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Chapter 5

Particularities of Oxidative Stress in Newborns

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Abstract

The oxidative stress at newborns is augmented by different conditions like preterm birth, asphyxia, respiratory distress, and intraventricular hemorrhage. Preterm neonates associate a more pronounced oxidative stress than healthy term newborns. Several neonatal conditions like respiratory distress (RDS), asphyxia, intraventricular hemorrhage, bronchopulmonary dysplasia, retinopathy, and necrotizing enterocolitis will increase the oxidative stress. The harmful effects of free radicals are linked to their capacity to react with polyunsaturated fatty acids of cell membranes, proteins, and nucleic acids. Free radicals will produce protein alteration with function loss and lipid peroxidation.

Keywords: oxidative stress, new born, prematurity

1. Introduction

Oxidative stress represents all injuries caused by reactive oxygen species (ROS) on biomolecules, inducing the destruction of membranes, enzymes, receptors, as well as alteration of cell function. The consequence of oxidative stress is a disruption of the physiological balance between pro-oxidants and antioxidants [1, 2].

Newborn possesses defense mechanisms such us molecules protection, limitation of ROS production, and mechanisms for repair and adaptation to endogenous and exogenous ROS overproduction.

Reactive oxygen species are involved in physiological processes such as physical exercise, hyperbarism, regulation of vascular tone, stimulation of cell growth and proliferation, stimulation of erythropoietin secretion, the learning and memory process, as well as in pathological processes: inflammation, aging, carcinogenesis [1–3].

In neonatal pathology, there are multiple circumstances that are associated with oxidative stress. An excess of reactive oxygen species in the context of immature, deficient antioxidant defense mechanisms may cause multisystemic diseases [2, 3].

1.1. Effects of reactive oxygen species on biomolecules

Reactive oxygen species will act on molecules, inducing their deterioration as follows:

- DNA lesions—action on the bases in the DNA structure—thymine, cytosine, adenine, guanine, deoxyribose, followed by cell damage and mutations
- Alteration of NADPH—inhibition of the nucleotide coenzyme activity
- Lipids and proteins—action on the covalent bond in their structure
- Glycoproteins—action on hyaluronic acid in their structure
- Lipid peroxidation—structural and functional changes in cell membranes

1.1.1. Effects of oxidative stress on proteins and amino acids

Reactive oxygen species (ROS) act on the side chain of amino acids. Through oxidation of amino acids in the structure of proteins, the following changes are induced: protein fragmentation, aggregation, and proteolytic degradation. Aldehydes resulting from lipid peroxidation and glycosylation will also act on proteins. The consequence will be the functional alteration of proteins, with the loss of their contractile, enzymatic, and transport function.

1.1.2. Effects of oxidative stress on lipids

The most extensively studied action on lipids is their cellular and extracellular peroxidation. Lipids and lipoproteins are involved, particularly those mainly composed of polyunsaturated fatty acids (PUFA) such as linoleic and arachidonic acids, abundantly in cholesterol esters, lecithin, and erythrocytic phospholipids. Lipoproteins can be oxidized by two pathways:

- Specific enzymatic oxidation—with the formation of prostaglandins, thromboxane, prostacyclin, leukotrienes, and isoprostanes
- Nonspecific enzymatic oxidation—a peroxidation process with the formation of products with a damaging effect.

As a result of lipid peroxidation, a disorganization of the cell structure occurs, lipids being part of the membrane structure. A more rigid membrane will develop, with implications on essential membrane proteins, such as Na^+-K^+-dependent ATPase. Thus, a change in the ion pump rate will occur.

Lipid peroxides alter the properties of cellular, mitochondrial, and lysosomal membranes, with the disappearance of osmotic, chemical, and electrical gradients. Thus, cell excitability

and metabolic processes are disturbed, and morphological lesions occur. In the nerve fiber, the myelin sheath is attacked. At pulmonary level, there is an alteration of endothelial membrane permeability, with the movement of lipids from the vascular space to the extravascular (interstitial) and intracellular space, and the development of pulmonary edema.

1.1.3. Effects of oxidative stress on carbohydrates

Glucose and monosaccharide oxidation leads to the formation of activated molecules that may interact with other molecules, generating new compounds. By polysaccharide oxidation, structural changes in deoxyribonucleic acid (DNA) may occur.

1.1.4. Effects of oxidative stress on nucleic acids

The number of oxidative attacks on DNA in humans is 10,000/cell. Thus, chromosome fragmentation occurs. Double-chain DNA is much more vulnerable than single-chain DNA. Altered fragments are eliminated as purine and pyrimidine bases by urinary excretion and they can be dosed. Oxidative DNA lesions accumulate with age. Aging and carcinogenesis are explained by incomplete DNA repair after oxidative attacks.

The superoxide anion resulting from the xanthine/xanthine oxidase system induces DNA breaks. The hydroxyl radical through its instantaneous reaction with nucleic acids also causes DNA breaks. These breaks should be normally repaired by cell enzymes, but due to their low fidelity, the repair process is inadequate through the inclusion of inappropriate bases in the DNA structure.

Oxygenated water also has effects on DNA. Through its reaction with DNA-bound metal ions, oxygenated water will induce the formation of hydroxyl radical, which will immediately fragment the DNA molecule.

2. Oxidative stress in newborns

The literature currently speaks about free radical disease in newborns, a term introduced by Sullivan [2], which includes the following pathogenic conditions: bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP), intra-periventricular hemorrhage (IPVH), necrotizing enterocolitis (NEC), and renal failure [1]. Premature newborns with a gestational age of less than 30 weeks and a weight of less than 1500 g, respectively, have a major risk to develop these disorders. At cerebral level, there is a predisposition to oxidative stress, due to the high amount of polyunsaturated fatty acids in the immature brain, particularly in neuronal membranes, but also due to the relatively high amount of protein-unbound iron [41]. Immaturity associated with premature birth and also oxygen therapy used for the treatment of respiratory distress significantly increase oxidative stress in premature newborns. The knowledge of oxygen toxicity mechanisms is important both for their prevention and to ensure a harmonious development of newborns in general and premature newborns in particular, given their low antioxidant defense capacity [1, 3].

2.1. Implication of oxidative stress in pulmonary disorders

Respiratory distress due to surfactant deficiency is one of the most frequent disorders in premature newborns. The higher the degree of prematurity, the higher is the incidence and severity of this disease. Due to the immature lung structure, surfactant deficiency, pulmonary fluid retention, and poorly developed bone and muscle structures, there is an increased susceptibility of the premature to pulmonary lesions. The mechanism of these pulmonary lesions is based on alveolar instability and pulmonary atelectasis.

Mechanical ventilation, in addition to its role of ensuring an adequate oxygen supply to the body, also has undesired effects. Thus, it interferes with inflammatory cell metabolism and pulmonary mediators. Pulmonary vascularization is supplied from the heart but is also a reservoir of neutrophils—1/3 of the total number of neutrophils outside the bone marrow is found in the lungs [38].

Animal experiments have evidenced that in the context of mechanical ventilation, the number of neutrophils and the level of mediators, platelet activating factors, thromboxane B2 in pulmonary lavage fluid, and TNF-alpha in alveolar macrophages increase [11]. In premature animals with RDS, mechanical ventilation will cause an increase in pulmonary inflammation mediators, an increase in granulocytes, and cytokine activation [8]. Ventilatory support may affect the alveolar-capillary barrier and induces a release of inflammation mediators from the alveolar space into the circulation. The translocation of endotoxins from the aerial space to the circulatory space will generate a systemic inflammatory response [8, 9].

Mechanical ventilation along with oxygen supplementation will generate oxidative stress, with protein oxidation under the action of ROS. The carbonyl group of proteins will react with 2,4-dinitrophenylhydrazine, and 2,4-dinitrophenyl-hydrazone will form, which is measured by spectrophotometry [16]. Prematures who develop bronchopulmonary dysplasia (BPD) have significantly higher levels of protein carbonyls during the first week of life [1, 16].

Newborns requiring mechanical ventilation are more exposed to free radical production as a result of oxygen therapy exposure, inflammatory response, and ischemia reperfusion [10, 49].

Experimentally, it has been demonstrated on animals that the synthesis of enzymatic and consequently, antioxidant systems occurs at the end of gestation. Hemolysis after birth associated with a low level of iron-binding antioxidants can favor protein damage [5].

Protein carbonylation in the tracheobronchial fluid is a marker of oxidative stress at pulmonary level, while the exhalation of lipid peroxidation products (ethane, pentane) is a marker of lipid peroxidation in the entire body. As a result, a high level of exhaled peroxidation products is associated with extrapulmonary morbidity [9, 10].

Enzymes protecting against oxidant agents in the lungs, such as catalase, can be the target of oxidative attack inducing their inactivation. Liposoluble free radical scavengers such as tocopherol or beta-carotene have a protective effect against oxidative attack on lipids, but not on proteins. At the end of the first week of life, a depletion of these scavengers occurs as a result of the rapid increase in ROS production under the action of pulmonary inflammatory response. Thus, lipid peroxidation products are released through oxidation of polyunsaturated

fatty acids. During the first 3–4 days of life, pulmonary proteins are the first target of free radicals. In the context of RDS, edema develops as a result of increased membrane permeability. Due to its high protein content, its presence in the lung will make it the ideal target for the initiation of oxidative attack by ROS [30, 31]. The inactivation of α_1-proteases under the action of oxidative attack will disrupt the balance of the pulmonary protease-antiprotease system. In infants with RDS, ROS will interact with the surfactant as well as with other proteins and lipid structures, delaying the normalization of pulmonary function [2, 5, 38].

Patients with respiratory distress have high levels of hydrogen peroxide in the pulmonary condensate. Oxidized glutathione and the altered alpha-1 protein also show high levels in the pulmonary fluid. In addition, the antioxidant proteins catalase and ferritin are elevated, which could represent a compensatory response. Oxidative stress markers are also increased in patients with sepsis, in those infected with HIV [40].

With the increase in the survival rate of newborns with extreme prematurity, the incidence of chronic pulmonary disorders has also increased, not only as an undesired consequence of RDS, but also of mechanical ventilation used for its treatment. The most frequent chronic pulmonary disease of premature infants with RDS and a history of mechanical ventilation is bronchopulmonary dysplasia. This is a chronic lung disease developing in newborns treated with oxygen and mechanical ventilation for a primary pulmonary disease. It affects between 20 and 60% of prematures, but it may also occur in term infants with severe respiratory distress [8].

Its etiopathogeny is complex. There is the hypothesis that bronchopulmonary dysplasia might start as an acute inflammation, which subsequently turns into a chronic lung disease under the action of free radicals [2, 5].

Preliminary studies have shown that pulmonary lesions can be improved by administration of antioxidants such as superoxide dismutase (SOD). The function of SOD is to convert the extremely toxic superoxide radical to less toxic hydrogen peroxide and water. Superoxide dismutase is also present naturally, but not as synthetic surfactant. Genetic engineering has demonstrated that in alveolar cells, SOD survives for a longer time period. An experimental animal with an SOD gene disrupted by genetic engineering, exposed to hyperoxia, will survive for a shorter time and will have more pulmonary lesions than an animal with an intact gene. Superoxide dismutase plays a major role in the prevention of pulmonary lesions in the context of hyperoxia. If SOD activity is increased in the newborn lung, inflammatory changes and pulmonary lesions can be prevented [9, 20, 21].

The incidence of bronchopulmonary dysplasia has increased not only with the extensive use of positive pressure ventilation for the treatment of neonatal respiratory distress, but also due to the increase of the survival rate by modern intensive care techniques in newborns with extreme prematurity. The Neonatal Research Network reported a 68% incidence of BPD in prematures with a gestational age of 22–28 WA.

Today, it is known that barotrauma, particularly at high inspiratory pressures, is a key factor in the development of pulmonary lesions independently of any other lesions generated by oxygen therapy. Epithelial alterations in the airways occur, as well as with an increase in capillary permeability with extravasation of protein substances. In addition to the implication

of high inspiratory pressure in the genesis of pulmonary lesions, increased tidal volume also plays an important role. High but also significantly decreased tidal volume may generate an accumulation of neutrophils and a release of toxic agents such as proteases and free radicals, as well as proinflammatory cytokines. An analysis of pathological anatomy data has allowed to evidence a correlation between the incidence of interstitial emphysema during the first week of life and the incidence of interstitial fibrosis or proliferation in newborns with bronchopulmonary dysplasia surviving for more than 28 days [22, 24].

Another factor that plays a role in the development of BPD is inflammation. Proinflammatory cytokines were detected in the tracheal aspiration fluid during the first 1–2 weeks of life in newborns who subsequently developed BPD. Recently, it has been demonstrated that amnionitis and fetal infection are risk factors for BPD; consequently, inflammation, even prenatal, plays a role in its genesis [3].

Premature infants, due to their pulmonary immaturity, have a high risk for BPD, because they require additional oxygen supply for a longer time period in the treatment of the lung disease, their intracellular antioxidant defense capacity is affected, and they have an increased susceptibility to infections.

Studies have demonstrated the presence of a high level of lipid peroxidation products on days 1–2 of life in newborns who develop BPD. The presence of a high level of protein peroxidation products in the tracheal aspirate of infants weighing less than 1500 g compared to those with a higher weight has also been demonstrated. This confirms the fact that antioxidant defense decreases with the increase of immaturity [9, 15, 16].

There is also a close correlation between the protein oxidation level and activated neutrophils. This supports the presence of a relationship among neutrophil accumulation, oxidative stress, and the development of BPD [1].

In randomized trials for the study of STOP-ROP, some authors monitored oxygen exposure and the evolution of retrolental fibroplasia and BPD [36]. Thus, in groups with 89–94% O_2 Sat, compared to those with 96–99% O_2 Sat, the influence of saturation on the progression of retrolental fibroplasia was not significant. In contrast, in the group with higher saturation, the BPD progression rate was higher (13.2%) compared to subjects exposed to lower saturation levels (8.5%) [23, 39].

Exposure to a FiO_2 of 100% on the first day practically doubles the risk of BPD. Excessive oxygen administration and/or barotrauma may increase the risk of BPD. However, a high PaO_2 is a cofactor, not a causal agent in the development of BPD [36].

Preterm birth associates vitamin A deficiency, which is important in pulmonary epithelial lesion repair. A number of studies have evidenced a significant reduction in the incidence of bronchopulmonary dysplasia under conditions of vitamin A administration (Shenai et al.) [34, 37], but there are also studies that could not demonstrate this fact (Perason et al.) [32, 33].

Selenium deficiency can also be considered in association with low glutathione levels. Darlow et al. found a significant relationship between plasma selenium levels and the incidence of bronchopulmonary dysplasia at the age of 28 days, but could not clarify whether these were a cause or an effect of BPD.

A study conducted in our department, which assessed lipid peroxidation by measuring malondialdehyde (MDA) levels on the first and third days of life found that MDA values in newborns with respiratory distress increased with the increase in the severity of respiratory distress (**Table 1**).

MDA values in the mentioned study tended to decrease on day 3 compared to day 1, but without a statistically significant difference.

Also, the lipid peroxidation process was more intense in the study group-newborns with different pathologies generating oxidative stress compared with the control group of healthy term newborns, without any oxidative-stress inducing disorders (**Figures 1** and **2**).

In the same study, we also monitored protein peroxidation in newborns with respiratory distress, and we found the presence of a significant correlation between protein carbonyl values on the third day of life and respiratory distress ($r = 0.56$; $p < 0.05$). For the evaluation of protein peroxidation in the mentioned study, we measured protein carbonyls on the first and third days of life using the Reznick spectrophotometric method with dinitrophenylhydrazine. The protein substrate in the lung is an optimal target for the action of ROS and the triggering of oxidation reactions of these proteins. In fact, it was demonstrated that in newborns with RDS, this protein oxidation process under the action of ROS also contributes to the pathogenesis of BPD. Under the action of reactive oxygen species, peroxidation of proteins and other lipid and protein structures occurs. Thus, the normalization of pulmonary function is delayed. Surfactant administration before the initiation of mechanical ventilation limits oxidative injury induced by mechanical ventilation.

2.2. Effects of oxidative stress at endothelial level

The effects of ROS at endothelial level during the neonatal period are found in the following morbid conditions: septic shock, systemic inflammation, acute ischemia, inducing considerable oxidative stress [5]. Reactive oxygen species contribute to the development of ischemic and inflammatory vascular lesions by an important efflux of oxidants to the ischemic tissue and the induction of new lesions in tissues and organs in the next reperfusion stage [29].

		RankSum1	RankSum2	U	p	n1	n2
RDS mild vs. severe	MDA DOL 1	255.0	273.0	84.0	0.1159	18	14
	MDA DOL 3	281.0	247.0	110.0	0.5611	18	14
RDS mild vs. moderate	MDA DOL 1	307.5	638.5	136.5	0.0283	18	25
	MDA DOL 3	379.0	567.0	208.0	0.6877	18	25
RDS severe vs. moderate	MDA DOL 1	75.5	114.5	23.5	0.1740	6	13
	MDA DOL 3	79.0	111.0	20.0	0.0956	6	13
MV yes vs. no	MDA DOL 1	376.5	1453.5	285.5	0.7229	13	47
	MDA DOL 3	407.5	1422.5	294.5	0.8454	13	47

RDS = respiratory distress syndrome, MV = mechanical ventilation, RankSum = rank sum, p = test sign, and n = group size.

Table 1. MDA values according to RDS severity.

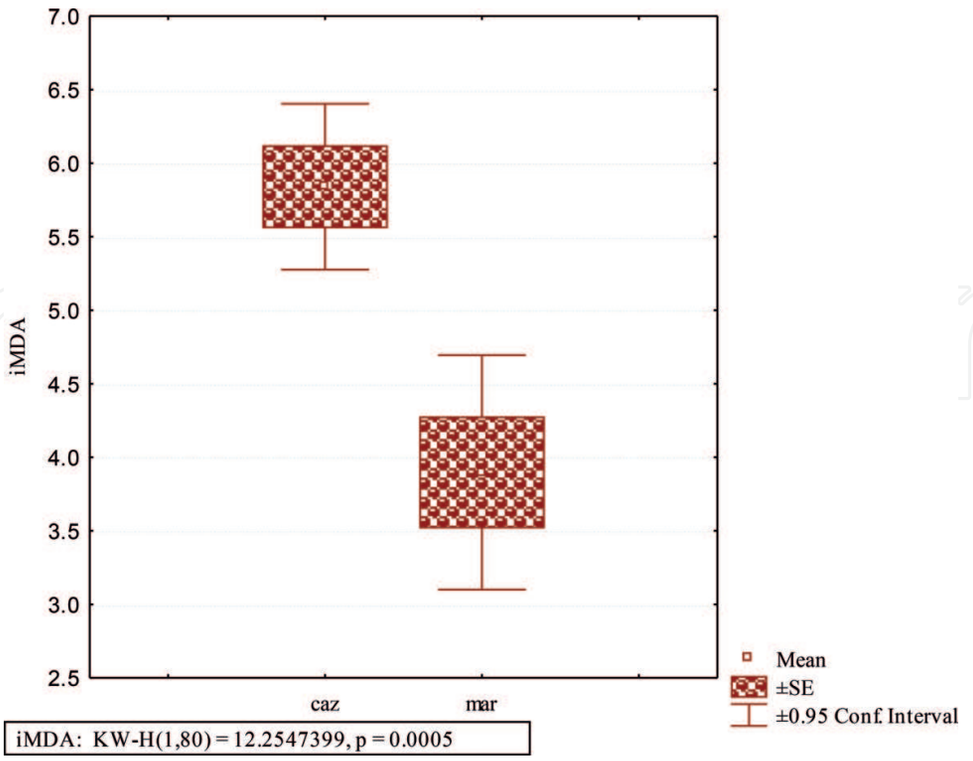

Figure 1. MDA values of case group DOL 1 vs. control group (caz = case, mar = control).

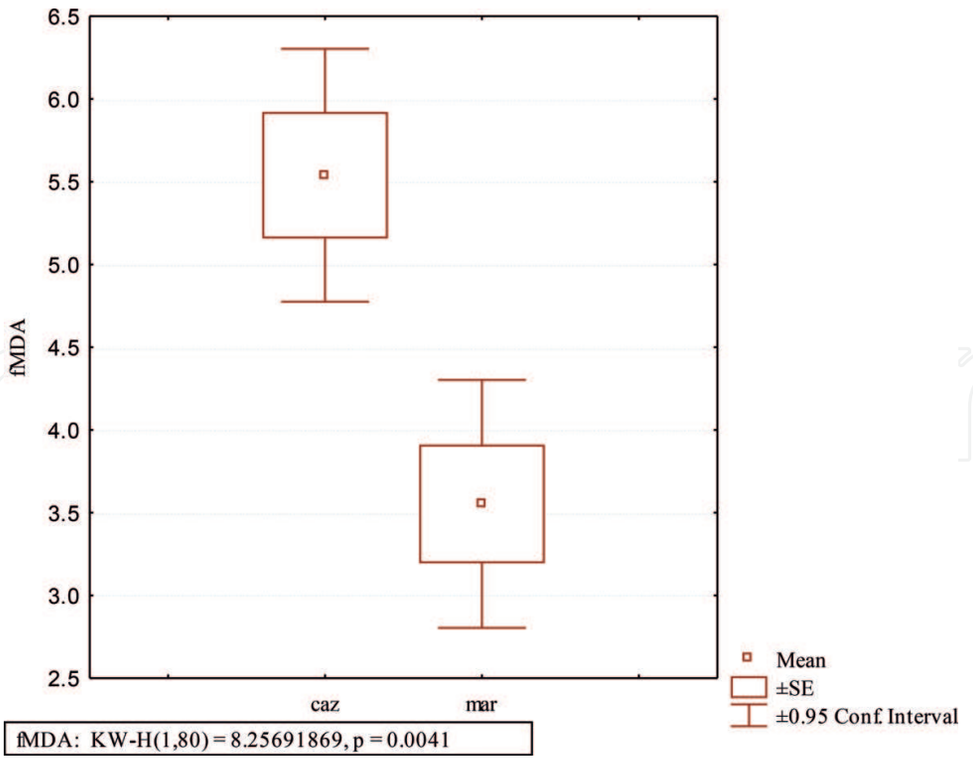

Figure 2. MDA values of case group DOL 3 vs. control group (caz = case, mar = control).

The vascular surface has the role to control critical processes for the functioning of organs. During inflammatory processes, the endothelium is exposed to oxidation [25]. It has been demonstrated that TNF-alpha stimulates the release of oxygenated water at the contact area between neutrophils and the endothelial cell, inducing endothelial cell retraction.

The exposure of endothelial cells to significant concentrations of exogenous oxidants will cause specific physiological effects. Endogenous oxidants have been recognized as a physiological signal component, probably triggered by lung injury, with the release of TNF-alpha, interleukin, growth factor β, and platelet growth factors. These will stimulate tyrosine phosphorylation, with the activation of ERK, extracellular signal-regulated kinase. DNA synthesis will occur accompanied by an increase in hydrogen peroxide levels. Oxidants mediate the effects of growth factors β and insulin, which will stimulate mitogen-activated proteins [27–28].

2.2.1. Oxidative stress in retinopathy of prematurity

The harmful effects of ROS at endothelial level also manifest in ROP [10, 23]. In the pathogenesis of ROP, the following factors are involved: self-regulation, which is absent in newborns, hyperoxygenation of the retina, which is frequent because antioxidant defense is reduced, particularly in the premature. Hyperoxygenation induces peroxidation of vasoactive isoprostanes. Vasoconstriction and vascular cell toxicity with ischemia and vascular proliferation occur [4, 7].

Glutathione is the most important intracellular antioxidant; however, its synthesis is reduced during intrauterine life and in prematures. In the vitreous fluid of the premature at risk for retinopathy, there is a high level of hypoxanthine with a role in the formation of free radicals [1, 12]. Papp et al. [6] found that the oxidized glutathione/reduced glutathione ratio is more than double in prematures with retinopathy compared to those without this disease. This is why the problem of using this ratio as a screening method for the detection of ocular involvement in premature infants was posed. The same authors found that in infants with active disease aged less than 3 months, reduced glutathione values were lower, and those of oxidized glutathione were higher, the greatest decrease in reduced glutathione occurring after the in vitro alteration of oxidative status.

2.2.2. Effects of oxidative stress in ulceronecrotic enterocolitis

In ulceronecrotic enterocolitis, there are multiple pathogenic mechanisms. One of the pathogenic links is hypoxic ischemic injury. Hypoxic ischemic injury in the mesentery is followed by a cascade of events, with intestinal mucosal reperfusion injury. Cytotoxic damage of vascular endothelial cells occurs, which in turn will cause ischemia and new cytotoxic effects through the formation of free radicals [41].

The regulation of mesenteric blood flow includes a reflex self-regulation mechanism. The peripheral autonomic nervous system plays a role in the regulation of mesenteric blood flow.

The initiated ischemia will induce transcapillary fluid passage and local edema. Reperfusion will exacerbate transcapillary fluid production and induce tissue destruction. This event is

mediated by factors released from the ischemic intestine, including endotoxins, histamine, prostaglandins, and superoxide anion, which result from oxygen metabolization. Superoxide will induce lipid peroxidation with the disruption of the integrity of capillaries and epithelial cells. Mucosal lesions characterized by edema, hemorrhage, ulceration, and necrosis may be induced experimentally by a combination of oxidants (hypoxanthine and xanthine oxidase) [14]. Superoxide dismutase can experimentally prevent or attenuate the described lesions. The protective effect of SOD was experimentally observed in animals with superior mesenteric artery occlusion. The sequential development of intestinal enzymes: SOD and xanthine oxidase can be a determining factor of neonatal intestinal susceptibility to ischemic mucosal injury [13, 45]. Regarding NEC prevention, many studies performed on animals show the beneficial effect of melatonin in preventing oxidative stress involved in the development of NEC lesions. Melatonin acts by reducing the level of inflammatory cytokines and by stimulating the activity of antioxidant enzymes. A decrease in TNF-α and IL-1β levels was found in animals treated with melatonin. Melatonin also counteracts the reduction of intestinal motility generated by lipopolysaccharides. Melatonin is an indolamine produced by the pineal gland, having serotonin as a precursor. It is synthesized by serotonin-rich enterochromaffin cells in the digestive tract. This synthesis takes place postprandially in the digestive tube and its level is 100 times higher than blood levels [45, 46].

The pathophysiological mechanism of NEC is based on a hypoxic-ischemic process similar to that found in postasphyxia brain injury. A number of studies have demonstrated that the association of melatonin with misoprostol, a gastric mucosal protective agent, is more beneficial than melatonin therapy alone. These studies on animals found that the administration of 10 mg/kg body weight melatonin for 3 days to animals with induced NEC-like lesions significantly reduced the severity of the disease by decreasing cytokine levels and stimulating antioxidant enzyme activity [45, 48].

2.2.3. Effects of oxidative stress in neonatal asphyxia and hypoxic ischemic encephalopathy

In the brain, there are some particularities that increase the vulnerability of this tissue to oxidative stress: cell membranes are rich in polyunsaturated fatty acids, the brain is poor in catalase and SOD, and there are some brain areas rich in iron. Nerve cell injury in the context of asphyxia will induce iron release [13]. Given the low antioxidant defense, the release of low molecular mass iron will allow the formation of hydroxyl radical and lipid peroxidation. By lipid peroxidation, free radicals induce molecular damage, including endothelial factor destruction. The low level of antioxidants in the serum seems to be directly involved in the genesis of cerebral hemorrhage [17]. Transferrin and ceruloplasmin levels can be indicators of the risk of cerebral hemorrhage in newborns with asphyxia at birth [17, 18]. In prematures with asphyxia, a decrease in these enzymes will precede cerebral hemorrhage. Ceruloplasmin acts as a strong ferroxidase, catalyzing iron oxidation to less reactive ions. The antioxidant defense capacity can be exceeded, making transferrin inadequate for binding, with the release of low molecular mass iron, which will subsequently induce lipid peroxidation. In case of iron overloading or severe oxidative stress, the antioxidant defense capacity is exceeded, making nontransferrin bonds available, with the release of low molecular mass iron, followed by lipid peroxidation. This may occur even if transferrin is not completely iron saturated [42].

Hypoxic ischemic brain injury is a long process that starts with the occurrence of the insult and continues during the recovery period, after reperfusion. This reperfusion process represents a paradoxical tissue response: the appearance of oxidative lesions in a hypoxic tissue, poorly perfused after circulatory stabilization, at the time of its perfusion with oxygenated blood. This stage of reperfusion will cause an excessive production of free radicals generating new lesions after the initial oxidative attack. In the premature, an increased risk of cerebral ischemia persists postnatally, during the first week of life [19, 26, 27].

In the reperfusion stage, ROS with a cell damaging effect are produced, in the first place by the conversion of xanthine dehydrogenase to xanthine oxidase. This process will result in the formation of thiol groups or proteolysis by activation of proteases in energetically depressed cells. In the reoxygenation stage, hypoxanthine is converted to uric acid under the action of xanthine oxidase, and superoxide and peroxide are released through the mediation of xanthine oxidase by molecular oxygen binding. Hydroxyl radical generated through the Fenton reaction will also be released, due to the catalytic effect of metals such as iron [42]. Perinatal hypoxic stress is a frequent cause of morbidity, mortality, and neurological damage in survivors. In the perinatal hypoxic context, several factors play a role in the pathogenesis of lesions: hypoxia with initial ROS formation, followed by ischemia-reperfusion, a stage at which arachidonic acid and phagocytes will be activated under the action of inflammation mediators. Thus, a vicious circle is created. ROS will be formed, followed by tissue lesions and the genesis of new free radicals [42, 43].

At CNS level, under asphyxia conditions, encephalopathy lesions are induced by activation of leukocytes or glial cells and release of new free radicals. Hypoxic lesions will be perpetuated by release of protein-unbound iron. Endothelial lesions, hemostasis abnormalities, and inflammatory lesions occur, as well as with an increase in anaerobic metabolism, lactic acid levels, brain damage as a result of oligodendroglial vulnerability, astrocyte dysfunction, N-methyl-D-aspartate receptor abnormalities, and synaptic damage [28, 29].

Mitochondrial DNA damage induces changes in respiratory chain proteins, with the formation of new free radicals and subsequent cell lesions. Neonatal cerebral hypoxia stimulates activin secretion, which in turn stimulates erythropoietin, resulting in the production of nucleated red blood cells [35, 43].

The types of lesions that occur in the brain as a result of hypoxia are variable. In the extremely immature brain, preoligodendrocytes and cell precursors of oligodendrocytes are affected. As the brain matures, the resistance of oligodendrocytes to oxidative stress increases due to an increase in antioxidant defense, as well as to the protein structure involved in programmed cell death.

After the hypoxic episode, there is an increase in the density of glutamate receptors, and mitochondrial calcium accumulation occurs, which will trigger apoptosis. Caspase 6 and, subsequently, caspase 3 are activated. NMDA receptor activation depresses the mitochondrial respiratory process and induces apoptosis—a process which is not found in adults, known as the NMDA paradox [41, 48].

Studies conducted by Peeters and Schulte regarding the activity of glutathione peroxidase in the cerebrospinal fluid of newborns with asphyxia and the neuron-specific enolase value evidenced a correlation between the glutathione peroxidase value and gestational age, as well

as between the neuronal enolase value and the neurological evolution of perinatal asphyxia cases. The influence of the genetic factor on postischemic evolution was also demonstrated, as well as on the presence of a correlation with patient's sex, males being more predisposed to develop lesions compared to females.

With the stabilization of newborns with asphyxia at birth, in the reoxygenation stage, the exposure of the diseased cell to a new oxidative attack follows. The use of 100% oxygen in resuscitation is an important source for the formation of ROS. Hyperoxia causes an increase in the activity of antioxidant enzymes such as catalase or superoxide dismutase, as well as an activation of enzymes in the glutathione reductase cycle: GSH-reductase and GSH-S-transferase. Some studies demonstrated that the urinary level of N-acetyl-glucosaminidase is correlated with the value of oxidized glutathione and is higher in newborns exposed to a FiO_2 of 100%. Based on these findings, resuscitation guidelines were changed in 2010, indicating to start neonatal resuscitation with atmospheric air, followed by an increase by titration in the FiO_2 value depending on the improvement of blood oxygen saturation [1, 3, 43].

In the study conducted in our unit, we found that lipid peroxidation in newborns with asphyxia was maintained at a high level on the third day compared to the first day of life, without a statistically significant difference. This high plateau value on day 3 was attributed to perinatal hypoxic stress and to the reoxygenation-reperfusion process. None of the newborns with asphyxia received hypothermia treatment, because at the time of the study, this therapeutic method was not available in our unit. The analysis of lipid peroxidation after neonatal asphyxia by gestational age groups evidenced more intense peroxidation in premature infants compared to term infants with asphyxia. We explained this process by the association of neonatal asphyxia with respiratory distress in the case of prematures, which involved oxygen therapy and respiratory support for its treatment, factors that increased oxidative stress and implicitly, malondialdehyde values. Regarding protein peroxidation, its markers are found in high amounts in the plasma of prematures with asphyxia. Their high values are maintained until the seventh day of life [43]. In newborns with hypoxic ischemic encephalopathy in our study, protein peroxidation was more intense compared to the other newborns. Hypoxic injury is aggravated by protein-unbound iron release. In its presence, as part of the Fenton reaction, hydroxyl radicals are released, which will exert a strong toxic effect on the brain. ROS toxicity in newborns is enhanced by the increased production of ROS, their rapid tissue growth, and impaired antioxidant defense. In the developing brain, endothelial lesions, hemostasis abnormalities, inflammatory reaction, an increase of anaerobic metabolism occur, which are followed by lactic acid accumulation. Oligodendroglial injury, astrocyte dysfunction, and synaptic abnormalities will result [43, 44].

Oxidative stress markers have an important predictive value for neuronal injury in newborns at risk. The correlation between the values of oxidative stress markers and imaging electrophysiological brain changes, near-infrared spectroscopy (NIRS) aspects, and the degree of neurological impairment is not currently described in the literature. This is why further studies are required in this respect. Also, research on therapies protecting against cerebral oxidative stress after perinatal asphyxia has not reported a clearly beneficial medication at this stage of knowledge. The only therapy currently applied with positive, beneficial results in perinatal asphyxia is controlled hypothermia. In the near future, studies are needed which

should attempt to identify free radical scavengers that can be administered to newborns and could be useful in limiting oxidative stress, particularly in prematures, in whom antioxidant defense is impaired due to the end of pregnancy before term [44, 47].

3. Conclusion

Oxidative stress at newborns has role in pathogenesis of different neonatal diseases. The oxidative stress is more severe in preterm than in term neonates. The antioxidant defense of preterm less developed than in term neonates, mainly the enzymatic antioxidant defense.

In the near future, studies are needed which should attempt to identify free radical scavengers that can be administered to newborns and could be useful in limiting oxidative stress, particularly in prematures, in whom antioxidant defense is impaired due to the end of pregnancy before term.

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