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PECULIARITIES OF METABOLISM (BY THE LEVEL OF NITRATES, NITRITES, MALONDIALDEHYDE AND SIALIC ACIDS) IN 6-9 MONTHS OLD INFANTS BORN FROM MOTHERS WITH METABOLIC SYNDROME, WHO HAD HYPOXIC-ISCHEMIC ENCEPHALOPATHY IN THE EARLY NEONATAL PERIOD

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Summary

According to the World Health Organization, asphyxia and hypoxic-ischemic encephalopathy remain the leading neurological causes of disability and mortality in newborns. The degree of brain damage and subsequent neurological complications is determined by a number of factors, including duration of hypoxia/ischemia, cerebral circulation, neonatal central nervous system immaturity, and maternal somatic status.

***The aim** of the study was to determine the metabolic characteristics (by nitrate, nitrite, malondialdehyde and sialic acid levels) of infants aged 6-9 months born to mothers with metabolic syndrome who had hypoxic-ischemic encephalopathy in the early neonatal period, and the effect of eNOS (G894T) and IL-1b (C3953T) gene variants on the metabolism of these metabolites.*

***Materials and methods.** To this end, a prospective case-control study was conducted in 30 children aged 6-9 months. The main study group consisted of 16 children born to mothers with metabolic syndrome who had an Apgar score of less than 7 points at 5 minutes and diagnostic clinical and neurosonographic signs of hypoxic-ischemic encephalopathy, as determined during a consultation with a pediatric neurologist at 6-9 months of age. The comparison group consisted of 14 randomly selected relatively healthy children aged 6-9 months, born to mothers without metabolic syndrome and without manifestations of hypoxic-ischemic encephalopathy in the early neonatal period.*

The concentration of nitrites was determined by determining the diazo compounds formed in the reaction with sulfanilic acid, and then the reaction was carried out with α-naphthylamine (Grisse-Ilosway reagent). The concentration of free malondialdehyde was determined by the spectrophotometric method. The concentration of sialic acids in urine was determined by the reaction with acetic-sulfuric acid reagent (Hess reaction). Molecular genetic analysis was carried out using buccal epithelium as biological material. Determination of IL1B 3953C>T (rs1143634) and eNOS G894T (rs1799983) polymorphic variants was carried out by polymerase chain reaction using the commercial kit «DreamTaq Green PCR Master Mix» (Thermo Scientific, USA) and specific oligonucleotide sequences (Metabion, Germany).

***Statistical analysis.** Traditional methods of parametric and non-parametric statistics were used to process quantitative values; non-parametric methods were used for the analysis of qualitative characteristics expressed mainly in percentages. Under the normal distribution of data, the main statistical characteristics were used, namely: the average value (M) to determine the central tendency; the standard error of the mean (m) for the precision of the estimate of the mean, the confidence interval (CI) for determining the 95 % interval of the mean. Hypotheses regarding the equality of general means were tested using a two-tailed Student's t-test. Comparison of relative or percentage values was performed using Fisher's exact two-sided test. Relationships between count variables were determined using Poisson multiple regression analysis. Statistical analysis was performed using the STATA 14.0 software package.*

The study was conducted in compliance with the 'Rules for Ethical Principles for Scientific Medical Research Involving Human Subjects' approved by the Declaration of Helsinki (1964-2013), ICH GCP (1996), EEC Directive 609 (24.11.1986), Order of the Ministry of Health of Ukraine No. 690 of 23.09.2009 and confirmed by the conclusion of the Biomedical Ethics Commission of Poltava State Medical University (Protocol No. 233 of 21.11.2024), which approved the study. Written consent was obtained from the parents of the newborns.

The study was conducted within the framework of the joint budget research work of the Department of Pediatrics № 1 with Neonatology of the Poltava State Medical University on the topic «Development of clinical and laboratory criteria, methods for predicting and preventing metabolic disorders in children of early age», state registration number 0120U102856, term of execution 2020-2024.

Research results. *A study of the indicators of nitric oxide exchange in the group of examined children showed that the concentration of nitrites was significantly higher in children with hypoxic ischemic encephalopathy in the period of 6-9 months of life, 2.69 nmol/l compared to healthy children, 1.39 nmol/l ($p<0.001$). Similar differences were obtained in the concentration of nitrates (5.41 vs 2.62; $p<0.001$). We did not obtain significant differences in the levels of nitrates, nitrites and malondialdehyde depending on the genotypes of the IL1B gene (C3953T, rs1143634), but we found a higher (at the level of $p<0.1$) concentration of sialic acids in children with the CC genotype than in children with CT or TT genotype. When studying the eNOS gene (G894T, rs1799983), we did not find significant differences in the levels of nitrates, nitrites, sialic acids and malondialdehyde in children with different genotypes. The study revealed a reliable direct relationship (at the level of $p<0.1$) between the level of nitrates in the urine of children of the examined groups and the concentration of sialic acids (Coef. 0.753, $p=0.062$).*

***Conclusion.** The results of the study indicate an increase in the levels of nitrates (5.41 vs. 2.62; $p<0.001$) and nitrites (2.69 vs. 1.39; $p<0.001$) in children of mothers with metabolic syndrome in the recovery period of hypoxic ischemic encephalopathy, which can indicate the progression of pathological processes caused by hypoxic damage or testify to the adaptive mechanisms of recovery after brain tissue damage. A reliable direct relationship was also found between the level of nitrates in urine in children of the examined groups*

and the concentration of sialic acids (Coef. 0.753, p=0.062). At the same time, these changes were not associated with polymorphic variants of the eNOS (G894T, rs1799983) and IL1B (C3953T, rs1143634) genes. Thus, determining the concentration of nitrates and nitrites can be implemented in clinical practice for early diagnosis and prediction of possible complications in children with hypoxic ischemic encephalopathy, because the increased level of nitrites, nitrates and sialic acids can serve as a marker of distant consequences of hypoxic damage. This allows us to consider them as promising predictors of a child's suboptimal neuropsychological development.

Key words: *Neonates; Prognosis; Nitrates and Nitrites; Malondialdehyde; Sialic Acids; eNOS (G894T, rs1799983) and IL1B (C3953T, rs1143634) genes; Follow-up; Neonatal Morbidity; Hypoxic-Ischemic Encephalopathy; Endothelial Dysfunction; Adverse Outcomes.*

Introduction

According to the World Health Organization, asphyxia and hypoxic ischemic encephalopathy (HIE) remain the leading neurological causes of disability and death in newborns. The prevalence of HIE in high-income countries is approximately 1-4 per 1000 newborns. In low-income countries, the incidence is much higher, 10-20 cases per 1000 newborns. Nearly 35 % of infants with HIE develop neurological lesions such as: speech, mental and motor development delays, epilepsy, cerebral palsy, and 25 % of infants die within the first two years of life [1].

The degree of brain damage and subsequent neurological complications are determined by a number of factors, in particular, the duration of hypoxia/ischemia, immaturity of the central nervous system of newborns, cerebral circulation and metabolism, somatic status of mothers, etc. It has been proven that changes in cerebral blood flow in HIE are closely related to the action of nitric oxide (NO). The role of NO in the pathogenesis of ischemic brain damage is controversial: it can exhibit both neuroprotective and neurotoxic properties, depending on the isoform of NO synthase (NOS) and the type of cells producing NO. Under oxidative stress, NO produced by nNOS (neuronal NOS) leads to neuronal death, causing mitochondrial damage, energy loss, and subsequent disruption of calcium homeostasis [2]. The endothelial form of NOS has a neuroprotective function, which is realized through NO-mediated enhancement of brain perfusion [3].

The production of NO is regulated by the *eNOS* gene, by changing its expression or the activity of the eNOS enzyme. The presence of G894T variants of the *eNOS* gene reduces NO and contributes to endothelial dysfunction. A decrease in the level of NO leads to vasoconstriction, slowing of blood flow and subsequent hypoxia. NO2 and NO3 anions are the final products of nitric oxide metabolism in the human body [4]. A decrease in nitrate and nitrite levels due to NO deficiency potentially indicates vascular ischemia and vasospasm, as well as the severity of oxidative stress. Endothelial dysfunction, manifested by the loss of neurovascular protective functions of NO, can significantly contribute to the development of cognitive disorders.

Reoxygenation and reperfusion of previously ischemic tissues also play a role in the pathophysiological pathways of the HIE development, which leads to the activation of a cascade of free-radical oxidation reactions, in particular, the formation of free oxygen radicals [5]. Free radicals can cause cell damage through lipid peroxidation (LPO), which can be assessed by measuring malondialdehyde (MDA) levels [6]. Its long half-life and reactivity allow it to act both inside and outside cells, and interaction with proteins and DNA determines its role in various pathophysiological processes. In traumatic injury of the central nervous system (CNS), damage caused by reactive oxygen species determines the onset of LPO and is characterized by elevated levels of MDA. This metabolite reacts with several functional groups on proteins, lipoproteins and DNA. The interaction of MDA with DNA demonstrates potential genotoxicity – the formation of DNA adducts that cause mutations and changes in gene expression. Along with the activation of POL in the development of HIE, inflammation also plays an important role [7], its activation together with an increase in the concentration of pro-inflammatory cytokines, can occur under the influence of NO [8,9]. It is known that under conditions of hypoxia and inflammation, the cytokine interleukin-1 beta (IL-1β) activates inducible NO synthase (iNOS), which dramatically increases NO production and, accordingly, potentiates brain damage after ischemic perinatal asphyxia [10].

To elucidate the role of inflammation in the development of HIE, our attention was drawn to sialic acids, because these compounds are the key or switch that controls the innate immune response in the CNS, formed in microglia [11]. Today, it is known that microglial cells play a key role in brain development, maturation and homeostasis, responding to infection, trauma or other pathological conditions, turning into macrophage-like cells with the function of innate immune protection, which can be regulated by sialylation [12, 13].

The above-mentioned changes can be realized to a greater extent in mothers with metabolic syndrome (MS), since maternal metabolic disorders create an intrauterine metabolic environment that negatively affects fetal development and probably plays an important role in programming metabolic disorders in adult life [14].

Considering the importance of nitrogen metabolism disturbances, lipid metabolism and inflammation in the development of HIE, the study of key metabolites of these pathophysiological pathways on the background of the study of gene variants encoding these proteins and affecting their functional state is extremely relevant.

The aim and tasks of the research. To investigate the peculiarities of metabolism (by the level of nitrates, nitrites, MDA and sialic acids) in infants 6-9 months of age born from mothers with MS, who had HIE in the early neonatal period and also find the associations between the genotypes of the *eNOS* (G894T), *IL-1β* (C3953T) genes and the above-mentioned metabolites.

Materials and methods. To achieve the aim, a prospective «case-control» study was conducted, which included 30 children aged 6-9 months who are registered

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in the follow-up office of the Poltava Regional Children's Clinical Hospital. The main group of the study consisted of 16 children who were born from mothers with MS and who had an Apgar score less than 7 points on 5th minute, and also had diagnostic clinical and neurosonographic signs of hypoxic-ischemic damage to the CNS, which were found during consultation with a pediatric neurologist in aged 6-9 months of life. The comparison group consisted of 14 randomly selected relatively healthy children aged 6-9 months who were born from mothers without MS and who had no manifestations of HIE in the early neonatal period. The distribution of children by gender did not differ significantly, in particular, there were 7 (50 %) girls in the main group, and 7 (43.75 %) in the comparison group.

The criteria for assigning mothers to the main group (presence of MS) were a singleton pregnancy and the presence of three or more of the following criteria: hypertriglyceridemia >1.7 mmol/l, high blood pressure >130 per 85 mm Hg, obesity during the first half of pregnancy, low level of high-density lipoprotein (HDL): <1.3 mmol/l, high fasting blood glucose > 5.5 mmol/l.

Inclusion criteria for the main group of children were: clinical and neurosonographic signs of hypoxic-ischemic injury to the central nervous system, gestational age of 25-36 (6/7) weeks, birth weight up to 2500 g, and the presence of informed parental consent for participation in the clinical study.

Exclusion criteria of the study were the presence of congenital malformations, genetic diseases, and parents' refusal to participate in the study.

The study was conducted in accordance with the Declaration of Helsinki. The Bioethics Committee of Poltava State Medical University (Protocol № 233 of November 21, 2024) approved the study. Parents of the children provided informed consent to participate in the study, after which they gave informed consent to the study.

The material for the study of nitrates, nitrites, MDA and sialic acids was urine, which was collected from children at the age of 6-9 months during a visit to the follow-up observation center.

Methods of determination of nitrates and nitrites in urine. The concentration of nitrites was determined by determining the diazo compounds formed in the reaction with sulfanilic acid, and then the reaction with α-naphthylamine (Griess-Ilosvay reagent) was carried out, resulting in the formation of red derivatives (azo dyes). The color intensity is proportional to the nitrite concentration. The concentration of nitrates was determined by the increase in the concentration of nitrites after the reduction of nitrates to nitrites by sulfuric hydrazine. To determine the concentration of nitrates and nitrites, aliquots of 0.2 ml of urine were used [15, 16].

The technique of spectrophotometric determination of the concentration of free malondialdehyde. The principle of the method is based on the fact that free MDA specifically reacts with 1-methyl-2-phenylindole in a mixture of methanol and acetonitrite with the formation of a chromogen (carbocyanine dye) of orange color with maximum light absorption at a wavelength of 586 nm [17].

The method of determining sialic acids in urine by reaction with acetic-sulfuric acid reagent (Hess reaction). The principle of the method is based on the fact that sialic acids, which are derivatives of neuraminic acid, are released as a result of the hydrolysis of urine glycoproteins and form a colored compound when heated with an acetic-sulphuric acid reagent (Hess reaction). The content of sialic acids was determined by plotting the dependence of absorption on concentration [18].

Molecular genetic analysis. The buccal epithelium was used as the biological material for the study. The material was collected using disposable sterile brushes and stored in tubes with the preservative «DNA/RNA Shield» (Zymo Research, USA). A commercial kit «Quick-DNA Mini Prep Plus Kit» (Zymo Research, USA) was used for DNA isolation. Determination of *IL1B* 3953C>T (rs1143634) [19] and *eNOS* G894T (rs1799983) [20] polymorphic variants was carried out by polymerase chain reaction (PCR) using the commercial kit «DreamTaq Green PCR Master Mix» (Thermo Scientific, USA) and specific oligonucleotides sequences (Metabion, Germany). The appropriate temperature regime was ensured with the help of the «FlexCycler BU» amplifier (Analytik Jena, Germany). Amplification products of the *IL1B* and *eNOS* genes were subjected to hydrolytic cleavage according to the RFLP (restriction fragment length polymorphism) reaction using restriction endonucleases «TaqI (10U/μL)» and «MboI (10 U/μL)», (Thermo Fisher Scientific, USA) in accordance. Evaluation of restriction fragments was carried out in an agarose gel (agarose «CSL-AG500», Cleaver Scientific Ltd, Great Britain; buffer «10xTVE Electrophoresis Buffer», Thermo Scientific, USA; molecular weight marker «GeneRuler 100 bp DNA Ladder», Thermo Scientific, USA) by adding ethidium bromide as a dye.

Statistical analysis. Traditional methods of parametric and non-parametric statistics were used to process quantitative values; non-parametric methods were used for the analysis of qualitative characteristics expressed mainly in percentages. Under the normal distribution of data, the main statistical characteristics were used, namely: the mean value (M) to determine the central tendency; confidence interval (CI) to determine the 95 % interval of the mean. Hypotheses regarding the equality of general means were tested using a two-tailed Student's t-test. Comparison of relative or percentage values was performed using Fisher's exact two-sided test. Relationships between count variables were determined using binary and multiple Poisson regression analyses. Statistical analysis was performed using the STATA 14.0 software package.

The study was conducted in compliance with the 'Rules for Ethical Principles for Scientific Medical Research Involving Human Subjects' approved by the Declaration of Helsinki (1964-2013), ICH GCP (1996), EEC Directive 609 (24.11.1986), Order of the Ministry of Health of Ukraine No. 690 of 23.09.2009 and confirmed by the conclusion of the Biomedical Ethics Commission of Poltava State Medical University (Protocol No. 233 of 21.11.2024), which approved the study. Written consent was obtained from the parents of the newborns.

The study was conducted within the framework of the joint budget research work of the Department of Pediatrics № 1 with Neonatology of the Poltava State Medical University on the topic «Development of clinical and laboratory criteria, methods for predicting and

preventing metabolic disorders in children of early age», state registration number 0120U102856, term of execution 2020-2024.

Research results

The study of nitric oxide metabolism indicators in the groups of examined children showed that the concentration of nitrites in the main group children in the period of 6-9 months of life was significantly higher than in the children of the comparison group (2.69 nmol/l vs. 1.39 nmol/l, p <0.001). As evidenced by the research results presented in Table 1, similar differences were obtained in the concentration of nitrates (p<0.001). We also pay attention to the slightly higher level of sialic acids in the children of the main group, compared to the children of the comparison group. And although the confidence level is p<0.1, we believe that further studies on a larger cohort of patients are needed to clarify the final role of sialic acids in the development and prolongation of hypoxic-ischemic damage of the CNS in babies 6-9 months of age. As for MDA, we did not find any reliable differences in its concentration among the children of the examined groups.

Table 1

Levels of nitrites, nitrates, MDA and sialic acids in children of the examined groups, M (95 % CI)

Indicators	Main group (n=16)	Comparison group (n=14)	p
Nintrites in urine (nmol/l)	2,69 (2,23-3,16)	1,39 (1,12-1,65)	<0,001
Nitrates in urine (nmol/l)	5,41 (4,52-6,03)	2,62 (2,13-3,11)	<0,001
MDA (μmol/l)	2,25 (1,52-2,97)	1,96 (1,05-2,88)	0,6088
Sialic acids (mmol/l)	0,28 (0,16-0,41)	0,15 (0,07-0,24)	0,0803

Considering the importance of eNOS and IL-1β in the development of HIE, the study of gene variants encoding these proteins and affecting their functional state is extremely relevant. Thus, there is convincing evidence that excessive production of IL-1β is a leading component of the development of inflammation and subsequent brain damage in newborns with encephalopathy [21]. Increased levels of IL-1β were observed in newborns with asphyxia, which was accompanied by impaired cerebral metabolism and subsequent developmental delay [22 23].

The next step of our research was the study of associations between the level of nitrates in urine and the concentration of MDA and sialic acids (Table 2). The study revealed a reliable direct relationship between MDA and sialic acids (p=0.014) and a relationship at the level of reliability p<0.1 between the concentration of nitrates in the urine of children of the examined groups and the concentration of sialic acids (Coef. 0.753, p=0.062) by binary Poisson logistic analysis. It should be noted that a reliable direct relationship between MDA and sialic acids was found in multiple Poisson regression analysis after correction for the gestational age of the child (Coef. 1.27 (95 % CI 0.25-0.29; p=0.014).

To study the relationship between *IL1B* gene variants (C3953T, rs1143634) and the concentration of nitrates, nitrites, MDA and sialic acids in the urine of children, two groups were formed. The first group (n=11) consisted of children carrying the T allele of the *IL1B* gene (C3953T, rs1143634), and the second group (n=19) consisted of children with the CC genotype. To study the relationship between *eNOS* gene variants (G894T, rs1799983) and the concentration of nitrates, nitrites, MDA and sialic acids in the urine of children, two groups were also formed. The first group (n=21) consisted of children carrying the T allele of the *eNOS* gene (G894T, rs1799983), and the second group (n=9) consisted of children with the GG genotype.

We found no significant differences in the levels of nitrates, nitrites, and MDA in the urine of examined children depending on the genotypes of the *IL1B* gene (C3953T, rs1143634), but attention should be paid to the higher (at the level of p <0.1) concentration of sialic acids in children with the CC genotype, than in children with CT or TT genotype (Table 3).

Concerning the *eNOS* (G894T, rs1799983) gene, we found no significant differences in the levels of nitrates, nitrites, sialic acids, and MDA in children with different variants of the *eNOS* (G894T, rs1799983), *IL1B* (C3953T, rs1143634) genes (Table 4)

Table 2

The relationship between the concentration of nitrates in urine and the level of MDA and sialic acids (by binary Poisson regression)

	Nitrates			Nitrites			MDA		
	Coef.	(95 % CI)	p	Coef.	(95 % CI)	p	Coef.	(95 % CI)	p
MDA	0,055	-0,062-0,173	0,359	0,047	-0,12-0,21	0,576	-	-	-
Sialic acids	0,753	-0,038-1,545	0,062	0,67	-0,45-1,80	0,239	1,28	0,26-2,29	0,014

Table 3

The concentration of nitrates, nitrites, MDA and sialic acids in the urine of examined children depending on the genotype of the *IL1B* gene (C3953T, rs1143634), M (95 % CI)

Indicators M (95 % CI)	Genotype CT or TT (n=11)	Genotype CC (n=19)	p
Nitrites in urine (nmol/l)	2,07 (1,45-2,69)	2,09 (1,61-2,58)	0,9493
Nitrates in urine (nmol/l)	4,09 (2,83-5,34)	4,12 (3,14-5,11)	0,959
MDA (μmol/l)	2,00 (1,05-2,95)	2,18 (1,46-2,9)	0,7455
Sialic acids (mmol/l)	0,15 (0,06-0,24)	0,27 (0,16-0,38)	0,077

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Table 4

The concentration of nitrates, nitrites, MDA and sialic acids in the urine of examined children depending on the genotype of the *eNOS* gene (G894T, rs1799983), M (95 % CI)

Indicators	Genotype GT or TT (n=21)	Genotype GG (n=9)	p
Nitrates in urine (nmol/l)	2,09 (1,68-2,52)	2,06 (1,19-2,92)	0,929
Nitrites in urine (nmol/l)	4,15 (3,32-4,98)	4,01 (2,25-5,78)	0,8780
MDA (μmol/l)	2,26 (1,53-2,98)	1,78 (1,00-2,56)	0,3406
Sialic acids (mmol/l)	0,19 (0,13-0,26)	0,29 (0,07-0,51)	0,3626

Discussion

Nitric oxide is an important regulator in many systems, including vascular endothelium and smooth muscle cells, macrophages, and neurons [24]. Violation of NO synthesis or metabolism is a key factor in the pathophysiological mechanisms of the development of a number of diseases in infants. A decrease in NO production leads to persistent pulmonary hypertension in newborns [25]. An excess of NO is associated with septic shock [26]. In addition, NO may play a key role in the development of neonatal hypoxic-ischemic brain injury [27].

Our previous studies clarified the role of *eNOS* gene polymorphism and nitrogen metabolism disorders in the development of hypoxic-ischemic damage of the CNS, in particular, a reliable connection between an increase in the level of nitrates and nitrites in the urine with the development of this condition in the early neonatal period was proven, while the percentage of infants with a polymorphic gene was significantly higher among premature infants with hypoxic-ischemic lesions of the central nervous system than among infants without this condition [28]. The results of the meta-analysis showed that the G894T variant affects the production of nitric oxide, in particular, it was determined that carriers of the T allele had lower levels of NO (which is equivalent to lower levels of nitrates/nitrites) [29]. Research by Sofowora et al. indicates that the clinical significance of this genetic variant can be manifested only in the presence of endothelial dysfunction [30].

According to other scientists, in children with asphyxia, in response to hypoxia, production (NO2–+NO3–) increases, which helps maintain calcium homeostasis of cells and protects them from damage [31]. But during a prolonged hypoxic state, the production of (NO – + NO –) from L-arginine weakens. We obtained significantly higher levels of nitrates and nitrites in urine in children aged 6-9 months with HIE than in healthy children. In our opinion, a higher level of nitrite and nitrate concentrations in children with HIE during the first half of life may indicate the prolongation of increased activity of nitric oxide in the body, which is an important indicator of the functioning of the endothelial system. This change can be a direct evidence of the adaptation of the endothelial system to postnatal conditions, the improvement of metabolic processes and the development of the immune response. Our results, combined with the data of other authors, indicate increased excretion of nitric oxide in older age [32]. This result may indicate that children with a complicated neonatal period may have increased NO metabolism for adequate perfusion. In the future, such activation may be a compensatory response of the body to tissue damage caused by the initial hypoxic-ischemic injury, indicating the presence of long-term endothelial dysfunction.

However, the low concentration of nitrites in blood plasma during the first minutes and hours after birth can be caused by several factors. Premature babies have a deficiency of L-arginine, which leads to a decrease in NO synthesis. Newborns also have a higher concentration of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NOS, compared to adults [33]. Thus, low L-arginine and elevated ADMA may result in low eNOS activity in neonates, contributing to reduced plasma nitrite levels after birth. A decrease in nitrate and nitrite levels due to NO deficiency potentially indicates vascular ischemia and vasospasm, as well as the severity of oxidative stress. Endothelial dysfunction, manifested by the loss of neurovascular protective functions of NO, can significantly contribute to the development of cognitive disorders.

In a study by Schlenzig et al. a higher concentration of MDA was found in infants who required artificial lung ventilation [34]. Research by Seif El Dein et al. established a correlation between the stages of severity according to the Sarnat scale and the level of MDA in blood serum. Newborns with an elevated level of MDA had more frequent convulsions, stage II-III according to the Sarnat scale, and a longer stay in the hospital [35]. Studies by Fulia et al. claims that newborns who suffered asphyxia had significantly higher levels of MDA and NO in blood plasma than healthy infants [36]. Now there is the convincing evidence, that enhanced NO formation, due to the expression of an inducible isoform of NO synthase, plays an important role in asphyxia, shock and inflammation [37]. We obtained a reliable relationship between the level of MDA and the concentration of sialic acids in multiple Poisson regression analysis after correction for the gestational age of the child. This confirms the opinion regarding the unity of two important pathophysiological pathways – LPO and inflammation [38], that is, the activation of LPO switches macroglia from a homeostatic state to an activated one by increasing the activity of sialic acids [11].

In our study, the level of sialic acids in babies with the CC genotype of the *IL1B* gene (C3953T, rs1143634) was slightly higher than in babies who are carriers of the T-allele of this gene. Today, it is known that sialic acids are components of glycoproteins and glycolipids that are part of cell membranes, and their release into the bloodstream and excretion with urine may be a sign of tissue damage during hypoxia. Clinical and experimental studies also demonstrate that sialic acids are the key regulator of immune cell biology, from hematopoiesis to

effector functions, while this metabolite with powerful biophysical characteristics is dynamically modulated during CNS development [12, 13, 40]. Sialic acids can inhibit complement activation by modulating key functions of the complement system during immune control and homeostasis of the innate immune response of the CNS [11, 43], bind and buffer neurotrophic factors, growth factors, neurotransmitters and cytokines [45, 46]. Recent studies have shown that sialylation is critical for the protection of extraembryonic fetal tissue against maternal complement attack [47], although the exact mechanism of the protective effect on the fetus is still unclear. That is, we assume that carriers of the T-allele may be more susceptible to the effects of trauma, infection, and other pathological conditions.

Regarding the peculiarities of the patient cohort – the impossibility of repeated blood sampling from newborn children for reasons of patient safety and considering the principles of bioethics, determining the indicators of nitric oxide metabolism, the concentration of MDA and sialic acids in urine can be a useful method for assessing oxidative stress in infants and monitoring the response to the latest implemented methods treatment of HIE.

Conclusion

Thus, the results of the study indicate an increase in the levels of nitrates (5.41 vs. 2.62; p<0.001) and nitrites (2.69 vs. 1.39; p<0.001) in children from mothers with metabolic syndrome in the recovery period of HIE, which may indicate the progression of pathological processes caused by hypoxic damage or indicate adaptive mechanisms of recovery after brain tissue damage. A reliable direct relationship was also found between the level of nitrates and the concentration of sialic acids (Coef. 0.753, p=0.062), MDA and the concentration of sialic acids in urine in children of the examined groups (Coef. 1.27 (95 % CI 0.25 (–0.29; p=0.014). However these changes were not associated with polymorphic variants of the *eNOS* (G894T, rs1799983) and *IL1B* (C3953T, rs1143634) genes.

Therefore, determining the concentration of nitrates and nitrites can be implemented in clinical practice for early diagnosis and prediction of possible complications in children with HIE, since an elevated level of nitrites, nitrates and sialic acids can serve as a marker of distant consequences of hypoxic damage. This allows us to consider them as promising predictors of a child's suboptimal neuropsychological development.

Conflict of interest. The authors declare no conflict of interest.

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ОСОБЛИВОСТІ МЕТАБОЛІЗМУ (ЗА РІВНЕМ НІТРАТІВ, НІТРИТІВ, МАЛОНОВОГО ДІАЛЬДЕГІДУ ТА СІАЛОВИХ КИСЛОТ) У НЕМОВЛЯТ 6-9 МІСЯЧНОГО ВІКУ, ЯКІ НАРОДИЛИСЯ ВІД МАТЕРІВ З МЕТАБОЛІЧНИМ СИНДРОМОМ І ЯКІ МАЛИ ГІПОКСИЧНО-ІШЕМІЧНУ ЕНЦЕФАЛОПАТІЮ В РАННЬОМУ НЕОНАТАЛЬНОМУ ПЕРІОДІ

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Відділ розвитку програми медичних гарантій Департаменту розвитку стратегії загального охоплення медичними послугами Національної служби здоров'я України[1] (Київ, Україна),
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Резюме.

За даними Всесвітньої організації охорони здоров'я основними неврологічними причинами інвалідності та смертності новонароджених дітей залишаються асфіксія та гіпоксично-ішемічна енцефалопатія. Ступінь ушкодження головного мозку та подальші неврологічні ускладнення визначаються низкою факторів, зокрема тривалістю гіпоксії/ішемії, мозковим кровообігом, незрілістю центральної нервової системи новонароджених, соматичним статусом матерів.

Метою дослідження було встановити особливості метаболізму (за рівнем нітратів, нітритів, малонового діальдегіду та сіалових кислот) у немовлят 6-9 місячного віку, які народилися від матерів з метаболічним синдромом, і які мали гіпоксично-ішемічну енцефалопатію в ранньому неонатальному періоді, а також вплив варіантів генів eNOS (G894T), IL-1b (C3953T) на обмін даних метаболітів.

Матеріали та методи. Для досягнення мети проведено проспективне дослідження «випадок-контроль», в яке включено 30 дітей віком 6-9 місяців. Основну групу дослідження склали 16 дітей, які народилися від матерів з метаболічним синдромом і які мали оцінку за шкалою Апгар менше за 7 балів на 5 хвилині, а також мали діагностичні клінічні та нейросонографічні ознаки гіпоксично-ішемічної енцефалопатії, що були виявлені при консультуванні дитячим неврологом у віці 6-9 місяців життя. Групу порівняння складали 14 рандомно відібраних відносно здорових дітей віком 6-9 місяців, які народилися від матерів без метаболічного синдрому і які не мали проявів гіпоксично-ішемічної енцефалопатії в ранньому неонатальному періоді.

Концентрацію нітритів визначали шляхом визначення діазосполук, що утворилися у реакції з сульфаніловою кислотою, а потім проводили реакцію з α-нафтиламіном (Реактив Грісса-Ілосвая). Концентрацію вільного малонового діальдегіду визначали методом спектрофотометричного визначення. Концентрацію сіалових кислот в сечі визначали за реакцією з оцтово-сірчанокислим реактивом (реакція Гесса). М*олекулярно-генетичний аналіз було* проведено з використанням букального епітелію в якості біологічного матеріалу. Визначення поліморфних варіантів *IL-1β 3953C>T* (rs1143634) та *eNOS G894T* (rs1799983) проводилось методом полімеразної ланцюгової реакції за допомогою комерційного набору «DreamTaq Green PCR Master Mix» (**T**hermo Scientific, США) та специфічних олігонуклеотидних послідовностей (Metabion, Німеччина). Статистичний аналіз. Для обробки кількісних величин використовували традиційні методи параметричної та непараметричної статистики: для аналізу якісних ознак, що виражалися в основному у відсотках, було застосовано непараметричні методи. За нормального розподілу даних використовували основні статистичні характеристики, а саме: середнє значення (M) для визначення центральної тенденції; стандартну похибку середнього значення (m) для точності оцінки середньої, довірчий інтервал (ДІ) для визначення 95 % інтервалу середньої. Гіпотези щодо рівності генеральних середніх перевіряли з використанням двостороннього t-критерію Стьюдента. Порівняння відносних, або виражених у відсотках, величин виконували за допомогою критерію точного двостороннього критерію Фішера. Зв'язки між лічильними перемінними визначали за допомогою множинного регресійного аналізу за Пуассоном. Статистичний аналіз проводили за допомогою пакету прикладних програм STATA 14.0.

Дослідження виконане із дотриманням «Правил етичних принципів проведення наукових медичних досліджень за участю людини», затверджених Гельсінською декларацією (1964-2013 рр.), ICH GCP (1996 р.), Директиви ЄЕС № 609 (від 24.11.1986 р.), наказу МОЗ України № 690 від 23.09.2009 р. та підтверджено висновком Комісії з питань біомедичної етики Полтавського державного медичного університету (Протокол № 233 від 21.11.2024 року) схвалила дослідження. На проведення досліджень було отримано письмову згоду батьків новонароджених дітей.

Дослідження виконано в рамках НДР кафедри педіатрії № 1 із неонатологією Полтавського державного медичного університету «Розробити клініко-лабораторні критерії, методи прогнозування та запобігання метаболічних порушень у дітей раннього віку», реєстраційний номер 0120U102856, термін виконання 2020-2024 рр.

Результати дослідження. Дослідження показників обміну оксиду азоту у групі обстежуваних дітей показало, що у малюків з гіпоксично-ішемічною енцефалопатією в період 6-9 місяців життя концентрація нітритів була значно вищою 2,69 нмоль/л порівняно зі здоровими дітьми 1,39 нмоль/л ($p<0,001$). Аналогічні відмінності отримані і в концентрації нітратів (5,41 vs 2,62; $p<0,001$). Нами не було отримано достовірних відмінностей у рівнях нітратів, нітритів та малонового діальдегіду залежно від генотипів гену *IL1B* (C3953T, rs1143634), проте ми виявили вищу (на рівні $p<0,1$) концентрацію сіалових кислот у дітей з генотипом CC, ніж у дітей з генотипом CT або TT. При дослідженні гену *eNOS* (G894T, rs1799983) ми не отримали достовірних відмінностей у рівнях нітратів, нітритів, сіалових кислот та малонового діальдегіду у дітей з різними генотипами. Дослідження виявило достовірний прямий зв'язок (на рівні $p<0,1$) між рівнем нітратів в сечі у дітей обстежених груп та концентрацією сіалових кислот (Coef. 0,753, $p=0,062$).

Висновок. Результати дослідження свідчать про підвищення рівнів нітратів (5,41 vs 2,62; $p<0,001$) та нітритів (2,69 vs. 1,39; $p<0,001$) у дітей від матерів з метаболічним синдромом у відновному періоді гіпоксично-ішемічної енцефалопатії, що може вказувати на прогресування патологічних процесів, викликаних гіпоксичним ураженням або ж свідчити про адаптивні механізми відновлення після ушкодження тканин головного мозку. Також було виявлено достовірний прямий зв'язок між рівнем нітратів в сечі у дітей обстежуваних груп та концентрацією сіалових кислот (Coef. 0,753, $p=0,062$). При цьому ці зміни не асоціювалися з поліморфними варіантами генів *eNOS* (*G894T*, rs1799983) та *IL1B* (C3953T, rs1143634). Таким чином, визначення концентрації рівня нітратів та нітритів можуть бути впроваджені в клінічну практику для ранньої діагностики та прогнозування можливих ускладнень у дітей з гіпоксично-ішемічною енцефалопатією, оскільки підвищений рівень нітритів, нітратів та сіалових кислот

може слугувати маркером віддалених наслідків гіпоксичного ураження. Це дозволяє розглядати їх як перспективні предиктори неоптимального нервово-психічного розвитку дитини.

Ключові слова: новонароджені; прогнозування; нітрати та нітрити; малоновий діальдегід; сіалові кислоти; гени *eNOS* (G894T, rs1799983) та *IL1B* (C3953T, rs1143634); катамнестичне спостереження; неонатальна захворюваність; гіпоксично-ішемічна енцефалопатія; ендотеліальна дисфункція; несприятливі наслідки.

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