop the terminal stage of AKI (Acute Kidney Injury).

A rise in Cystatin C concentration by the end of the early neonatal period in this category of children indicates that the restoration of glomerular filtration has been disrupted, despite the glomerular apparatus's anatomical and functional immaturity.

Even with regular RI values, hypoxia causes arteriovenous shunting with blood supply to juxtamedullary nephrons and damage to the cortex, so the renal artery resistance index does not always represent the magnitude of renal tissue damage.

Conclusions.

1. Based on the findings, it can be concluded that SGA newborns are a high-risk category for developing AKI, which must be considered when treating this population of children. Preventing impaired polypragmasia and

water-salt metabolism, particularly when nephrotoxic drugs are used, is a critical issue in modern neonatology.

2. The use of more sensitive markers to avoid extreme AKI is needed since assessing renal functions solely on the basis of creatinine does not allow for an objective assessment of the degree of damage to the tubular apparatus.

Prospects for further research. Further study of the KIM-1, NGAL and Cystatin C biomarkers in children with various kidney diseases in the early childhood and adolescence period is planned.

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ВИЗНАЧЕННЯ СТУПЕНЯ ПОШКОДЖЕННЯ НИРОК У НОВОНАРОДЖЕНИХ З МАЛОЮ МАСОЮ ТІЛА В ЗАЛЕЖНОСТІ ВІД СПІВВІДНОШЕННЯ ЇХ АНТРОПОМЕТРИЧНИХ ПОКАЗНИКІВ ТА ГЕСТАЦІЙНОГО ВІКУ

Ахундова А. А.

Резюме. Новонароджені із затримкою внутрішньоутробного розвитку займають особливе місце в сучасній неонатології, оскільки ці діти найбільш чутливі до впливу різноманітних патологічних факторів як у внутрішньоутробному, так і в неонатальному періодах. Вони піддаються тривалому впливові гіпоксії у внутрішньоутробному періоді, що зумовлює тяжкий перебіг різноманітних захворювань органів та систем в ранньому неонатальному віці, зокрема нирок. Ураження нирок у недоношених новонароджених часто залишається недіагностованим на фоні інших захворювань, таких як РДС (респіраторний дистрес синдром) та ССЗ (серцево-судинні захворювання). У нашій роботі ми, при використанні KIM-1 (Kidney Injury Molecule-1), NGAL (Neutrophil Gelatinase Associated Lipokalin) та Cystatin C біомаркерів, провели порівняльний аналіз тяжкості пошкодження нирок у новонароджених з ішемічною нефропатією (ІН). Також визначалися рівень креатині ну крові та індекс резистентності (ІР) ниркової артерії. До дослідження було включено 72 новонароджених з малою масою тіла при народженні (33 МГВ новонароджених (малі для гестаційного віку) та 39 ВГВ (що відповідають гестаційному вікові)). Вони були розділені на три групи в залежності від ступеня ІН (діти з ІН I, II або III ступеня), при цьому кожна група, в свою чергу, була розділена на дві підгрупи. Новонароджені з ІН I ступеня (N=36) були розділена на більш дрібні групи МГВ (N=16) та ВГВ (N=20); новонароджені з ІН II ступеня (N=20) були розділені на МГВ (N=9) і ВГВ (N=11) та новонароджені з ІН III ступеня (N=16) були розділені на підгрупи МГВ (N=8) и ВГВ (N=8). Наші результати показали,що рівень креатині ну підвищувався у дітей підгруп МГВ при більш тяжких ступенях ІН, на більш пізню добу, в той час як рівень KIM-1 був високим при всіх ступенях ІН, з перших днів життя. Рівень NGAL також був вище у МГВ новонароджених з ІН II-го та III-го ступеня з перших днів життя, а рівень Cystatin C у цієї ж категорії малюків підвищувався лише при тяжкому ступені ІН. ІР був високим у всіх підгрупах основної групи. Таким чином, МГВ новонароджені складають групу ризику у виникненні ГНН (гостра ниркова недостатність) і рання діагностика ІН у цих новонароджених залишається днією з проблем сучасної медицини.

Ключові слова: KIM-1, NGAL, Cystatin C, ВГВ, МГВ, ішемічна нефропатія, ГНН.

DETERMINATION OF THE DEGREE OF THE KIDNEY INJURY IN LOW BIRTH WEIGHT INFANTS DEPENDING ON THE CORRESPONDENCE OF THEIR ANTHROPOMETRIC PARAMETERS TO GESTATIONAL AGE

Akhundova A. A.

Abstract. Newborns with intrauterine growth retardation occupy a special place in modern neonatology, since they are susceptible to prolonged exposure to hypoxia in the intrauterine period, and this causes a severe course of various diseases of organs and systems in the early neonatal age. Kidney damage in premature infants often remains undiagnosed in the presence of other diseases such as RDS (Respiratory Distress Syndrome) and heart disorders. In our investigation, we performed a comparative analysis of the severity of kidney damage in newborns with ischemic

nephropathy (IN) using KIM-1 (Kidney injury Molucule-1), NGAL (Neuthrophil Gelatinase Associated Lipokalin) and Cystatin C biomarkers. The level of creatinine in the blood and the resistance index (RI) of the renal artery were also determined. A total of 72 newborns were included in the study. SGA (small for gestational age) newborns comprised 33, while AGA (appropriate for gestational age) newborns comprised 39 of the total number of infants. They were split into three groups based on the intensity of their IN (children with IN of I, II, or III degrees), with each group divided into two subgroups. Newborns with I degree IN (N=36) were divided into smaller subgroups of SGA (N=16) and AGA (N=20); newborns with II degree IN (N=20) were divided into SGA (N=9) and AGA (N=11); and newborns with III degree IN (N=16) were divided into SGA (N=8) and AGA (N=8) subgroups. Our results revealed that the creatinine level increased in children of the SGA subgroups with more severe degrees of IN, at a later days, while the level of KIM-1 was high at all degrees of IN, from the first days of life. The NGAL level was also higher in SGA infants with II and III degrees from the first days of life, and the Cystatin C level in the same category of infants increased only with severe IN. IR was high in all subgroups of the main group. So SGA newborns constitute a risk group for the development of acute renal injury, and early diagnosis of IN in these newborns remains one of the problems in modern medicine.

Key words: SGA infant, AGA infant, AKI (Acute Kidney Injury), Ischemic Nephropathy, KIM-1, NGAL, Cystatin C.

DETERMINATION OF THE DEGREE OF THE KIDNEY INJURY IN LOW BIRTH WEIGHT INFANTS DEPENDING ON THE CORRESPONDENCE OF THEIR ANTHROPOMETRIC PARAMETERS TO GESTATIONAL AGE

Akhundova A. A.

Abstract. Aim. Using novel biomarkers of kidney damage, to compare the functional state of kidneys in SGA and AGA newborns with ischemic nephropathy (IN).

Materials and Methods. A total of 72 newborns were included in the study. SGA (small for gestational age) newborns comprised 33, while AGA (appropriate for gestational age) newborns comprised 39 of the total number of infants. They were split into three groups based on the intensity of their IN (children with IN of I, II, or III degrees), with each group divided into two subgroups. On days 1-3 and 7-10 of the patient's life, blood and urine samples were taken to determine the levels of KIM-1 and NGAL in the urine, as well as Cystatin C in the blood serum. The levels of biomarkers were determined by the enzyme-linked immunosorbent assay (ELISA).

Results. The difference in creatinine levels between the subgroups on days 1-3 and 7-10 was significant only in the II degree of nephropathy. In both the I and II degrees of nephropathy, the values of KIM-1 in SGA newborns substantially exceeded the values of this marker in the AGA subgroup. The highest concentrations of KIM-1 were found in the subgroups of III degree IN, with nearly equal values. The subgroup with SGA newborns had slightly higher levels of NGAL in the urine on days 1-3 with II degree IN and days 7-10 with III degree of IN. Statistically significant Cystatin C values were found in newborns with developmental delays on the 1^{st}-3^{rd} days of life and on the 7^{th}-10^{th} days of life.

Disscusion. Thus, relative to premature infants with a weight that corresponds to their gestational age, the tubular apparatus of the kidneys in newborns with intrauterine growth retardation is more vulnerable. High KIM-1 values, indicate the severity of tubular disorders in SGA newborns at the I degree of IN. Despite the lack of a statistically significant difference in creatinine levels between subgroups with III degrees of IN, the level of NGAL in infants who had a developmental delay was statistically significantly higher than in AGA newborns, indicating that this category of newborns may develop the terminal stage of AKI.A rise in Cystatin C concentration indicates that the restoration of glomerular filtration has been disrupted, despite the glomerular apparatus's anatomical and functional immaturity. Even with regular RI values, hypoxia causes arteriovenous shunting with blood supply to juxtamedullary nephrons and damage to the cortex, so the renal artery resistance index does not always represent the magnitude of renal tissue damage.

Conclusions.

Based on the findings, it can be concluded that SGA newborns are a high-risk category for developing AKI, which must be considered when treating this population of children.

The use of more sensitive markers to avoid extreme AKI is needed since assessing renal functions solely on the basis of creatinine does not allow for an objective assessment of the degree of damage to the tubular apparatus.

Key words: SGA infant, AGA infant, AKI (Acute Kidney Injury), Ischemic Nephropathy, KIM-1, NGAL, Cystatin C.

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