vă. Aceasta boală poate afecta permanent căile bronşice si poate duce la boli pulmonare obstructive cronice, o stare medicală gravă în care plămînii sunt deterioraţi iremediabil determinând micşorarea capacităţii respiratorii progresiv şi iremediabil. Tratamentul BC la gravide include adesea antibiotice, care nu pot vindeca boala, dar previn apariţia unei infecţii secundare.

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THE STATUS OF THE PRO-OXIDANT SYSTEM AND ANTIOXIDANT DEFENSE SYSTEM IN CRITICALLY ILL NEWBORNS: A PRELIMINARY STUDY

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SUMMARY

Key words: newborn, pro-oxidant system, antioxidant defense system, oxidative modification of proteins, malonodialdehyd, ceruloplasmin, catalase, gamma-glutamyl transpeptidase.

Background & objectives: *the objective of this research is to study the status of the pro-oxidant and antioxidant defense systems and relations between different components of these systems in critically ill newborns.*

Methods: *the basic group of observation included 25 ill term neonates who had clinical symptoms of disorders on their first week of life and received treatment in the Neonatal Intensive Care Unit. The control group included 37 apparently healthy term neonates. The study included detection of pro-oxidant system (oxidative modification of proteins (OMP), malonodialdehyd (MDA)) and antioxidant defense system (ceruloplasmin (CP), catalase (CT), gamma-glutamyltranspeptidase (GGTP)) at 48-72 hours of life.*

Results: *the obtained results showed that newborns from the basic group as compared to the children from control group had significantly increased OMP and MDA levels and CP activity and significantly increased CT and GGTP activities. The formation of qualitatively new relations between components of pro-oxidant and antioxidant systems and individual components in the middle of the antioxidant system in ill newborn was found in this study.*

Interpretation & conclusions: *we concluded that term newborns who had clinical symptoms of disorders on their first week of life demonstrated excessive activation of pro-oxidant processes, inadequate antioxidant protection and formation of new relations between components of these systems and individual components in the middle of the antioxidant system. The immature all systems and organs of these babies along with both intracellular oxygen free radical toxicity and extracellularly generated cytotoxic products of activated inflammatory cells need special medical protection as a part of postnatal therapeutic care.*

РЕЗЮМЕ

STATUTUL SISTEMELOR PRO-OXIDANT ŞI ANTIOXIDANT DE PROTECŢIE LA NOU-NĂSCUŢII CRITIC BOLNAVI: UN STUDIU PRELIMINAR

Cuvinte-cheie: nou-născut, sistem pro-oxidant, sistem antioxidant de protecţie, modificarea oxidativă a proteinelor, malondialdehida, ceruloplasmina, catalază, gama-glutamil transpeptidaza.

Context şi Obiective: *obiectivul acestei cercetări este de a studia statutul sistemelor pro-oxidant şi antioxidant de protecţie şi relaţiile dintre diferitele componente ale acestor sisteme la nou-născuţii critic bolnavi.*

Metode: *grupul de studiu a inclus 25 copii bolnavi născuţi la termen, care au avut semne clinice patologice în prima lor săptămână de viaţă şi care au primit tratament în secţia de terapie intensivă pentru nou-născuţi. Grupul de control a inclus 37 copii născuţi la termen şi aparent sănătoşi. Studiul a inclus detecţia sistemului pro-oxidant (modificarea oxidativă a proteinelor (MOP), malondialdehida (MDA)) şi sistemului antioxidant de protecţie (ceruloplasmina (CP), cataleza (CT), gama-glutamil transpeptidaza (GGT)) la 48-72 ore de viaţă.*

Rezultate: rezultatele obţinute au arătat că la nou-născuţii din grupul de studiu, comparativ cu copiii din grupul de control, a crescut în mod semnificativ nivelul MOP şi MDA şi activitatea CP, precum şi semnificativ a crescut CT şi activitatea GGTP. Am stabilit formarea relaţiilor calitativ noi între componentele sistemelor pro-oxidant şi antioxidant şi componentele individuale din interiorul sistemului antioxidant la nou-născuţii bolnavi.

Interpretare şi concluzii: *am concluzionat că copiii născuţi la termen, care au avut semne clinice patologice în prima lor săptămână de viaţă au demonstrat o activare excesivă a proceselor pro-oxidante, o protecţie antioxidantă inadecvată şi formarea relaţiilor noi între componentele acestor sisteme şi componente individuale în interiorul sistemului antioxidant. Imaturitatea sistemelor şi organelor la aceşti copii, toxicitatea oxigenului intracelular al radicalilor liberi şi produsele citotoxice generate extracelular de celulele inflamatorii activate necesită o protecţie medicală specială ca parte a îngrijirii terapeutice postnatale.*

Introduction. The rapid passage from the intrauterine to the extrauterine environment induces a drastic exposure of the neonates to environment oxygen, which implies a sudden systemic adaptation to the post-natal condition[1,2]. When they are born, once the first breath begins, neonates are exposed to an oxygen-enriched environment, which increases aerobic metabolic pathways. The first breaths of the neonate imply also an increase in reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation[3]. Under physiological circumstances, free radicals are kept under control by an adequate antioxidant defense system (ADS) whose activation depends upon the entity of the oxidative injury itself[4]. The ADS involves endogenous and exogenous, enzymatic (superoxide dismutase, catalase, glutathione peroxidase) and nonenzymatic (thiol antioxidants and melatonin), vitamins (vitamin A, E, C) and non-vitamin components (carotenoids and polyphenols) that work synergistically to neutralize free radicals[5]. When these defense mechanisms are inadequate, either due to increased ROS and RNS production or diminished antioxidant levels, oxidative stress (OS) occurs which leads to the damage of biological molecules, such as lipids, proteins, carbohydrates, and DNA, inflicting tissue injury and dysfunction[1-5].

OS is implicated in several neonatal diseases and, in 1988, Saugstad proposed the concept of 'oxygen radical diseases of neonatology', which distributes diffrent diseases although demonstrating diffrent symptoms belonging to the same entity and impairing neonatal vitality and growth, as well as affecting the physiological development of the individual. Newborn diseases in the human species, such as broncho-pulmonary dysplasia/chronic lung disease, periventricular leukomalacia, neonatal encephalopathy, retinopathy of prematurity, neonatal renal dysfunctions and necrotizing enterocolitis are included in this entity of oxygen radical disease[6-9].

For a clinician, it is important to have deep knowledge about the factors affcting maternal/neonatal oxidative status and the cascades of events occurring when the neonate is subjected to OS. Given these premises, a deep knowledge concerning the factors affecting maternal/neonatal oxidative status and the techniques of neonatal care/resuscitation represent a necessary tool to optimize neonatal survival rate and vitality[1].

The **objective** of this research is to study relations between different components of pro-oxidant system and antioxidant defense system in critically ill newborns who have received treatment in Neonatal Intensive Care Unit at 48-72 hours of life.

Material & Methods. This study was conducted on the base of the Neonatology Unit of the Clinical Maternity Hospital №2 of Chernivtsi and on the Department of Pediatrics, Neonatology and Perinatal Medicine of Bukovinian State Medical University (Ukraine) during December 2013 – March 2014.

Informed written consent was obtained from parents prior to enrollment of their babies in the study. All studies were conducted in compliance with the basic provisions of the GCP (1996), Council of Europe Convention on Human Rights and Biomedicine (1997), Declaration of Helsinki of the World Medical Association about Ethical Principles for Medical Research Involving Human Subjects (1964 - 2008).

62 term neonates were selected in this study. The

basic group of observation included 25 ill term neonates who had clinical symptoms of disorders on their first week of life and received treatment in Neonatal Intensive Care Unit (I group). The control group included 37 apparently healthy term neonates (II group). All the infants were in physiological term of gestation, the groups under observation did not differ reliably in the body weight and length as well as gender signs. The exclusion criteria of the study were gestational term less 37 week, birth weight less 2500g, evidence-based early neonatal sepsis and major congenital anomalies.

A predesigned and pretested proforma was used to collect the data such as gestational age, birth weight and relevant perinatal history. The ill neonates were scored on the basis of Score for Neonatal Acute Physiology Perinatal Extension (SNAP-PE) and Neonatal Therapeutic Intervention Scoring System (NTISS)[10]. Patients were examined at 48-72 hours of life.

The examination of children chromaticity was used that enabled to make examinations on small blood volume. Blood sampling was performed from peripheral vein in the amount of 1.0 ml, 0.1 ml (500 IU) of heparin was added which diluted in 0.4 ml of 0.9% NaCl (ratio 1:4). Heparinized blood was centrifuged at 3000 rpm/min during 3 min and separated of the plasma. The red blood cells were washed three times in isotonic NaCl. Then blood plasma and erythrocytes were frozen in plastic tubes at the temperature of 12°C.

Oxidative modification of proteins (OMP) was measured as described by E. Dubinina et al.[11] in the modification by I. Meschishen[12]. The blood plasma (0.2 ml) was used for examination. 0.85% NaCl (0.8 ml), 1M (2,4-dinitrophenyl)hydrazine (2,4-DPhH) (1.0 ml) dissolved in 2M hydrochloric acid, and 5% trichloroacetic acid (THO) (1.0 ml) were added in centrifuge tube. 1.0 ml of 2M hydrochloric acid instead of 2,4-DPhH was added in the control sample. Then the material to be examined was incubated during 1 hour at the temperature of 37°C and centrifuged at 3000 rpm/min during 10 min. The resulting precipitate was washed three times with 5.0 ml of 5% THO, each time carefully re-suspended precipitate with a glass rod. 5.0 ml of 8M urea solution was added to the resulting precipitate. Then it was incubated for 5 min in a boiling water bath until being dissolved. The groups of aldehydes and ketones of the aminoacid residues were formed as a result of the oxidation reaction of proteins which interacted with 2,4-DPhH and had different absorption spectrum. The optical density of the formed dinitrophenylhydrazones was registered on the photoelectrocolorimeter at wavelength 370 nm. The intensity of OMP were expressed as units of optical density/milliliter of erythrocytes (u.o.d./ml).

The content of lipid peroxidation was measured as Malonodialdehyde (MDA) level in erythrocytes[13]. The test was based on the reaction between MDA and thiobarbituric acid (TA) which leads of the formation trimethine colored complex at room temperature and acid value of pH. 1.3 ml of distilled water, 0.2 ml $FeSO_4$ x $7H_2O$ (139 mg in 100 ml H_2O) and 0.2 ml of red blood cells which had been previously washed three times in isotonic NaCl were added in the centrifuge tube. It was stirred thoroughly with a glass rod and 1 ml of 0.8% TA was intoduced 10 min. later. The sample was stirred with a glass rod, 0.3 ml of 60% trichloroacetic acid was added 5 min later and boiled in a water bath for 10 min. Samples were cooled, centrifuged at 3000 rpm/min and the optical density at 532 nm was determined. Calculations of the content of the MDA were performed taking into account the molar ratio extent 1.56×10^5 M^{-1} cm^{-1} and expressed in micromol/milliliter of erythrocytes (µmol/ml).

Plasma Ceruloplasmin (EC1.16.3.1, CP) level was measured as described by V.Kolb, V.Kamyshnikov[14]. 4.0 ml of acetate buffer (pH 5.5) were added to 0.05 ml of blood plasma and 0.5 ml solution of p-phenylenediamine (PPD). The tube was shaken and put to thermostat for 1 hour. After 1 hour of incubation 1.0 ml of NaF was added. At one time 4.0 ml of acetate buffer (pH 5.5) were added 0.05 ml of serum, 1.0 ml NaF and 0.5 ml of PPD in the control sample. After that the tubes were incubated for 30 minutes in a fridge. CP concentrations were expressed as milligram/liter (mg/l).

Plasma activity of Catalase (EC 1.11.1.6, CT) was determined by the method of M. Korolyuk et al.[15] 2 ml of 0.03% hydrogen peroxide were added to 0.05 ml of blood plasma. The reaction was carried out at the temperature of 37°C in thermostat for hemocoagulation with transparent walls. After 10 minutes 1.0 ml of 4% ammonium molybdate was added. At this time a control sample was put which was contributed with 0.05 ml of distilled water instead of blood plasma. Then $E_{410\text{-blank}}$ and $E_{410\text{exp}}$. against a control of distilled water on the spectrophotometer in the 1 cm cuvette in length 1 cm were measured.

CT activity was calculated according to the formula: CT= ($E_{410\text{-blank}}$ - $E_{410\text{exp}}$.)/Epsilon,

where $E_{410\text{-blank}}$ - indicator extents blank sample, $E_{410\text{exp}}$ - indicator extent test sample, Epsilon - $2,22 \cdot 10^4$-milimol$^{-1} \cdot$cm^{-1} (molar ratio of optical density of hydrogen peroxide).

CT activity was expressed in micromol/min·liter (µmol/min·l).

Plasma activity of gamma-GlutamylTranspeptidase (EC 2.3.2.2, GGTP) was measured by photometric method. The test was based on the transfer reaction of the L-glutamyn residue from chromogenic substrate (gamma-L-glutamyl p-nitroanilide) to the acceptor dipeptides (glicil-glicin) which was catalyzed by GGTP. Blood plasma (0,1 ml) was added to the

substrate-buffer component, then it was incubated at a temperature of 37°C during 15 min and acetic acid (6,0 ml) was added. After stopping the reaction optical density against a blank sample on the photometer was measured. GGTP activity was expressed in unit/liter (UN/l).

The analyses were conducted on the basis of the laboratory of the Department of Bioorganic and Biological Chemistry and Clinical Biochemistry, Bukovinian State Medical University.

Statistical processing of mathematical data was performed by means of the program Statistica 7.0 (StatSoftInc., USA) with the detection of median (Me) and interquartile range [Lq – lower quartile; Uq – upper quartile] for the selections with abnormal distribution. Non-parametric Mann-Whitney (MW) U-criterion was used for the comparison of two selections. Correlation between paraclinical parameters was assessed by calculating the Spearman's correlation coefficient. The difference of the parameters was considered to be statistically significant with $p<0,05$.

Results. No statistical differences exist in the body weight or in any other clinical characteristics of the two respective groups. Clinical characteristics of the studied population are reported in Table 1.

The results of this study showed significant differences in the frequency of pathologic factors during antenatal and intranatal periods in newborns from basic group as compared to the control group of observation. However, pathological intranatal period has a more significant impact on the formation of newborn's disorders in postnatal period. Table 2 shows the statistics about the features of gestational and intranatal periods in the groups of observation.

Table 1.
Clinical characteristics of the groups being under observation

	Basic group	Control group
Number of patients, n (%)	25 (100,0)	37 (100,0)
Term of gestation, week	38,28±1,15	38,78±1,34
Sex (boys), n (%)	18 (72,0)	25 (67,5)
Sex (girls), n (%)	7 (28,0)	12 (32,5)
Body weight, g	3276,8±501,76	3374,87±442,87
Body length, cm	52,68±2,23	53,24±2.54

Data expressed as Mean±SD or percent.

Table 2.
Clinical characteristics of antenatal and intranatal periods in the groups of observation

	Basic group	Control group
Number of patients, n (%)	25 (100,0)	37 (100,0)
Number of pregnancy: first, n (%)	12 (48,0)	17 (45,9)
Number of pregnancy: second, n (%)	6 (24,0)	15 (40,5)
Number of pregnancy: third and more, n (%)	7 (28.0)	5 (13.6)
Number of delivary: first, n (%)	17 (68,0)	18 (48,6)
Number of delivary: second, n (%)	5 (20,0)	16 (43,2)
Number of delivary: third and more, n (%)	3 (12,0)	3 (8,2)
Age of mother > 30 year, n (%)	10 (40,0)*	5 (13,6)
Disoders of cardiovascular system, n (%)	8 (32,0)	11 (29,7)
Disoders of gastrointestinal tract, n (%)	5 (20,0)	5 (13,6)
Disoders of urinary system, n (%)	7 (28,0)	10 (27,0)
Disoders of endocrine system, n (%)	3 (12,0)	2 (5,4)
Infectious diseases (TORCH), n (%)	12 (48,0)	18 (48,6)
Threatened spontaneous abortion, n (%)	20 (88,0)*	14 (37,8)
Threatened premature birth, n (%)	7 (28,0)*	3 (8,2)
Anemia in pregnant women, n (%)	14 (56,0)	22 (59,4)
Preeclampsia, n (%)	5 (20,0)	4 (10,8)
Fibromyoma of uterus, n (%)	3 (12,0)*	-
Poor obstetric history: spontaneous abortion, n (%)	12 (48,0)*	2 (5,4)
Poor obstetric history: induced abortion, n (%)	3 (12,0)	1 (2,7)
Poor obstetric history: premature birth, n (%)	1 (4,0)	1 (2,7)
Poor obstetric history: ectopic pregnancy, n (%)	2 (8,0)	1 (2,7)
Type of delivery:Vaginal, n (%)	16 (64,0)*	34 (91,9)
Type of delivery: Elective caesarean section, n (%)	1 (4,0)	2 (5,4)
Type of delivery: Emergency caesarean section, n (%)	8 (32,0)*	1 (2,7)
Amniotic fluid: Clear, n (%)	16 (64,0)*	37 (100,0)
Amniotic fluid: Meconium, n (%)	9 (36,0)*	-

The weaknesses of labor, n (%)	5 (20,0)*	1 (2,7)
Discoordinating labors activity, n (%)	1 (4,0)*	-
Fetal distress during labor, n (%)	5 (20,0)*	-
Vacuum extraction of the fetus, n (%)	1 (4,0)*	-
Umbilical cord entanglement, n (%)	2 (8,0)	2 (5,4)
Kainer pregnancy optimality score, points	38,23±3,02*	40,5±2,27
Kainer delivery optimality score, points	17,54±2,30*	20,25±1,37

Data expressed as Mean±SD or percent. * p<0,05 compared with the control.

The results of our study showed that all infants from the basic group of observation had more than one associated contributing condition which could lead to the development of multipleorgan dysfunction syndrome in early neonatal period. The frequency of each associated contributing condition is presented in Table 3.

Table 3.

Clinical characteristics of postnatal period of the basic group

Number of patients, n(%)	25 (100,0)
The basic pathology:	
Sever asphyxia (score Apgar on fifth minute of life less than 3 points), n (%)	4 (16,0)
Moderate asphyxia (score Apgar on fifth minute of life 4-6 points), n (%)	1 (4,0)
Hypoxic-ischemic encephalopathy, n (%)	10 (40,0)
Meconium aspiration syndrome, n (%)	6 (24,0)
Respiratory distress syndrome, n (%)	3 (12,0)
Hemolytic disease of the newborn, n (%)	2 (8,0)
The concomitant pathology:	
Hypoxic-ischemic encephalopathy: depression syndrome, n (%)	16 (64,0)
Hypoxic-ischemic encephalopathy: excitation syndrome, n (%)	4 (16,0)
Hypoxic-ischemic encephalopathy: convulsive syndrome, n (%)	6 (24,0)
Hypoxic-ischemic encephalopathy: syndrome of vegetative-visceral disorders, n (%)	12 (48,0)
Respiratory failure (score Dowens>7 points), n (%)	25 (100,0)
Cardiovascular failure, n (%)	15 (60,0)
Renal failure, n (%)	2 (8,0)
Liver failure, n (%)	2 (8,0)
Hemorrhagic syndrome, n (%)	9 (36,0)
Disorders of the gastrointestinal tract, n (%)	20 (80,0)
Neonatal jaundice, n (%)	7 (28,0)
Morpho-functional immaturity, n (%)	6 (24,0)
Score SNAP-PE, points	54,0±2,11
Score NTISS, points	24,0±0,55

Data expressed as Mean±SD or percent.

In present study the levels of OMP and MD were found to be significantly increased in patients from basic group compared to control group. In the ill infants OMP level was 1,03 u.o.d./ml [0,99; 1,05], in the healthy babies – 0,89 u.o.d./ml [0,81; 0,94], p<0,05. MDA level in the main group was 22,98 µmol/ml [19,99; 24,25], in the control group – 20,44 µmol/ml [19,04; 22,11], p<0,05.

The results of the examinations conducted have shown a considerable imbalance between different components of antioxidant system in children who was received treatment in NICU as compared with healthy infants. A significant decrease of CT and GGTP activities and significant increase of CP concentration has been found during examination. Thus, CT activity in the basic group constituted 11,85 µmol/min·l [8,85; 14,69], in the control group – 14,19 µmol/min·l [12,23; 16,98], p<0,05; GGTP activity – accordingly to the group of observations 121,56 UN/l [101,79; 177,84] and 161,38 UN/l [102,39; 169,46], p<0,05. CP concentration in the ill newborns was 274,75 mg/l [191,63; 285,25], p<0,05, in the healthy babies – 215,26 mg/l [122,5;228,38 mg/l], p<0,05.

Correlation between paraclinical parameters was assessed by calculating the Spearman's correlation coefficient. The results of our studies showed the formation of aqualitatively new relations between components of pro-oxidant and antioxidant systems and individual components in the middle of the antioxidant system at the disturbed conditions of the early neonatal period (Table 4).

Correlation between OMP, MD, CT, GGTP and CP in the groups of observation (Spearman rank R)

Group	OMP Basic	OMP Control	MD Basic	MD Control	CT Basic	CT Control	GGTP Basic	GGTP Control
OMP	1,0	1,0	-0,6546*	0,1461	0,8911*	-0,2816	0,8074*	0,2899
MD			1,0	1,0	-0,6071*	-0,3378	0,5766*	0,4554*
CT					1,0	1,0	0,7208*	-0,4844*
GGTP							1,0	1,0
CP	0,3853	0,5971	0,2162	0,2667	0,4688*	-0,5761	0,5636*	0,5855*

* $p<0,05$. OMP – Oxidative modification of proteins, MD – Malonodialdehyde, CT – Catalase, GGTP – gamma-GlutamylTranspeptidase, CP – Ceruloplasmin.

Discussion. Free radicals are highly reactive molecules containing one or more unpaired electrons. They donate or gain electrons from other molecules in an attempt to pair their electrons and generate a more stable species[16]. Free radicals are normally produced in living organisms and are divided into two broad categories: ROS and RNS. When produced in physiological concentrations, ROS behave as important mediators of almost all cell functions. On the other hand, when produced in excess, they induce OS, responsible for cell and tissue injury[3,17].

Free radical reactions may cause alteration of macromolecules, such as polyunsaturated fatty acids and proteins. Free radicals produced during lipid peroxidation have some very local effects, because of their short life, but the breakdown products of lipid peroxides may serve as 'oxidative stress second messengers', due to their prolonged half-life and their ability to diffuse from their site of formation, compared to free radicals. Those breakdown products, mostly aldehydes, such as MDA, have received a lot of attention because they are most of the time reactive compounds. They have been considered for a long time as toxic end-products of lipid peroxidation[18].

MDA production by nonenzymatic processes remains poorly understood despite its potential therapeutic value, because this MDA is believed to originate under stress conditions and has high capability of reaction with multiple biomolecules such as proteins or DNA that leads to the formation of adducts, and excessive MDA production have been associated with different pathological states. On the other hand, MDA does not reflect the balance between promoters of peroxidation and antioxidants; during its measurement, also substances other than MDA may be detected, such as thromboxanes, by-products that are not necessarily relevant to peroxidation, thus making MDA method not so much reliable[18]. Previously, some authors have measured MDA, one of the by-products of lipid oxidation and commonly used as an indicator of OS, in the venous blood of healthy and ill mother with different pathology (pre-eclampsia, diabetes mellitus, metabolic syndrome), umbilical arterial and venous blood of the newborn[19]. In our study the levels of MDA were found to be significantly increased in critically ill patients as compared to healthy babies at 48-72 hour after birth. The high lipoperoxidation of membrane lipids in these newborn may lead to an alteration in the functional properties of the lipid bilayer of cell membranes, with consequent deep changes in its permeability and develop of the pathological cascade of OS.

As to proteins, their oxidation may trigger alterations in the stereo-isomeric structure of the membrane pores or may damage the DNA inducing fragmentation, base modifications and strand breaks which, in turn, result in mutations and oncogenesis. Prof. Giuseppe Buonocore found that total hydroperoxides and advanced oxidative protein products increased from birth to seven days of life in both preterm and term babies, indicating that an OS occurs early in life and newborns are particularly susceptible to oxidative damage[20]. Also Barbara Marzocchi and colleges had showed that oxidation of albumin can be expected to decrease plasma antioxidant defenses and increase the likelihood of tissue damage due to OS in the newborns[21]. The results of our study confirmed that infants who developed and were born with hypoxia have an increase of the intensity of OMP.

According to the literature analyzed, the consequences of adduct formation on the protein level may be associated with numerous cytotoxic consequences including the disruption of cell signalling, altered gene regulation, inhibition of enzyme activity, mitochondrial dysfunction, impaired energy metabolism, altered tertiary structure and finally loss of cytoskeletal formation in critically ill children[18].

Antioxidants may act inhibiting free radical generation, preventing the oxidation of substrates or may behave as scavengers, neutralizing free radicals, transforming them into chemically stable products[3,22]. During physiological passage from the fetal to the neonatal transition and labour, hypoxia and OS may develop in a newborn, also given its constitutive deficiency in antioxidants[6]. Many authors indicate a critical balance between free radical generation and antioxidant defense in newborn[23-25]. It is true, that in comparison with adults, increased concentrations of some anti-

oxidants such as ascorbate and bilirubin are found in newborns, however, only for a short period after birth. It is important to notice, that other major antioxidants such as vitamin E, b-carotene, melatonin and sulph-hydryl groups, of plasma metal binding proteins such as CP and transferrine and of erythrocyte superoxide dismutase show reduced activity in newborns[6].

CP, a copper-binding protein, prevents copper from participating in reactions with radicals and plays a major role in iron metabolism as ferroxidase enzyme, which catalyzes oxidation of ferrous (Fe^{2+}) to ferric (Fe^{3+}) ions for binding to transferrin. Transferrin and CP levels have been found to be low in infants, resulting in higher circulating Fe^{2+}. Patcharee Boonsiri and colleges found that plasma CP levels on the 1st day was significantly lower than the level on the 3rd day in both premature and full-term infants. They also detected that the plasma CP levels in full-term group were higher than in the premature group on 1 and 3 days after birth. It is interesting that a correlation between plasma CP and gestational age was found, perhaps because hepatic function with respect to CP synthesis is more developed in adults than in infants. However, Moison et al. found that plasma CP in premature infants with or without respiratory distress syndrome was not significantly different. The other authors confirmed that plasma CP level in premature infants with asphyxia was lower than infants without asphyxia[26]. In our study we got opposite results: plasma CP levels in critically ill term infants was higher than in healthy newborns at 48-72 hour after birth. The results found may be indicative of a sufficient activation of this part of ADS in term infants during intensive care.

It should be noted that in present research a low activity of two other components of ADS (CT and GGTP) in ill term newborns was found. According to the literature, CT a 240-kD tetramericheme protein, is one of the major intracellular antioxidant enzyme responsible for detoxifying the hydrogen peroxide produced under physiological conditions to oxygen and water. The enzyme consists of 4 protein subunits, each of which contains ferric ions of the heme group that undergo oxidation following interaction with the first molecule of H_2O_2 to produce Fe^{+4} in a structure called compound 1. A second molecule of H_2O_2 serves as an electron donor and results in the destruction of the two H_2O_2 molecules involved to produce an oxygen molecule[27].

GGTP belongs to the N-terminal nucleophile hydrolase superfamily, enzymes that cleave the γ-glutamyl amide bond of glutathione to give cysteinylglycine. The released γ-glutamyl group can be transferred to water (hydrolysis) or to amino acids or short peptides (transpeptidation). GGTP plays a key role in the gamma-glutamyl cycle by regulating the cellular levels of the antioxidant molecule gluta-thione, hence it is a critical enzyme in maintaining cellular redox homeostasis[28,29]. GGTP is involved in many physiological disorders related to OS. Tiina M. Asikainen and Carl W. White have showed that low GGTP is one of many pathological factors which lead to the development of bronchopulmonary dysplasia[30].

Conclusions. Based on our measurements, we conclude that term newborns with clinical symptoms of disorders on their first week of life and receive treatment in Neonatal Intensive Care Unit have excessive activation of pro-oxidant processes, inadequate antioxidant protection and formation of new relation between components of pro-oxidant and antioxidant systems and individual components in the middle of the antioxidant system against oxidative stress at birth. The immature all systems and organs of these babies along with both intracellular oxygen free radical toxicity and extracellularly generated cytotoxic products of activated inflammatory cells need special medical protection as a part of postnatal therapeutic care.

The perspectives for further studies. Further studies with larger number of cases using tests of pro-oxidant and antioxidant components necessary to determine the role of the pathological processes of oxidative stress in the damage to various organ systems in infants and improvement of appropriate antioxidant therapy.

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