Predictors of adverse outcomes in preterm infants in the early neonatal period

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Abstract

Introduction. Extremely and very preterm infants are a special cohort of newborns with inherent functional immaturity and specific pathological conditions which are accompanied by hypoxia, reoxygenation, tissue hypoperfusion, increased risk of oxidative stress. Premature infants are prone to oxidative deterioration which plays a key role in the pathogenesis of severe diseases such as respiratory distress syndrome, necrotizing enterocolitis, periventricular leukomalacia, patent ductus arteriosus, bronchopulmonary dysplasia, and retinopathy of prematurity.

Objective. The aim of the research was to estimate the predictors of adverse outcomes in preterm infants with gestational age less than 32 weeks in the early neonatal period by assessment of oxidative stress markers.

Material and methods. A total amount of 46 preterm infants with gestational age less than 32 weeks in the early neonatal period were included in the research. According to the results of early outcomes newborns were divided into two groups: I group – 12 infants with adverse outcomes and II group - 34 preterm infants with favorable outcomes. Twenty healthy newborns were included in the control group (III group). Clinical examination of infants and laboratory determination of oxidative stress markers were done for all participants.

Results. Asphyxia, early onset sepsis, intraventricular hemorrhage in preterm infants less than 32 weeks of gestation had increased the risk of mortality in the early neonatal period. The level of 8-isoprostane, ROS, TBP, LHP was significantly higher in neonates with adverse outcomes (group I). It was found that the level of 8-isoprostane more than 595.4 mg/ml, ROS level above 0.483 IU, TBP higher than 5.14 µmol/l and LHP levels above 5.91 U/m were associated with unfavorable outcomes in the early neonatal period. Decreased activity of the antioxidant system (catalase, SOD) increases the risk of early neonatal mortality.

Conclusions. The activation of oxidative stress in preterm infants may be a predictor of adverse outcomes in the early neonatal period.

Keywords: **preterm infant; oxidative stress; early neonatal period; adverse outcomes; favorable outcomes.**

Introduction

Prematurity is the leading cause of death among newborns and it comes in second among children below five years old, after pneumonia and, at the same time, prematurity increases the risk of serious lifetime disabilities [1, 2]. Extremely and very preterm infants are a special cohort of newborns with their inherent functional immaturity and specific pathological conditions which are accompanied by hypoxia, reoxygenation, tissue hypoperfusion, increased risk of oxidative stress [3, 4].

Oxidative stress plays a key role in the pathogenesis of several newborn diseases, such as respiratory distress syndrome, bronchopulmonary dysplasia, patent ductus arteriosus, necrotizing enterocolitis, retinopathy of prematurity, and periventricular leukomalacia [5]. Premature infants are prone to oxidative deterioration due to decreased activity of antioxidant

enzymes such as catalase and glutathione peroxidase. An imbalance between prooxidants and antioxidants leads to oxidative damage [6].

Immaturity of the respiratory, digestive, immune and antioxidant systems, with, at the same time, numerous medical invasive interventions in preterm infant (resuscitation, ventilation, parenteral nutrition, blood transfusions) contributes to the deepening of oxidative, metabolic stress and causes the development of "oxygen-radical diseases of newborns" [7, 8].

Objective

The aim of the research was to estimate the predictors of adverse outcomes in preterm infants with gestational age less than 32 weeks in the early neonatal period by assessment of oxidative stress markers.

Materials and methods. A total amount of 46 preterm infants with gestational age less than 32 weeks in the early neonatal period were included in the research. Among them there were 18 infants with extremely low birth weight (less than 1000 g) and gestational age of 28 weeks and less, and 28 infants with very low birth weight (more than 1000 g) and gestational age 29-32 weeks.

According to the results of early outcomes preterm infants were divided into two groups: 12 infants were included in group I (infants who died in the early neonatal period); 34 surviving preterm infants who were discharged for outpatient care were included in group II (with favorable outcomes). The control group (group III) included twenty newborns who were born at term, in satisfactory condition, without burdened perinatal history.

The investigation included clinical examination of infants and laboratory determination of markers of oxidative stress (8-isoprostane, reactive oxygen species (ROS)), thiobarbituric acid products (TBP) of lipid peroxidation, lipid hydroperoxides (LHP) and antioxidant system (catalase and superoxide dismutase (SOD) activity). The 8-isoprostane concentration in the urine was determined by enzyme-linked immunoassay (ELISA KitBuffer, Cayman Chemical, Michigan, USA) according to the manufacturer's instructions. ROS in blood mononuclear cells were evaluated by a flow laser cytometer. The level of TBA-active products, LHP in umbilical cord blood was determined by spectrophotometry at the appropriate wavelength. Catalase and SOD activity in umbilical cord blood was determined by spectrophotometric method.

Statistical analysis was performed using the computer programs Microsoft Excel and MedCalc. Quantitative parameters were presented in as medians (Me) and lower (Lq) and upper (Uq) quartiles. Nonparametrical tests were used to check differences between 2 groups (Mann-Whitney test) and 3 groups (Kruskal-Wallis test). Qualitative parameters were

compared using two-tailed Fisher exact test. Odds ratio (OR) and its 95 % confidence interval (95% CI) were calculated. In order to find cut-off points of predictive markers ROC-analysis was performed with ROC-curve assessment.

Results and Discussion

Twelve preterm infants (group I) died in the early neonatal period: 7 (58.3%) infants with extremely low birth weight and 5 infants (41.7%) with very low birth weight. The gestational age of infants in group I (with unfavorable outcomes) was 26.50 (25.50; 30.00) weeks, the gestational age of infants in group II - 30.00 (27.00; 31.00) weeks, (p= 0.076).

Birth weight of the newborns in group I was 935.00 (710.00; 1560.00) g, in group II - 1300.00 (900.00; 1700.00) g, without significant differences. According to the gender distribution boys (75.00%) predominated in group I, and there was a uniform gender distribution in group II (boys - 52.94%, girls - 47.06%).

Analysis of morbidity in the comparison groups has showed that early onset sepsis with multiple organ dysfunction, intraventricular hemorrhage III-IV degree, asphyxia were significantly more common in group I (p <0,05), and were mainly the main causes of the early infant mortality (Fig. 1).
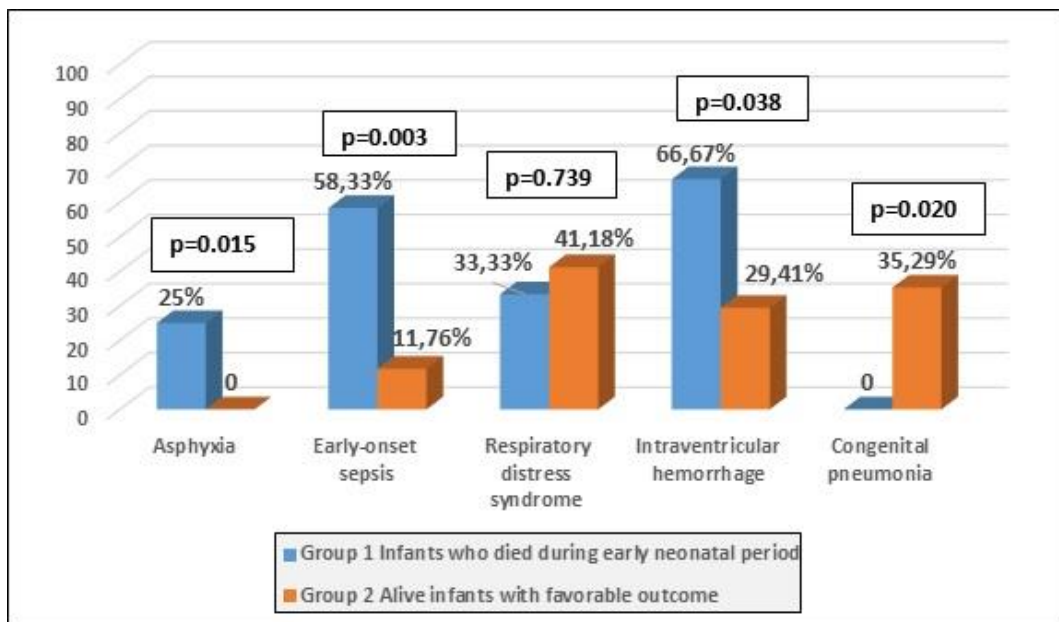

Figure 1. Clinical characteristic of the study groups

Assessment of the odds ratio has showed that asphyxia, early onset sepsis, intraventricular hemorrhage in preterm infants less than 32 weeks of gestation increase the risk of mortality in the early neonatal period (Fig.2).
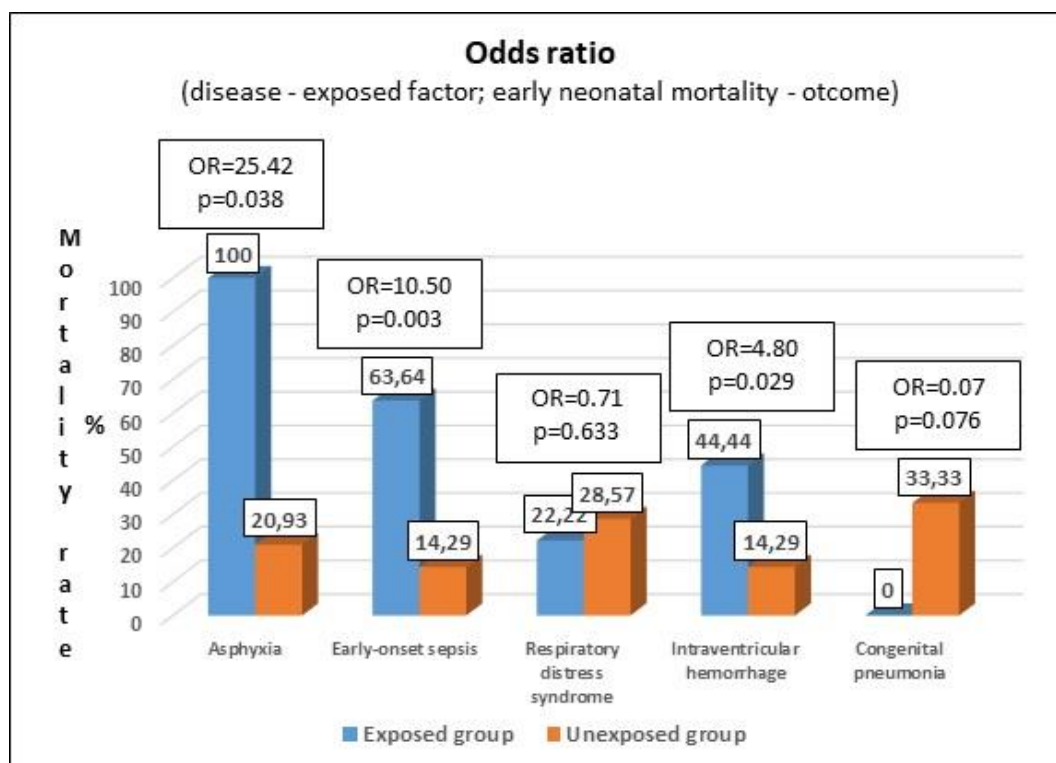
Figure 2. Morbidity influence on the mortality rate in the early neonatal period

Analysis of oxidative stress markers in preterm infants less than 32 weeks of gestation was conducted. Evaluation of oxidative stress indicators in the groups with favorable and unfavorable outcomes revealed significant differences (Table 1).

Table 1 - Indicators of oxidative stress in infants with favorable and unfavorable outcomes in the early neonatal period (Me (Lq; Uq))

Groups	8-isoprostane, mg/ml	Reactive oxygen species (ROS), IU	TBP, μmol/l	LHP, U/ml
I group (unfavorable outcomes) (n=12)	625.45 (584.35; 689.90)	0.50 (0.40; 0.57)	6.28 (5.19; 6.70)	6.50 (5.10; 6.79)
II group (favorable outcomes) (n=34)	577.10 (532.20; 607.50)	0.47 (0.43; 0.52)	5.04 (4.68; 5.75)	5.06 (4.17; 5.88)
Control group (III group), (n=20)	209.80 (149.14; 255.09)	0.22 (0.18; 0.27)	3.28 (2.91; 3.56)	3.57 (3.19; 3.85)
Kruskal–Wallis test	H=41.55; $p<0.001$*	H=34.39; $p<0.001$*	H=27.06; $p<0.001$*	H=29.19; $p<0.001$*
p	$\mathbf{p_{1-2}<0.01}$* $p_{1-3}<0.001$* $p_{2-3}<0.001$*	$p_{1-2}>0.05$ $p_{1-3}<0.001$* $p_{2-3}<0.001$*	$\mathbf{p_{1-2}<0.01}$* $p_{1-3}<0.001$* $p_{2-3}<0.001$*	$\mathbf{p_{1-2}<0.01}$* $p_{1-3}<0.001$* $p_{2-3}<0.001$*
Note. * - statistically significant result				

It was found that the level of 8-isoprostane, ROS, TBP, LHP in preterm infants (groups I and II) were higher compared to the control group (p_{1-3}<0.001, p_{2-3}<0.001). The comparison of indicators between the groups I and II has revealed a significant increase of prooxidative markers in newborns with unfavorable outcomes: 8-isoprostane level (625.45 [584.35; 689.90] and 577.10 [532.20; 607.50], p_{1-2}<0.01), TBP indices (6.28 [5.19; 6.70] and 5.04 [4.68; 5.75], p_{1-2}<0.01), LHP level (6.50 [5.10; 6.79] and 5.06 (4.17; 5.88), p_{1-2}<0.01) (tabl.1).

Increased levels of 8-isoprostane, TBP, LHP in preterm infants with an unfavorable outcomes indicate a severe pathological process, the development of deep tissue hypoxia, hypoperfusion, systemic lesions of organs and systems. Immediately after birth, the sudden increase in oxygen supply leads to overproduction of reactive oxygen species and down-regulation of antioxidants. This condition induces the enhancement of cytokines and inflammatory mediators (interleukin-6, interleukin-8, TNF-α) expression and leads to the inflammation, infection, formation of "free radical disease of the newborn" with prognostic adverse outcomes [9, 10].

An assessment of the cut-offs of prooxidant markers levels in the diagnosis of unfavorable outcomes in the early neonatal period was conducted. It was found that the level of 8-isoprostane more than 595.4 mg/ml was associated with mortality in 72.73% of cases (95% CI 39.0–94.0) with a specificity of 81.82% (95% CI 69.1- 90.9). ROS level increasing more than 0.483 IU with a sensitivity of 63.64% (95% CI 30.8–89.1) and a specificity of 78.18% (95% CI 65.0–88.2) leads to unfavorable outcomes in the early neonatal period.

Level of TBP higher 5.14 μmol/l also leads to mortality with high specificity of 90.91% (95% CI 58.7–99.8) and sensitivity of 72.73% (95% CI 59.0–83.9). It was found that the LHP levels above 5.91 U/ml was associated with the death in the early neonatal period (sensitivity 81.82% (95% CI 48.2–97.7), specificity 87.27% (95 % CI 75.5–94.7) (table 2, fig.3).

Indicators of the antioxidant system (SOD and catalase) differed significantly in newborns of the study groups (premature infants with adverse and favorable outcomes, control group) in the early neonatal period (tabl.3), which is consistent with the literature data [11, 12].

Decreased activity of the antioxidant system have increased the risk of early neonatal mortality: catalase (OR 91.67; 95% CI: 4.85-1733.61; p=0.003), SOD (OR 14.00; 95% CI: 2.89-67.72; p=0.001). The indicators of antioxidant system did not differ significantly in preterm infants with a favorable outcomes and newborns of control group, (p>0.05) (table 3).

Table 2 - Relationship between prooxidant markers and the risk of unfavorable outcomes in the early neonatal period

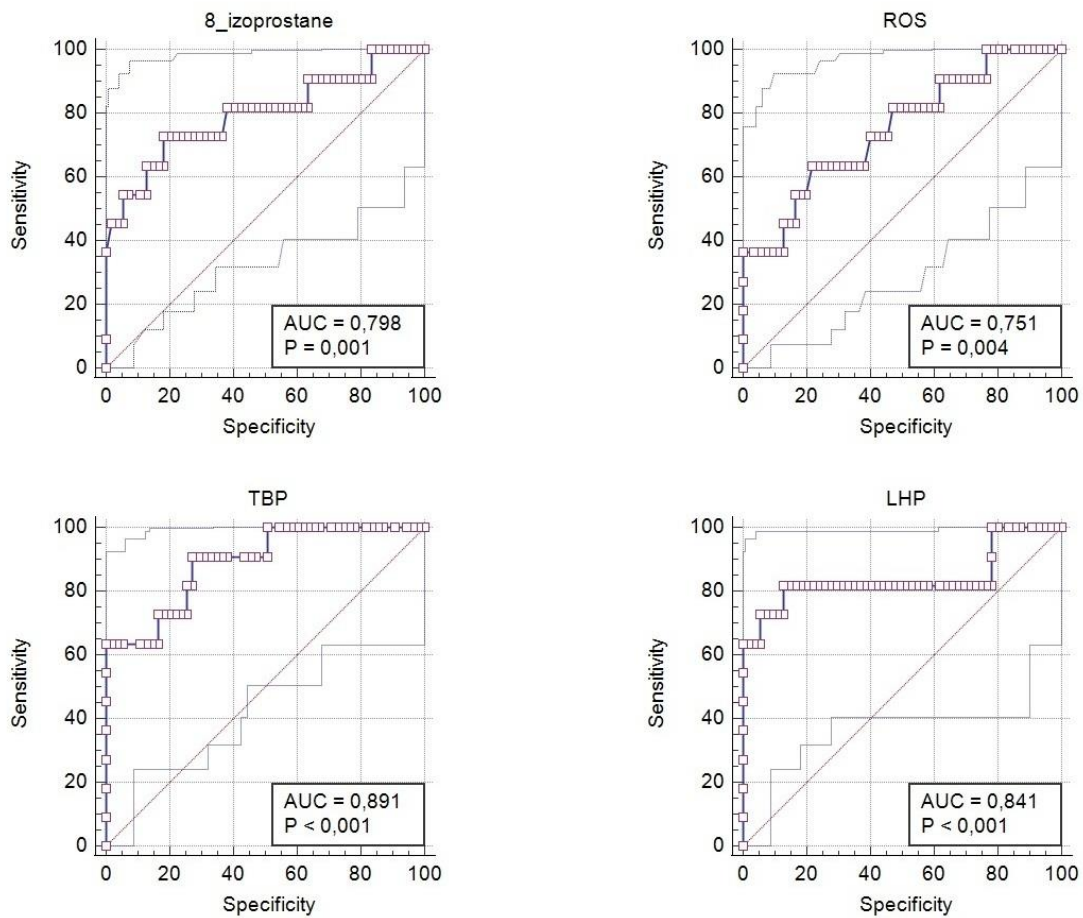| Parameters	8-isoprostane	ROS	TBP	LHP
AUC - ROC Curve	0.798	0.751	0.891	0.841
95 % CI AUC	0.681–0.887	0.629–0.849	0.790–0.954	0.731–0.920
p	<0.001	0.004	<0.001	<0.001
Youden index	0.546	0.418	0.634	0.691
Criterion	>595.4	>0.483	>5.14	>5.91
Sensitivity	72.73	63.64	90.91	81.82
Specificity	81.82	78.18	72.73	87.27

Fig 3. ROC curves of the impact of prooxidant markers on the mortality in the early neonatal period

Analysis of antioxidant markers ROC curves with statistical significance (p<0.001) have allowed to distinguish SOD and catalase levels, which leads to unfavorable outcomes in preterm infants.

Table 3 - Indicators of the antioxidant system in the umbilical cord blood in newborns of the study groups (Me (Lq; Uq))

Groups	Superoxide dismutase, mmol/hour*l	Catalase, IU/ml
I group (unfavorable outcomes) (n=12)	1.38 (1.30; 1.89)	0.38 (0.36; 0.40)
II group (favorable outcomes) (n=34)	2.46 (2.12; 2.95)	0.47 (0.42; 0.50)
Control group (III group), (n=20)	2.62 (2.34; 2.88)	0.47 (0.45; 0.49)
Kruskal–Wallis test	H=18.10; p<0.001*	H=17.25; p<0.001*
p	**p_{1-2}<0.001*** p_{1-3}<0.001* p_{2-3}>0.05	**p_{1-2}<0.001*** p_{1-3}<0.001* p_{2-3}>0.05
Note. * - statistically significant result		

Thus, mortality occurs in 81.82% of cases (95% CI 48.2-97.7) if the level of SOD ≤1.44 mmol/hour*l; specificity - 98.18% (95% CI 90.3–100.0). At the same time, the level of catalase ≤0.424 IU/ml is absolutely associated with mortality in preterm infants (sensitivity 100% (95% CI 71.5-100.00), specificity 74.55% (95% CI 61.0–85.3) (table 4, fig. 4).

Table 4 – Relationship between antioxidant markers and the risk of unfavorable outcomes in the early neonatal period

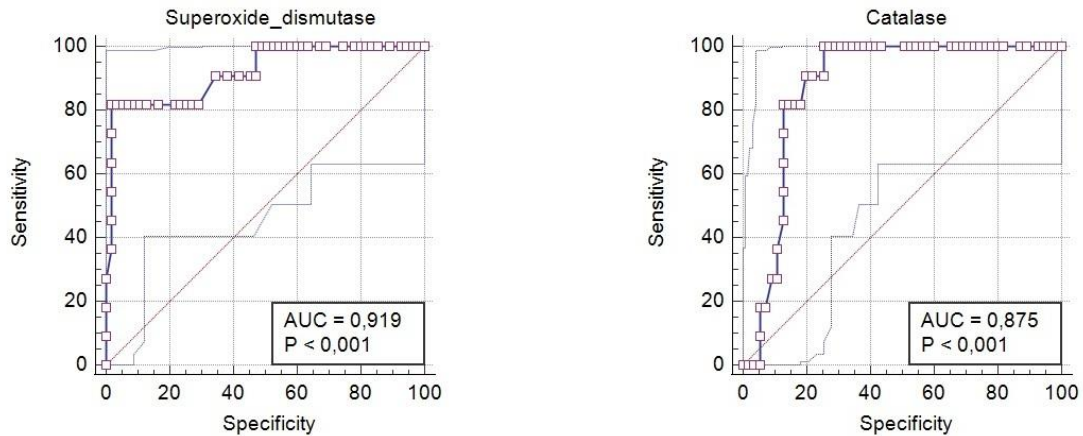| Parameters	Superoxide dismutase	Catalase
AUC - ROC Curve	0.919	0.875
95 % CI AUC	0.825–0.972	0.771–0.944
p	<0.001	<0.001
Youden index	0.800	0.746
Criterion	≤1.44	≤0.424
Sensitivity	81.82	100.00
Specificity	98.18	74.55

Fig 4. ROC curves of the impact of antioxidant markers on the mortality in the early neonatal period

Conclusion. There is an activation of oxidative stress with high prooxidant levels and reduced activity of the protective antioxidant system in preterm infants with the gestational age less than 32 weeks.

The activation of oxidative stress may play a significant role in the pathogenesis of severe diseases in preterm infants and may be a predictor of adverse effects in the early neonatal period.

Further Research Prospects

Further researches are recommended to study the role of oxydative stress markers on long-term outcomes of preterm infants and the effectiveness of oxydative stress correction.

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