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THE EFFECT OF ENOS GENE POLYMORPHISM
AND NITRIC OXIDE METABOLISM
INDICATORS ON THE NEONATAL
CONSEQUENCES IN PREMATURE BABIES
BORN FROM MOTHERS WITH METABOLIC
SYNDROME

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Summary

Metabolic syndrome is considered to be a cluster of disorders that directly contribute to the development of cardiovascular disease and are characterized by chronic systemic inflammation. Numerous epidemiological data indicate that an adverse intrauterine environment, caused by the peculiarities of the nutritional status or placental insufficiency in a woman with metabolic syndrome, can “program” the susceptibility of the fetus to further development of cardiovascular and metabolic diseases, has an impact on cognitive and behavioral development. Nitric oxide (NO) plays a critical role in the pathogenesis of components of the metabolic syndrome. Children born prematurely have a high incidence of brain damage, which can lead to motor, cognitive, behavioral, social and sensory disorders.

The purpose of the research was the study of the effect of the eNOS gene polymorphism and indicators of nitric oxide metabolism on the neonatal consequences in prematurely born children from mothers with metabolic syndrome.

Material and methods. A study was conducted in which 100 premature infants were included. Two groups were formed: the main group (n=34), which included preterm infants (birth weight 2145.29±148.19 g and gestational age 33.18±0.55 weeks) of mothers with metabolic syndrome, and the comparison group (n=66), which included preterm infants (birth weight 2295.99±101.45 and gestational age 34.03±0.45 weeks) of mothers without metabolic syndrome. The children underwent a genetic study – determination of the polymorphism of the eNOS gene, as well as the level of nitrites, nitrates and nitrosothiols in the urine.

By decision of the bioethics commission No. 217 dated 12.06.2023, the materials of the scientific work comply with the Rules of Humane Treatment of Patients.

Traditional methods of parametric and nonparametric statistics were used; nonparametric methods were used to analyze qualitative characteristics expressed mainly in percentages. The methods of parametric statistics were used to check the normality of the distribution of quantitative characteristics using the Kolmogorov-Smirnov criterion.

Statistical processing of the obtained results was carried out using the package of application programs EXCEL-2003® and STATA version 11 for Windows (StataCorp, Texas, USA).

The work was carried out as part of the scientific and experimental work of the Department of Pediatrics #1 with Neonatology of the Poltava State Medical University “To develop clinical and laboratory criteria, methods of predicting and preventing metabolic disorders in young children (state registration number 0120U102856).

Results. The most common diseases in the infants of the studied groups were the consequences of intrauterine hypoxia (44.1%) and respiratory failure requiring artificial lung ventilation (50.0%), although no significant differences were found in the prevalence of these conditions. We identified the presence of significant associations between the consequences of intrauterine hypoxia and the levels of nitrates (OR 1.19; 95% CI 1.01-1.40; p=0.042), nitrosothiols (OR 1.19; 95% CI 0.99-1.42; p=0.050) and the polymorphic genotype 4aa/ab of the eNOS gene (OR 0.28; 95% CI 0.12-0.67; p=0.004). Analysis of systemic hemodynamics revealed no significant differences in baseline values between preterm infants with and without intrauterine hypoxia, but we did observe an association with urine output on day 3 of life.

To finally clarify the complex influence of indicators of nitrate metabolism on the development of intrauterine hypoxia and to predict the development of consequences of this condition in premature infants, the following indicators are included in the regression prognostic model: the level of nitrates, nitrites, 4aa/4ab genotype and urine output on the third day of life. As the research results show, there is a direct reliable relationship with nitrates and an inverse relationship with nitrites, 4aa/4ab genotype and urine output. This prediction model has high operating characteristics – the area under the ROC curve is 0.8168.

Some mechanisms of the influence of maternal metabolic syndrome on the development of relevant disorders in newborns are known, including disorders of nitric oxide synthesis, endothelial dysfunction, and oxidative stress. In our study, the consequences of intrauterine hypoxia were reliably associated with an increase in the concentration of urinary nitrates and a decrease in nitrites, as well as the absence of the 4aa/ab genotype, which is associated with reduced release of nitric oxide. There is evidence that nitric oxide can have both protective and deleterious effects, depending on factors such as nitric oxide synthase isoform and duration of exposure to hypoxia.

Conclusions. In preterm infants born to mothers with metabolic syndrome, elevated urinary nitrate levels and the absence of the eNOS 4aa/ab genotype increase the likelihood of suffering the consequences of intrauterine hypoxia.

Key words: Premature Babies; Mothers with Metabolic Syndrome; eNOS Gene; Nitric Oxide Metabolism; Systemic Hemodynamics; Morbidity.

Introduction

The metabolic syndrome (MS) is considered to be a cluster of disorders that directly contribute to the development of cardiovascular disease and are

characterized by chronic systemic inflammation. Numerous epidemiological data indicate that an adverse intrauterine environment, caused by specific nutritional status or placental insufficiency in a woman with MS, may “program”

the susceptibility of the fetus to further development of cardiovascular and metabolic diseases, and has an impact on cognitive and behavioral development [1, 2]. Pregnant women with MS are at increased risk for eclampsia, preterm delivery and other complications [3-5]. Nitric oxide (NO) plays a critical role in the pathogenesis of MS components and is involved in several mitochondrial signaling pathways that control cellular respiration and apoptosis. Children born prematurely have a high rate of brain damage, which can lead to motor, cognitive, behavioral, social and sensory disorders. The increase in the number of children who survive despite small gestational age is accompanied by an increase in suboptimal results of nervous system development [6]. Among the most frequent factors causing damage to the nervous system in the perinatal period is hypoxia as a result of an unfavorable course of pregnancy and childbirth [7]. The frequency of adverse neurological consequences varies: up to 17% of premature babies have severe anomalies, and in extremely low-birth-weight infants, only 42% of developmental deviations can be defined as moderate [8]. Most of the pathological phenomena underlying hypoxic lesions are the result of impaired cerebral perfusion and oxygen transport to the brain. However, the pathophysiological consequences of hypoxic-ischemic “stroke” (this is the term used in most foreign sources) are complex and develop gradually over a period of time, which makes it difficult for medical professionals to determine timely and adequate treatment options [9]. There is an increasing number of publications on energy deficits at the neuronal level in infants with asphyxia and hypoxic-ischemic encephalopathy [10]. Currently, the options for timely diagnosis and treatment of premature infants with hypoxic lesions of the central nervous system are limited. Many studies point to mitochondrial dysfunction and activation of autophagy [11]. In the search for methods for early diagnosis of the consequences of intrauterine hypoxia (IUH) in premature infants, we focused on indicators of nitrate metabolism. Nitric oxide (NO) is one of the universal regulators of physiological functions of the body with a wide range of action. The vasodilatory effect of NO, combined with the effect of cGMP on decreasing the concentration of Ca²⁺ in the cytosol of muscle cells and increasing Na⁺, has been studied in detail. There is also an effect of NO on the calcium transport systems of the mitochondria, which in case of imbalance quickly leads to energy deficit and activation of free radical oxidation processes. Excessive NO, which can be produced by iNOS, on the other hand, plays a key role in neuronal damage by disrupting mitochondrial function and contributing to the accumulation of mitochondrial substrate [12].

NO is the end product of l-arginine conversion by constitutive and non-constitutive isoforms of NOS. To date, 3 isoforms of NOS are known: neuronal or neural (nNOS), endothelial (eNOS) and inducible (iNOS). nNOS and eNOS are constitutional types of enzymes and ensure the synthesis of nitric oxide under normal conditions; iNOS is activated in response to pathogenic stimuli and produces significantly higher levels of NO, playing an important role in tissue inflammation and body defense [13]. Changes in the expression of different NOS isoforms, absence or overproduction of NO lead to an imbalance of active forms

of nitrogen and oxygen. The main links of NO conversion are protein nitrosylation with formation of S-nitrosothiols, NO oxidation to NO₂- and NO₃-. Nitrosothiols are one of the forms of NO deposition and an important source of NO under physiological conditions. Therefore, the cellular level of NO depends on the activity of NO synthases, the activity of nitrite and nitrate reductases, and the presence of a sufficient pool of deposited NO. There are studies showing that NO is produced in higher intravascular levels in neonates than in adults [14]. A decrease in nitrate and nitrite levels due to NO deficiency may indicate vascular ischemia and vasospasm [15]. Endothelial dysfunction, manifested by the loss of neurovascular protective functions of NO, may contribute significantly to the development of cognitive dysfunction [16].

The purpose of the research was the study of the effect of *eNOS* gene polymorphism and indicators of nitric oxide metabolism on the course of the neonatal consequences in premature babies born from mothers with metabolic syndrome.

Material and methods. A study was conducted in which 100 premature infants were included. Two groups were formed: the main group (n=34), which included preterm infants (birth weight 2145.29±148.19 g and gestational age 33.18±0.55 weeks) of mothers with MS, and a comparison group (n=66), which included preterm infants (birth weight 2295.99±101.45 and gestational age 34.03±0.45 weeks) of mothers without MS. Exclusion criteria were the presence of congenital malformations, genetic pathology, parental refusal to participate in the study. The premature infants included in the study were cared for and treated in hospitals of the Poltava region. The results of clinical examination, hemodynamic parameters (heart rate, blood pressure), urine output level on the 1st and 3rd day of life were recorded in the medical record of the inpatient. The conditions in the pathogenesis of which the vascular component plays an important role were selected as neonatal consequences, in particular respiratory failure (RF) requiring the use of artificial lung ventilation, intraventricular hemorrhage (IVH), early consequences of intrauterine or intranatal hypoxia (consequences of IUH), and food intolerance.

All children underwent a genetic study – determination of the rs61722009 polymorphism of the eNOS gene to account for possible genetically determined variations in NO concentration. Two alleles were identified in the 27 bp repeat of intron 4 of the eNOS gene, the larger of which, eNOS-4b, has 5 of the 27 bp tandem repeats (GAAGTCTAGACCTGCTGC(A/G)GGGGTGAG) and the smaller of which, eNOS-4a, has 4 repeats and is associated with reduced NO production [17].

The material for the study was the peripheral blood of newborns. Blood samples were collected in a volume of 0.25 ml. After receiving the samples, they were stored at a temperature of -20°C until the study was conducted. Isolation of DNA samples from the obtained material was carried out using a commercial kit of reagents “Quick-DNA Universal Kit”, then molecular genetic research was carried out by the method of polymerase chain reaction (PCR).

The subject of the biochemical research was urine. The concentration of low molecular weight nitrosothiols was determined by calculating the difference in the concentration of nitrites (NO₂⁻) before and after the oxidation of nitrosothiol complexes (S-NO) to nitrites with a solution of mercuric chloride (HgCl₂). A 0.2 ml aliquot of urine was collected for the study [18]. The concentration of nitrites was determined by determining the content of diazo compounds formed in the reaction with sulfanilic acid, and then the reaction was carried out with α-naphthylamine (Griess-Ilosvay reagent), resulting in the formation of red derivatives (azo dyes) [19]. The concentration of nitrates was determined by the increase in the concentration of nitrites after reduction of nitrates to nitrites with sulfurous hydrazine. Aliquots of 0.2 ml of urine were used to determine the concentration of nitrates and nitrites.

Traditional methods of parametric and nonparametric statistics were used; nonparametric methods were used to analyze qualitative characteristics expressed mainly in percentages. The methods of parametric statistics were used to check the normality of the distribution of quantitative characteristics using the Kolmogorov-Smirnov criterion.

In case of normal distribution of the data, the main statistical characteristics were used: the mean (M) to determine the central tendency, the standard error of the mean (m) to determine the accuracy of the mean estimate, the confidence interval (CI) – to determine the 95% interval of the mean.

The hypothesis of equality of general means was tested by means of two-sample t-test.

For non-normal distributions, the median (Me) and interquartile range (Q) were used to determine the central tendency.

Relative values or values expressed as percentages were compared using the χ² (chi-squared) test, and quantitative indicators with non-normal distributions in unrelated samples were compared using the Wilcoxon run-sum test.

Simple and multiple logistic regression analysis was used to prove the relationship between individual

indicators, odds ratios (OR), predictive coefficients (β) and their confidence intervals were calculated. After identifying reliable risk factors, prognostic models were developed using multiple logistic analysis with further evaluation of the operational characteristics of the specified models and identification of the model with the largest C coefficient (area under the ROC curve) using the STATA 14.0 application package.

Statistical processing of the obtained results was performed using the EXCEL-2003® application package and STATA version 11 for Windows (StataCorp, Texas, USA).

According to the decision of the Bioethics Committee 217 dated 12.06.2023, the materials of the scientific work comply with the rules of humane treatment of patients in accordance with the requirements of the Tokyo Declaration of the World Medical Association, the International Helsinki Recommendations, the Declaration of Human Rights, the Convention of the Council of Europe on Human Rights and Biomedicine, the laws of Ukraine, the regulations of the Ministry of Health of Ukraine and the requirements of the Code of Ethics of a Physician in Ukraine.

The work was carried out as part of the scientific and experimental work of the Department of Pediatrics #1 with Neonatology of the Poltava State Medical University “To develop clinical and laboratory criteria, methods of predicting and preventing metabolic disorders in young children (state registration number 0120U102856).

Results and their discussion. Babies born to mothers with MS were significantly more likely to be born by cesarean section (CS) than were babies born to mothers without MS – 44.1% versus 10.6%, p=0.0002. We found no significant differences between the groups in the number of neonatal conditions studied, particularly the consequences of acute respiratory distress syndrome, RF requiring the use of ALV, IVH, and food intolerance (Table 1). The most common conditions in the infants of the studied groups were the consequences of IUH and RF requiring ALV. In the majority of cases, intrauterine hypoxia of the fetus was the reason for CS.

Table 1

Neonatal consequences and indicators of nitric oxide metabolism in children of the examined groups

Indicators	Main group, n=34	Comparison group, n=66	p
RF, that required ALV, n (%)	17 (50.0)	23 (34.8)	0.143
Food intolerance, n (%)	4 (11.8)	6 (9.1)	0.317
IVH, n (%)	4 (11.8)	13 (19.7)	0.673
Consequences of intrauterine hypoxia (P 20.9), n (%)	15 (44.1)	35 (53.1)	0.398
Nitrates (nmol/l), Me (Q ₁ -Q ₃)	3.95 (2.12-5.78)	4.86 (2.43-6.08)	0.587
Nitrites (nmol/l), Me (Q ₁ -Q ₃)	1.22 (0.91-2.13)	1.52 (0.92-2.73)	0.418
Nitrosothiols (μmol/l), Me (Q ₁ -Q ₃)	2.89 (2.61-4.70)	2.82 (2.61-5.7)	0.950
4aa/4ab genotype, n (%)	13 (38.2)	23 (34.8)	0.738

Note: RF – respiratory failure, ALV – artificial lung ventilation, IVH – intraventricular hemorrhage

The study of nitric oxide metabolism showed no significant differences in the levels of nitrites, nitrates, and nitrosothiols between the studied groups of children born to mothers with and without MS. Also, no differences were

found in the distribution of children into groups depending on the 4aa/ab polymorphism of the eNOS gene. Therefore, the presence of maternal MS does not affect the nitrate metabolism of preterm infants immediately after birth.

According to numerous studies, the presence of maternal MS causes intrauterine hypoxia of the fetus [7], which is an indication for cesarean section, which in turn worsens the infants' adaptation to extrauterine life after birth. Therefore, we investigated the existence of a relationship between neonatal outcomes and indicators of nitric oxide metabolism levels (nitrates, nitrites, and nitrosothiols) in preterm infants.

We found the presence of reliable associations between the consequences of IUH and the levels of nitrates, nitrosothiols and the polymorphic genotype 4aa/ab of the eNOS gene (Table 2). As for other neonatal conditions,

no reliable relationship was found between them and the levels of nitrates, nitrites and nitrosothiols. The presence of the 4aa/ab polymorphic genotype of the eNOS gene was reliably associated with the development of clinical manifestations of IUH in a premature infant, i.e. it reduced the probability of the infant having clinical consequences of intrauterine hypoxia, and was not associated with the development of other neonatal conditions. A reliable direct relationship between the development of IUH in preterm infants and the levels of nitrates and nitrosothiols was found according to a simple logistic regression analysis (Table 2).

Table 2

The relationship between the eNOS gene polymorphism and indicators of nitric oxide exchange with neonatal outcomes according to simple logistic regression analysis

	Nitrates in urine	Nitrites in urine	Nitrosothiols in urine	4aa/4ab genotype
RF	OR 1.06 95% CI 0.91-1.24 p=0.422	OR 1.13 95% CI 0.81-1.57 p=0.464	OR 1.03 95% CI 0.90-1.19 p=0.651	OR 0.93 95% CI 0.40-2.14 p=0.865
IVH	OR 0.96 95% CI 0.78-1.18 p=0.766	OR 0.76 95% CI 0.39-1.33 p=0.434	OR 1.03 95% CI 0.90-1.19 p=0.651	OR 1.2 95% CI 0.29-2.54 p=0.781
Consequences of IUH	OR 1.19 95% CI 1.01-1.40 p=0.042	OR 1.23 95% CI 0.87-1.72 p=0.236	OR 1.19 95% CI 0.99-1.42 p=0.050	OR 0.28 95% CI 0.12-0.67 p=0.004
Food intolerance	OR 1.2 95% CI 0.32-4.6 p=0.781	OR 0.85 95% CI 0.46-1.56 p=0.593	OR 0.9 95% CI 0.66-1.23 p=0.503	OR 0.85 95% CI 0.46-1.56 p=0.593

Note: RF – respiratory failure, IVH – intraventricular hemorrhage, IUH – intrauterine hypoxia

At the same time, we did not find any relationship between the 4aa/ab genotype and indicators of nitric oxide metabolism, in particular nitrates (OR 1.08, p= 0.318), nitrites (OR 1.11, p=0.548) and nitrosothiols (OR 1.07, p=0.352).

Furthermore, the peculiarities of nitric oxide exchange in premature infants were analyzed according to the presence or absence of IUH. The median values of nitrates, nitrites and nitrosothiols in premature infants with IUH were significantly higher than those in infants without

IUH. Since one of the most important functions of NO in the human body is the regulation of blood flow by influencing vascular tone and the state of the endothelium, we studied the peculiarities of hemodynamic indicators and renal function in this cohort of patients (Table 3). Analysis of systemic hemodynamics showed no significant differences in the baseline values of preterm infants with and without IUH, but we noted a <0.1 association with hourly diuresis on day 3, although this value remained within the normal range.

Table 3

Indicators of nitric oxide exchange and systemic hemodynamics among children depending on the presence or absence of intrauterine hypoxia consequences

Prognostic variables	Newborns with IUH, n=50	Newborns without IUH, n=50	OR 95% CI	p
Nitrates in urine (nmol/l) Me (Q ₁ -Q ₃)	4.86 (3.95-4.08)	3.04 (1.8-6.08)	1.19 (1.00-1.4)	0.017
Nitrites in urine (nmol/l) Me (Q ₁ -Q ₃)	1.82 (1.22-2.18)	0.22 (0.61-2.74)	1.22 (0.87-1.72)	0.031
Nitrosothiols in urine (μmol/l) Me (Q ₁ -Q ₃)	3.92 (2.75-5.94)	2.75 (2.61-3.47)	1.19 (0.99-1.42)	0.020
Heart rate, 1 st day after birth (per min.) M±m	148.08±1.91	150.78±1.65	0.98 (0.95-1.01)	0.287
Heart rate, 3 rd day after birth (per min.) M±m	149.12±1.86	151.24±1.93	0.99 (0.95-1.02)	0.426
Mean blood pressure, 1 st day after birth (mmHg) M±m	33.92±1.06	33.06±1.01	1.02 (0.96-1.07)	0.555
Mean blood pressure, 3 rd day after birth (mmHg) M±m	35.24±0.92	35.26±0.92	0.99 (0.94-1.06)	0.988
Urine output, 1 st day after birth (ml/kg/h) M±m	2.07±0.25	2.3±0.2	0.92 (0.72-1.19)	0.535
Urine output, 3 rd day after birth (ml/kg/h) M±m	3.03±0.16	3.5±0.18	0.72 (0.51-1.02)	0.067

In order to finally clarify the complex influence of nitrate metabolism indicators on the development of IUH and to predict the development of its consequences in premature infants, the following indicators are included in the regression prognostic model: the level of nitrates, nitrites in urine, the *4aa/4ab* genotype and hourly urine

output on the third day of life. As the research results show, there is a direct and reliable relationship with nitrates and an inverse relationship with nitrites, the *4aa/4ab* genotype and urine output (Table 4)

This predictive model has high operational characteristics – the area under the ROC curve is 0.8168.

Table 4

Odds ratio and prognostic coefficients of the prognostic model regarding the development of the consequences of intrauterine hypoxia

	OR (95% CI)	β (95% CI)	p
Nitrates (nmol/l)	4.75 (2.05-10.99)	1.56 (0.72-2.39)	0.000
Nitrites (nmol/l)	0.06 (0.01-0.32)	-2.79 (-4.43-(-1.14)	0.001
<i>4aa/4ab</i> genotype (yes)	0.11 (0.03-0.37)	-0.43 (-0.082-(-0.99)	0.000
Urine output (ml/kg/h)	0.65 (0.17-5.68)	-0.42 (-0.82-(-0.02)	0.037

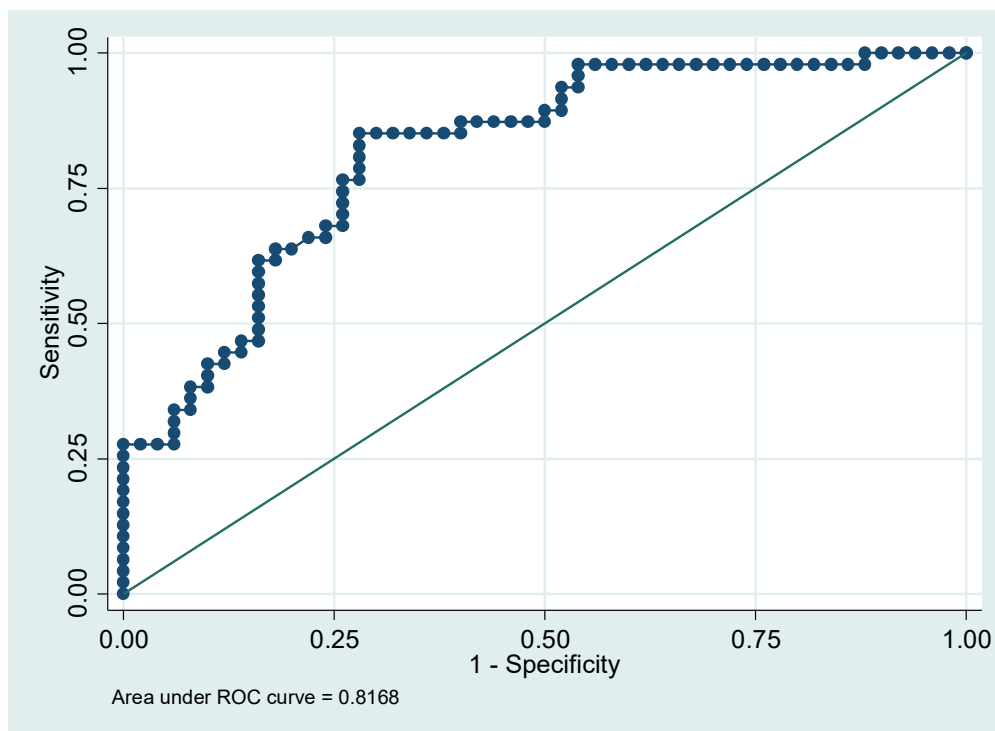


Fig. 1. ROC-curve for predicting the development of clinical manifestations of the intrauterine hypoxia consequences in premature infants

As a result of the research conducted, no association was found between maternal MS and indicators of nitrate metabolism in preterm infants. However, a recent meta-analysis showed the programming effect of maternal MS during pregnancy on the metabolic conditions of newborns [20], and a number of earlier cohort studies analyzed the risks of developmental disorders in children of mothers with MS [21, 22]. Some mechanisms of the influence of maternal MS on the development of relevant disorders in newborns are known, including disorders of NO synthesis, endothelial dysfunction and oxidative stress [23, 24]. In particular, the influence of very-low-density lipoproteins (VLDL) on NO bioavailability, redox homeostasis, and mitochondrial function has been experimentally demonstrated [25]. High serum glucose concentrations cause vascular endothelial dysfunction leading to decreased activation of endothelial nitric oxide synthase (eNOS) and increased reactive oxygen species,

which explains the decreased synthesis and bioavailability of NO and increased NO consumption [26]. And the work of Grasemann C demonstrated the influence of intrauterine exposure to maternal hyperglycemia and a high-fat diet on changes in the pulmonary metabolism of L-arginine/NO in the offspring [27]. Although we did not find significant differences in nitric oxide metabolism disorders in preterm infants depending on the presence of MS in their mothers, we believe that further studies of this plan in a larger cohort of patients are needed. In our study, the consequences of IUH were significantly associated with an increase in the concentration of nitrates in the urine, and a decrease in nitrites, and the absence of the *4aa/4ab* genotype, which is associated with reduced NO excretion. There is an evidence that NO can have both protective and deleterious effects depending on factors such as NOS isoform and time after the exposure to hypoxia [28]. Immediately after hypoxic-ischemic brain injury, the release of NO from eNOS is

protective, mainly promoting vasodilation; therefore, NO is of particular importance for the autoregulation of cerebral vascular tone. However, NO produced by inducible nitric oxide synthase (iNOS) may have neurotoxic effects to which the immature brain is particularly sensitive. It should be noted that iNOS is more easily and intensively expressed in neonates after the hypoxic event, in addition, this category of patients has reduced reserves of the antioxidant system [29]. Probably, the increased level of nitrates in the urine of newborns, which significantly increases the chances of having the consequences of intrauterine hypoxia, is a confirmation of this fact.

An example of the application of our research can be not only the prediction of the development of IUH, which may be insignificant according to clinical manifestations, but also to find out the status of the child regarding the exchange of NO, since more and more works are appearing that prove the effectiveness of the use of NO modulators, such as inhaled nitric oxide (iNO) and sildenafil are clinically used for the treatment of pulmonary hypertension in premature infants [30, 31], with caveats regarding the lack of clear indications. There are concerns about side effects in small-for-gestational-age preterm infants and in mothers who receive nitric oxide donors during pregnancy, whose efficacy in prolonging pregnancy has been demonstrated in a recent meta-analysis [32].

Conclusions. Among the premature infants born to mothers with MS, such pathological conditions as the consequences of intrauterine hypoxia (44.1%) and RF requiring mechanical ventilation (50.0%) were the most common, although no significant differences in their

prevalence were found. Significant associations were found between the outcomes of intrauterine hypoxia and levels of nitrates (OR 1.19; 95% CI 1.01-1.40; $p=0.042$), nitrosothiols (OR 1.19; 95% CI 0.99-1.42; $p=0.050$), and polymorphic genotype 4aa/ab of the eNOS gene (OR 0.28; 95% CI 0.12-0.67; $p=0.004$). Analysis of systemic hemodynamics showed no significant differences in the baseline values of preterm infants with and without IUH, but we noted a $p < 0.1$ association with urine output on day 3 after birth, although this value remained within the normal range. According to the results of the multiple logistic regression analysis, in preterm infants born to mothers with MS, an increase in the level of urinary nitrates and the absence of the eNOS 4aa/ab genotype increased the likelihood of having the consequences of intrauterine hypoxia.

Prospects for further research. Considering the complexity and the lack of a complete diagnostic algorithm for the possible effects of hypoxia on the central nervous system in premature infants, it is hypothetically possible, after conducting studies on a larger sample of patients, to consider the determination of urinary nitrates and nitrosothiols as a minimally invasive screening method. In addition, the effect on the expression of different isoforms of NOS may be an option for the development of neuroprotective therapy in neonates with the consequences of intrauterine hypoxia.

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ВПЛИВ ПОЛІМОРФІЗМУ ГЕНУ ENOS ТА ПОКАЗНИКІВ ОБМІНУ ОКСИДУ АЗОТУ НА НЕОНАТАЛЬНІ НАСЛІДКИ У ПЕРЕДЧАСНО НАРОДЖЕНИХ ДІТЕЙ ВІД МАТЕРІВ З МЕТАБОЛІЧНИМ СИНДРОМОМ

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Резюме

Метаболічний синдром розглядається як кластер порушень, які безпосередньо сприяють розвитку серцево-судинних захворювань і характеризуються хронічним системним запаленням. Численні епідеміологічні дані свідчать про те, що несприятливе внутрішньоутробне середовище, спричинене особливостями нутритивного статусу або плацентарною недостатністю у жінки з метаболічним синдромом, може «програмувати» сприйнятливості плода до подальшого розвитку серцево-судинних та метаболічних захворювань, має вплив на когнітивний і поведінковий розвиток. Оксид азоту (NO) відіграє вирішальну роль у патогенезі компонентів метаболічного синдрому. Діти, народжені передчасно, мають високу частоту пошкоджень мозку, що може призвести до моторних, когнітивних, поведінкових, соціальних, сенсорних розладів.

Мета і завдання дослідження. Вивчення впливу поліморфізму гену *eNOS* та показників обміну оксиду азоту на неонатальні наслідки у передчасно народжених дітей від матерів з метаболічним синдромом.

Матеріал та методи дослідження. Було проведено дослідження, до якого увійшли 100 передчасно народжених дітей. Для досягнення мети було сформовано дві групи: основну (n=34), в яку увійшли передчасно народжені діти (вага при народженні 2145,29±148,19 г та гестаційним віком 33,18±0,55 тижнів) від матерів з метаболічним синдромом, та групу порівняння (n=66), в яку увійшли передчасно народжені діти (вага при народженні 2295,99±101,45 та ГВ 34,03±0,45 тижн) від матерів без метаболічного синдрому. Дітям було проведено генетичне дослідження – визначення поліморфізму гену *eNOS*, а також рівня нітритів, нітратів та нітрозотіолів сечі.

Рішенням комісії з біоетики № 217 від 12.06.2023 підтверджено, що матеріали наукової роботи відповідають Правилам гуманного поводження з пацієнтами

Використовували традиційні методи параметричної та непараметричної статистики. Для аналізу якісних ознак, що виражалися в основному у відсотках, були застосовані непараметричні методи. За допомогою методів параметричної статистики здійснювались перевірка нормальності розподілу кількісних ознак з використанням критерію Колмогорова-Смірнова. Статистична обробка отриманих результатів проводилася за допомогою пакета прикладних програм EXCEL-2003® і STATA версії 11 для Windows (StataCorp, Техас, США).

Робота виконана у межах НДР кафедри педіатрії № 1 та неонатології Полтавського державного медичного університету «Розробити клініко-лабораторні критерії, методи прогнозування та запобігання метаболічних порушень у дітей раннього віку» (державний реєстраційний номер 0120U102856).

Результати дослідження. Найпоширенішими захворюваннями серед немовлят обстежених груп виявились наслідки внутрішньоутробної гіпоксії (44,1%) та дихальна недостатність, що потребувала штучної вентиляції легень (50,0%), хоча достовірних відмінностей у поширеності цих станів знайдено не було. Ми ідентифікували наявність достовірних асоціацій між наслідками внутрішньоутробної гіпоксії та рівнем нітратів (ВШ 1,19; 95% ДІ 1,01-1,40; p=0,042), нітрозотіолів (ВШ 1,19; 95% ДІ 0,99-1,42; p=0,050) та поліморфним генотипом 4aa/ab гену *eNOS* (ВШ 0,28; 95% ДІ 0,12-0,67; p=0,004). Аналіз системної

гемодинаміки виявив відсутність достовірних відмінностей у базових показниках передчасно народжених немовлят з наслідками внутрішньоутробної гіпоксії та без неї, але ми звернули увагу на наявність зв'язку з погодинним діурезом на третю добу.

Для остаточного з'ясування комплексного впливу показників нітратного обміну на розвиток внутрішньоутробної гіпоксії та прогнозування розвитку наслідків даного стану у передчасно народжених дітей в регресійну прогностичну модель включено такі показники: рівень нітратів, нітритів, генотип *4aa/4ab* та погодинний діурез на третю доби життя. Як свідчать результати досліджень, існує прямий достовірний зв'язок з нітратами та зворотній з нітритами, генотипом *4aa/4ab* та погодинним діурезом. Дана прогностична модель має високі операційні характеристики – площа під ROC кривою становить 0,8168.

Деякі механізми впливу метаболічного синдрому у матерів на розвиток відповідних порушень у новонароджених відомі, і серед них порушення синтезу оксиду азоту, ендотеліальна дисфункція, окислювальний стрес. У нашому дослідженні наслідки внутрішньоутробної гіпоксії достовірно асоціювались зі збільшенням концентрації нітратів у сечі, та зменшенням нітритів, і відсутністю генотипу *4aa/ab*, який пов'язаний зі зменшенням виділенням оксиду азоту. Є свідчення про те, що оксид азоту може мати як захисну, так і шкідливу дію залежно від таких факторів, як ізоформа синтази оксиду азоту і час впливу гіпоксії.

Висновки. У передчасно народжених дітей від матерів з метаболічним синдромом підвищення рівня нітратів сечі та відсутність генотипу *eNOS 4aa/ab* збільшує шанси мати наслідки внутрішньоутробної гіпоксії.

Ключові слова: передчасно народжені діти; матері з метаболічним синдромом; ген eNOS; метаболізм оксиду азоту; системна гемодинаміка; захворюваність.

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