

# РЕЗУЛЬТАТИ ДИСЕРТАЦІЙНИХ ТА НАУКОВО-ДОСЛІДНИХ РОБІТ RESULTS THESIS AND SCIENTIFIC-RESEARCH

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DIAGNOSTIC ASPECTS OF PERSISTENT  
PULMONARY HYPERTENSION IN  
PREMATURE INFANTS WITH OXIDATIVE  
STRESS

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## Summary

**Introduction.** Persistent pulmonary hypertension is a problem that leads to high morbidity and mortality in preterm infants. In clinical studies, oxidative stress (OS) contributes to the development of pulmonary hypertension (PH). The most specific biomarker of OS in preterm infants is urinary 8-hydroxy-2-deoxyguanosine (8-OHdG).

**The aim of the study.** To determine the clinical and diagnostic relationship between the value of 8-hydroxy-2-deoxyguanosine and the level of mean trunk pulmonary artery pressure in premature infants with respiratory distress syndrome and asphyxia in the early neonatal period.

**Material and methods.** Determination of 8-OHdG (ng/ml) by enzyme-linked immunosorbent assay (ELISA) and PH by echocardiography (EchoCG) in 60 premature infants at 26-32 weeks of gestation at 1 and 3-5 days of life in 2 groups: I - 32 children with respiratory distress syndrome (RDS); II - 28 children with RDS in combination with perinatal asphyxia.

**Results.** The average value of pulmonary artery pressure (mmHg) in the group II of children in comparison with group I was higher both in 1 and 3-5 days of life. The level and dynamics of 8-OHdG correlated with the severity of PH, which required longer respiratory support in group II. Sexual dimorphism of 8-OHdG levels and dynamics was noted.

**Conclusions.** Perinatal asphyxia in preterm infants with RDS on the 1st day of life complicates the course of PH, as indicated by a higher level of the urinary 8-OHdG and correlated to mPAP. Gender characteristics of the dynamics of 8-OHdG levels in children with perinatal pathology reveal reduced adaptability and reactivity of boys to OS at birth.

Dynamics in the form of a decrease in the level of 8-OHdG in the urine is a favorable prognostic sign of pulmonary hypertension. Gender characteristics of the dynamics of 8-OHdG levels in children with perinatal pathology reveal reduced adaptability and reactivity of boys to OS at birth.

**Key words:** Pulmonary Hypertension; Preterm Infants; 8-hydroxy-2-deoxyguanosine.

## Introduction

Persistent pulmonary hypertension (PPH) is a syndrome that complicates the early neonatal period and leads to high morbidity and mortality in premature newborns. The mechanisms of transformation of the cardiovascular, respiratory, and central nervous system in the cohort of preterm infants with PPH are still poorly explored. The studies on the role of the PPH in the remodeling of the cardiorespiratory system focus on searching for the best strategy in managing this pathology, especially in the population of premature infants.

PPH develops as a result of persistent vasospasm resulting from hypoxia, acidosis, hypothermia, hypoglycemia, and polycythemia. Reactive oxygen species, like superoxide and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), induced by hypoxia, lead to vasoconstriction [1, 2].

The value of the urinary 8-OHdG is one of the most specific markers of nucleotide damage due to OS in cohort of premature infants [3-5].

**Aim of the study** is to establish the clinical correlation between the value of 8-OHdG and the level of a mPAP in premature infants with RDS and asphyxia in the early neonatal period.

## Material and methods

We analyzed the observations of 60 premature infants

at gestational age of 26/1–32/6 weeks, all of them were nursed in the intensive care unit for premature newborns due to the modern protocols.

The correlation between mean pulmonary artery pressure and the value of the urinary 8-hydroxy-2-deoxyguanosine in infants with respiratory distress syndrome and respiratory distress syndrome associated with perinatal asphyxia was studied.

Due to the presence of associated pathology, newborns were divided into two groups; group I consisted of 32 children with RDS without associated asphyxia, group II - 28 children with RDS associated with asphyxia at birth. Gender differentiation in groups is presented in the table 1. The inclusion criteria for both groups were the presence of PPH. Diagnosis was made according to clinical signs, and the definitive diagnosis of PPH was established by echocardiography monitoring according to the method described in European recommendations [5]. Echocardiography can also be used to determine the severity of pulmonary hypertension [2, 6].

A quantitative measurement of the value of 8-OHdG, ng/ml on the 1st day of life, and on the 3rd–5th day was provided by ELISA using the DNA Damage ELISA kit, Enzo Life Sciences (USA) as the manufacturer's instructions require.

Design of a study was discussed and approved at a session of the Ethical commission of the Kharkiv Medical

Academy of Postgraduate Education (Prot. № 5 from 18.12.2020). All parents gave an informed consent for examination of their children.

Statistical analysis was performed using Microsoft Excel 2019 software. Under the conditions of normal distribution of quantities, parametric statistical methods

were used to calculate the arithmetic mean (M) and the representativeness error of the mean (m). Using Fisher's exact test we analyzed – the quantitative parameters.

Pearson's linear correlation coefficient (r) was applied to find the correspondence between mean mPAP and the value of the urinary 8-OHdG.

**Table 1.**

**Clinical characteristics of patient groups, n (%), M±m**

	<b>Group I (n=32)</b>	<b>Group II (n=28)</b>
Gestational age, weeks	30,55±0,56	30,81±0,69
Birth weight (g)	1352 ±108	1650 ±149
Boys, n (%)	18 (56,3%)	17 (60,7%)
Girls, n (%)	14 (43,7%)	11 (39,3%)

**Results and discussions**

During the treatment, which included surfactant administration, choosing the most optimal tactics of respiratory therapy (high-frequency ventilation, traditional mechanical ventilation, non-invasive mechanical ventilation, CPAP), correction of metabolic acidosis, hypoglycemia, the following results were obtained.

In group I the average mPAP level, mmHg. of children on the 1st day of life was lower than in group II – 24,04±0.41 vs 27.60±0.32, p=0.037. In the group II regardless of the treatment, the average mPAP, mm Hg. of children increased

on the 3rd – 5th day of life and was 29,91±0,67. Yet in group I the average mPAP reduced during the treatment was 19.95±0.30, p<0,05.

Levels of the urinary 8-OHdG, ng/ml, were analyzed: on the first day of life in children of group II the index was 2,27±0,39; in the group I the indicator was lower - 1,83±0,29, p<0,05. By the 3rd-5th day of life, in children of group II, we noted an increase in the level of 8-OHdG - 4,10±0,42 while in group I the index decreased - 1,06±0,28, p<0,05.

The children in group II received longer respiratory support (in days) (7.33±0.61 vs 4.04±0.41, p<0,05 (Table 2).

**Table 2.**

**Dynamics of the oxidative stress and mPAP levels in newborns**

<b>Index</b>	<b>Group I (n=32)</b>	<b>Group II (n=28)</b>
Mean mPAP on the 1st day of life, mmHg	24,04±0,41	27,60±0,32*
Mean mPAP on the 3rd - 5th day of life, mmHg	19,95±0,30	29,91±0,67*
Dynamics, n (%)	-4,09 (-17,01%)	+2,31 (+7,72%)
8-OHdG level on the 1st day of life, ng/ml	1,83±0,29	2,27±0,39 *
8-OHdG level on the 3rd - 5th day of life, ng/ml	1,06±0,28	4,10±0,42*
Dynamics, n (%)	-0,77 (-42,07%)	+1,83 (+44,63%)
Respiratory support, days	4,04±0,41	7,33±0,61*

\*p <0,05

Regarding the gender characteristics, the following distribution of 8-OHdG levels, ng/ml, was obtained: on the 1st day of life in boys of group I the level of the urinary 8-OHdG was higher than in girls. By the 3rd-5th day of life the indicator in both genders decreased, but in boys the dynamics of reducing the level of 8-OHdG in the urine

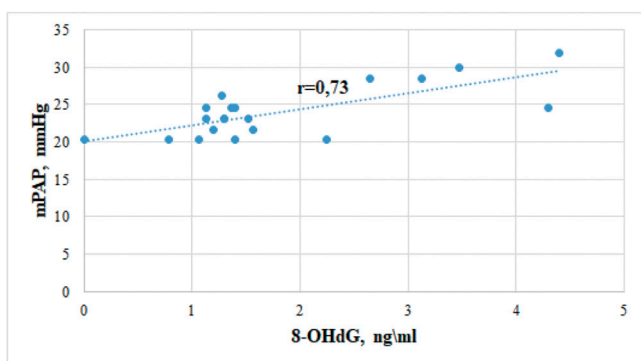
was lower than in girls. Regarding children of group II, the following results were obtained: on 1st day of life, the level of 8-OHdG was higher in boys. Then, by 3rd -5th days of life, the index increased by 37.94% in the population of boys and, despite the aggravating effects of asphyxia, decreased by 20.53% in girls (Table 3).

**Table 3.**

**Gender differences of the dynamics of 8-OHdG levels**

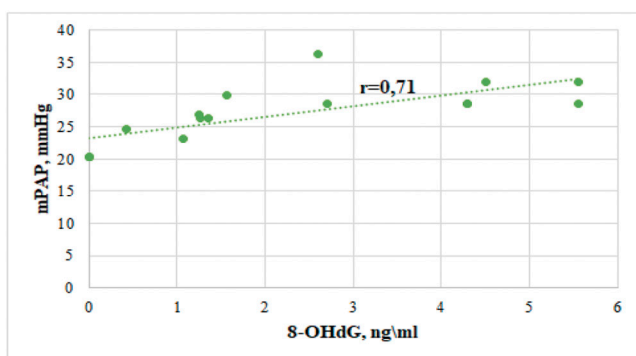
	<b>Group I (n=32)</b>		<b>Group II (n=28)</b>	
	<b>Boys (N=18)</b>	<b>Girls (n=14)</b>	<b>Boys (n=17)</b>	<b>Girls (n=11)</b>
1st day of life	1,87±0,32	1,77±0,27*	2,82±0,48	2,24±0,30*
3rd -5th days of life	1,14±0,14	0,90±0,21*	3,89±0,45	1,78±0,19*
Dynamics, n (%)	-0,73 (-39,03%)	-0,87 (-49,15)	+1,07 (+37,94%)	-0,46 (-20,53%)

\*p <0,05



**Fig. 1 Correlation between mPAP and 8-OHdG levels in children with RDS on the 1st day of life.**

In the group of children with RDS and with associated birth asphyxia on the first day of life, the correlation



**Fig. 3 Correlation between mPAP and 8-OHdG level in children with RDS and birth asphyxia on the 1st day of life.**

Modern perinatal technologies can significantly improve the quality of caring for low and extremely low birth weight infants and reduce the number of complications. However, PPH still remains a serious clinical problem for diagnostics and treatment, particularly in the population of premature infants.

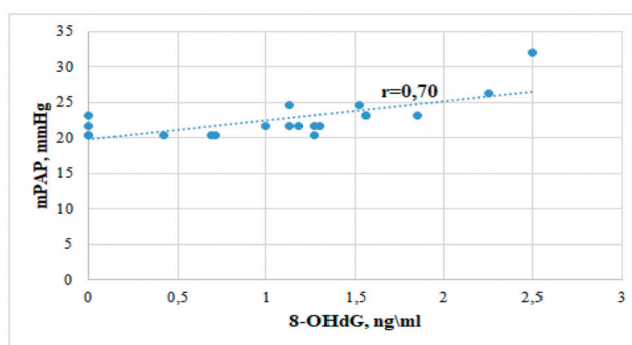
A fetus has high pulmonary vascular resistance due to low oxygen partial pressure in arterioles and alveoli. Normally, from the moment of the umbilical cord clamping and beginning of spontaneous breathing, the partial pressure of oxygen in the alveoli increases. Still, birth asphyxia contributes to maladaptation of the cardiorespiratory system, with a further mPAP increase and the progression of PH [7].

Another cause of PPH in preterm infants with RDS is abnormal pulmonary vasoconstriction as a result of unexpanded lungs. Therefore, therapeutic strategies usually included pulmonary recruitment and surfactant administration that helps to reduce increased mPAP [8].

In the group of children with RDS on the first day of life, the average mPAP was  $24,04 \pm 0,41$  mmHg, which a decreased to 17,01% by days 3-5.

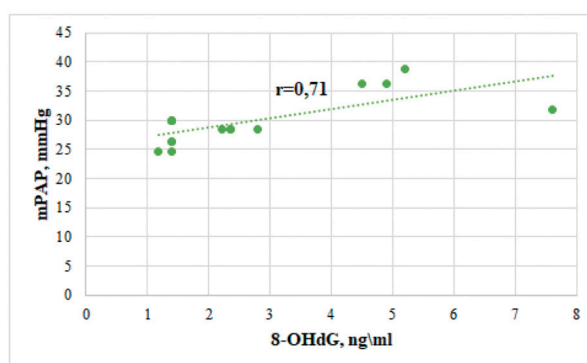
Thus, the level of mPAP in children with RDS and its dynamics depend on the influence of asphyxia, which is confirmed by the literature data [7].

Infants born with asphyxia and <32 weeks of gestation usually require primary resuscitation with positive pressure ventilation and supplemental oxygen, which releases large amounts of free radicals. In children <32 weeks of gestation, the production of antioxidants, such as superoxide



**Fig. 2 Correlation between mPAP and 8-OHdG levels in children with RDS on the 3rd-5th days of life.**

coefficient was 0,71,  $p=0,002$ ; on days 3rd-5th - 0,71,  $p=0,004$  (Fig. 3 and 4).



**Fig. 4 Correlation between mPAP and 8-OHdG level in children with RDS and birth asphyxia on days 3-5 of life.**

dismutase, catalase and glutathione - is reduced [3].

Hence, a decrease in the partial pressure of oxygen in blood, causes disruption in production of the nitric oxide synthase enzyme, which is necessary for the synthesis of nitric oxide (NO), known as the endogenous vasodilator. The key factor in reducing the bioavailability of endogenous NO in tissues is the activation of superoxide anions due to oxidative stress caused by asphyxia [8]. In addition, free radicals are the factors damaging the surfactant [8].

According to our data, the level of OS determined by the value of the urinary 8-OHdG (ng/ml) in premature infants in the group with RDS associated with asphyxia on the first day of life was  $2,27 \pm 0,39$ . By 3rd-5th days, there was noted an increase in the level of 8-OHdG by 44,63%.

In the group of children with RDS, the value of 8-OHdG on the first day of life was  $1,83 \pm 0,29$  ng/ml, and by days 3-5 it decreased by 42,07%.

Consequently, asphyxia contributes to an increased level of OS in children with perinatal pathology. The studied OS biomarker indicates the severity of the condition and course of PPH, which was noted in the duration of mechanical ventilation – infants with perinatal asphyxia required a longer respiratory support. Similar results were obtained by Z. Elkabany, et al., who established a direct moderate positive correlation between the level of the 8-OHdG and the number of days on mechanical ventilation ( $r=0,574$ ,  $p=0,02$ ) [9].

The substantiated positive moderate correlation between the level of the urinary 8-OHdG and mPAP indicates a direct relationship between the level of mean pulmonary

artery pressure and the severity of oxidative stress which allows adopting it and applying in practical management of premature newborns with RDS. The gender characteristics of the levels and dynamics of 8-OHdG indicate a lower adaptability and reactivity of the antioxidant system in male newborns, which have already been verified in several previous studies [10].

### Conclusions

1. Perinatal asphyxia complicates the course of RDS in newborns due to stronger manifested PPH and a higher level of OS;
2. The value of the urinary 8-OHdG, which indicates the level of OS at birth, correlates with the mPAP in newborns

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## ДІАГНОСТИЧНІ АСПЕКТИ ПЕРСИСТУЮЧОЇ ЛЕГЕНЕВОЇ ГІПЕРТЕНЗІЇ У ПЕРЕДЧАСНО НАРОДЖЕНИХ ДІТЕЙ З ОКСИДАНТНИМ СТРЕСОМ

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### Резюме

**Вступ.** Персистуюча легенева гіпертензія (ПЛГ) – проблема, яка зумовлює високу захворюваність та смертність недоношених новонароджених. В експериментальних моделях та клінічних дослідженнях оксидативний стрес (ОС) сприяє розвитку легеневої гіпертензії (ЛГ) з подальшим ремоделюванням серцево-судинної системи. Найбільш чутливим та специфічним біомаркером ОС у недоношених новонароджених є 8-гідрокси-2-дезоксигуанозин (8-OHdG) у сечі.

**Мета дослідження.** Визначити клініко-діагностичний взаємозв'язок значення 8-гідрокси-2-дезоксигуанозину та рівня середнього тиску в стовбурі легеневої артерії у недоношених новонароджених з респіраторним дистрес-синдромом та асфіксією в ранньому неонатальному періоді.

**Матеріал і методи дослідження.** Проведено визначення 8-OHdG (нг/мл) методом імуноферментного аналізу (ІФА) та ЛГ методом ехокардіографії (ЕхоКГ) у 60 недоношених новонароджених у терміні гестації 26-32 тижні на 1 та 3-5 добу життя у 2-х групах: I – 32 дитини з респіраторним дистрес-синдромом (РДС); II – 28 дітей з РДС у подальшому з перинатальною асфіксією.

**Результати дослідження.** Середнє значення тиску у легеневої артерії (мм.рт.ст) у II групі дітей у порівнянні з I групою було вище як у 1, так і к 3-5 добі життя. Рівень та динаміка 8-OHdG корелювала з вираженістю ЛГ, що вимагало більш тривалої респіраторної підтримки у II групі. Відзначався статевий диморфізм рівня та динаміки 8-OHdG.

**Висновки.** Перинатальна асфіксія у недоношених новонароджених з РДС на 1 добу життя ускладнює перебіг ЛГ,

на що вказує більш високий рівень ОС за даними 8-ОНдG та його кореляції із середнім тиском у легеневій артерії (mPAP). Динаміка у вигляді зниження рівня 8-ОНдG у сечі є сприятливою прогностичною ознакою перебігу легеневої гіпертензії. Гендерні особливості динаміки рівнів у дітей із перинатальною патологією вказують на знижену адаптивність та реактивність хлопчиків до окисного стресу при народженні.

**Ключові слова:** легенева гіпертензія; передчасно народжена дитина, 8-гідрокси-2-дезоксигуанозин.

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