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# VIROLOGY

# Pro- and Antioxidant Status in Newborn with COVID-19

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There is practically no information on the state of oxidative stress reactions in newborns with coronavirus infections. At the same time, such studies are extremely important and can contribute to better understanding of the process of reactivity in patients of different ages. The content of pro- and antioxidant status indicators was assessed in 44 newborns with confirmed COVID-19. It was found that the content of compounds with unsaturated double bonds, primary, secondary, and final LPO products were elevated in newborns with COVID-19. These changes were accompanied by higher SOD activity and retinol level and reduced activity of glutathione peroxidase. Contrary to popular opinion, newborns can be a COVID-19-susceptible age group and require more close monitoring of metabolic reactions during the period of neonatal adaptation that is an aggravating background during infection.

**Key Words:** *COVID-19; oxidative stress; antioxidants; newborns; children*

COVID-19 is one of the most serious public health problems in recent years. Due to high mortality, as well as serious economic and social consequences, the studies of the pathogenetic mechanisms of the disease and new therapeutic targets are of paramount importance [1]. A still unclear aspect of the pathogenesis of COVID-19 is the large heterogeneity of response among patients, from asymptomatic course to severe symptoms [2]. Symptoms tend to become more aggressive and fatal among more vulnerable groups, including the elderly, patients with chronic diseases, patients receiving immunosuppressive therapy, and pregnant women [1,2]. It is believed that COVID-19 rarely affects infants, therefore, studies of body reactivity are mainly focused on adult patients [3]. The most common symptoms in patients with COVID-19 in the neonatal period are respiratory failure (in 73% of cases), fever (in 63% of cases), neurological (apathy, irritability), and gastrointestinal symptoms (in 50% of cases) [4]. At the same time, usually, COVID-19 in the neonatal period does not lead to death [1].

It is known that the metabolic activity under physiological conditions is accompanied by ROS generation [5]. The activation of cells that provide antimicrobial immunity, neutrophils and macrophages, and the production of proinflammatory cytokines depend on ROS [5-7]. The role of ROS in the development of an antiviral immune response through the formation of type I IFN is important [8]. ROS also play an important role as mediators of cell signaling pathways, which, however, does not entail a negative effect on cell structures due to compensation by antioxidant mechanisms [9]. Under conditions of hyperproduction, including COVID-19, ROS are able to stimulate inflammatory signaling cascades through protein kinases, transcription factors, and an increase in the genomic expression of proinflammatory regulators, which, in turn, leads to hyperactivation of the immune system [5]. These phenomena are accompanied by the development of oxidative damage to

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various macromolecules, including proteins, lipids, and nucleic acids [10].

There is practically no information on the state of oxidative stress reactions in newborns with coronavirus infections now. At the same time, such studies are extremely important and can contribute to better understanding of the reactivity process in patients of different ages and to the development of preventive measures in newborns.

The aim of this study was to evaluate the activity of indicators of pro- and antioxidant status in newborns with COVID-19.

## MATERIALS AND METHODS

We examined 44 newborns (main group) hospitalized in 2020-2021 in Ivano-Matreninsky City Children's Clinical Hospital with a confirmed diagnosis of COVID-19 (detection of SARS-CoV-2 in nasopharyngeal mucus by PCR). The mean age of children on the day of hospitalization was 4.0±3.1 days. Criteria for inclusion in the main group: children born alive, with a gestational age of more than 36 weeks, confirmed COVID-19 in a child, signed informed consent from the parents and/or legal representatives of the child.

The control group ($n$=80) was recruited at the Irkutsk City Perinatal Center and included healthy newborns. In terms of gestational age, anthropometric data and sex characteristics, the control group was comparable to the main one. Criteria for inclusion in the control group: children born alive, with a gestational age of more than 36 weeks, no diagnosis of COVID-19 in the child, signed informed consent from the parents and/or legal representatives of the child.

Exclusion criteria from both groups: unwillingness of the patient's parents and/or legal guardians to participate in the study at any stage, non-compliance of the patient with the inclusion criteria.

The experiment was carried out in accordance with Declaration of Helsinki of the World Medical Association (revised 2013). The study was approved by the Committee on Biomedical Ethics at the Scientific Centre for Family Health and Human Reproduction Problems (No. 6.1; June 19, 2020).

The boys were prevailing (66%; $n$=29) in the sex structure. Most of the children were Caucasian (84%, $n$=37). Body weight and length (Me (Q25-Q75)) at birth was 3210 (2720-3600) g and 51 (49.5-54) cm, respectively, body weight upon admission to the hospital was 3300 (2780-3660) g. Most of the newborns (89%; $n$=39) in the main group were born full-term, with a gestational age of 39 (38.25-40) weeks, were immediately breastfed and were breast-fed for the period of treatment in the hospital (66%, $n$=29). During the period of hospital treatment, the condition was assessed as moderate in 93% children ($n$=41) and severe in 7% ($n$=3). COVID-19 manifested as pneumonia in 25% newborns ($n$=11) and as an acute respiratory viral infection (ARVI) in the rest of the children in the group. The most common symptoms of ARVI were fever (34%; $n$=15) and runny nose (30%; $n$=13).

Blood for the study was taken from a peripheral vein (back of the hand) in the initial period of the disease (main group) or on the third day of life (control group) using disposable vacuum systems with a 23G needle in the morning before feeding. All children had analgesia (non-pharmacological methods of pain correction – non-nutritive sucking of 20% glucose solution *per os*). Plasma and erythrocyte hemolysate were used as the study material. The contents of substrates with unsaturated double bonds (DB), conjugated dienes (CD), ketodienes and conjugated trienes (KD and CT), TBA-reactive substances (TBARS), total antioxidant activity (AOA), SOD activity, α-tocopherol, retinol, reduced (GSH) and oxidized (GSSG) glutathione were analyzed [11]. The activity of glutathione peroxidase (GPX) was measured by ELISA using commercial kits from Randox. Measurements were performed on an SF-2000 spectrophotometer (Spektr), a BTS-350 spectrofluorophotometer (BioSystems), and an FLUORATE 02 ABLF-T (Lumeks). For ELISA, a MultiSkan ELX808 microplate reader (BioTek) was used.

The results were analyzed using Statistica 10.0 software (StatSoft, Inc.). The visual-graphical method and the Kolmogorov–Smirnov test with the Lilliefors and Shapiro–Wilk correction were used to evaluate normality of data distribution; Fisher's test was applied for evaluating the equality of general variances; Mann–Whitney test was used for the analysis of intergroup differences. The critical significance level was set at 5%.

## RESULTS

Analysis of the data in the main group showed the presence of statistically significant differences in number of LPO indicators (Fig. 1). Thus, newborns with COVID-19 had higher values of compounds with DB (by 1.3 times; $p$=0.004), primary, secondary, and end-products of LPO ($p$<0.0001): CD content was increased by 1.57 times, KD and CT by 2.14 times, and TBARS products by 1.73 times in comparison with the control.

In the main group, significant ($p$<0.0001) differences were found in the AOD system: SOD activity and retinol levels were elevated by 1.26 and 3.53 times, respectively, and GPX activity was reduced by 1.35 times lower (Fig. 2).

Newborns with COVID-19 had increased levels of LPO products at all stages of the process relative to

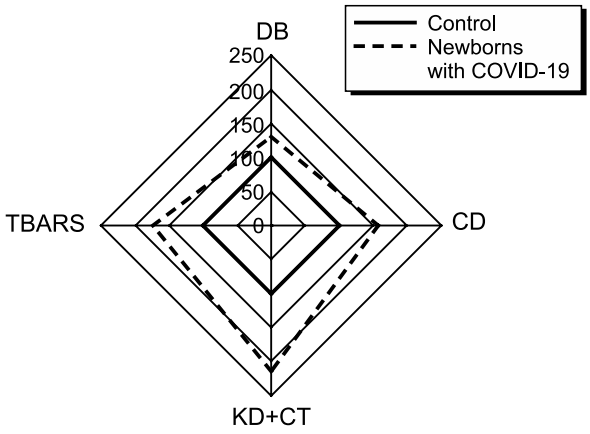


**Fig. 1.** The level of prooxidant status components in newborns with COVID-19. All presented indicators in newborns with COVID-19 significantly differed from the control (taken as 100%).

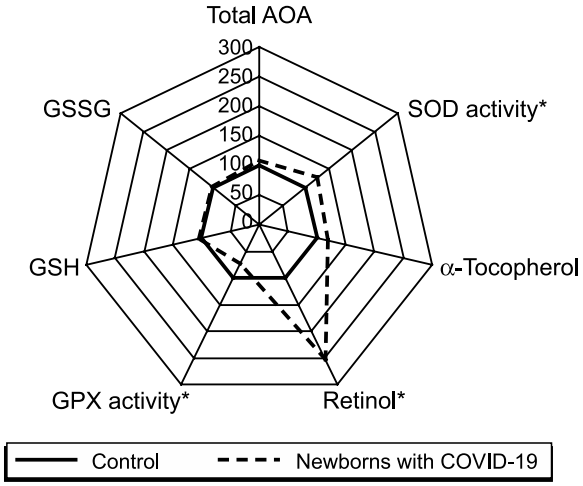


**Fig. 2.** The level of components of the antioxidant status in newborns with COVID-19. AOA: antioxidant activity. *Statistically significant differences from the control (taken as 100%).

control values. The transition from prenatal to postnatal period of life causes a significant increase in oxygen tension in the arterial blood, as well as activation of metabolic pathways that ensure adaptation of the newborn to the extrauterine environment [9]. Under these conditions, the balance in the LPO–AOD system is crucial for the maintenance of cell functions.

Any aggravating pathology (including COVID-19) in this period can have negative consequences in the future. It is known that toxic LPO metabolites generated during chemical interactions of ROS/reactive nitrogen species with polyunsaturated fatty acids and arachidonic acid derivatives can have a multifaceted damaging effect on cellular biostructures [8]. Our results agree with the data about activity of these reactions in children of small gestational age with respiratory viral infections [12]. It has been proven that ROS generation caused by respiratory viruses is associated with a cascade of pathological processes that have extremely negative consequences on the body [5].

Oxidative stress develops under conditions when generation of free radicals is not balanced by the activity of intracellular antioxidant systems. Our study revealed higher levels of some antioxidant defense factors (SOD and retinol) against the background of reduced GPX values in newborns with coronavirus infection. SOD, along with catalase and GPX, is a key enzyme that regulates the innate antioxidant response and potentiates accumulation of toxic metabolites at the primary stages of the chain reaction. The role of vitamin A and related retinols in the modification of the immune system due to the expression of the main antibodies that destroy viruses has been established [13]. It was also noted that the addition of vitamin A to therapeutic measures contributes to a significant improvement in the course of lower respiratory tract infection in children [7].

A possible explanation of increased functional activity of antioxidant factors in newborns of the main group can be that most of them are breast-fed from birth. It is known that breast milk contains antiproteases, including α-1-antitrypsin, α-1-antichymotrypsin, and an elastase inhibitor, which naturally limit the penetration of pathogens into the organism, thereby limiting systemic inflammation [14]. It also contains a variety of antioxidants, including catalase, GPX, and SOD, enzyme active site metals (*e.g.*, Cu, Zn, and Se), fat- and water-soluble vitamins (A, C, and E), metal-binding proteins (*e.g.*, lactoferrin), and melatonin [9]. Antioxidants are an important part of the anti-inflammatory system in human milk and can counteract the excess production of ROS caused by hyperoxia, reperfusion and/or inflammation, as well as tissue damage caused by ROS. As is known, the SARS-CoV-2 virus infects epithelial, endothelial cells, and alveolar macrophages, triggering the production of proinflammatory cytokines and chemokines, inflammatory macrophage proteins, which then attract immune cells, neutrophils, and macrophages to the site of infection [5]. Severe lung inflammation is usually caused by increased invasion of neutrophils and macrophages into the alveolar space, which promotes further inflammation and creates a proinflammatory feedback loop that leads to damage to the endothelial and epithelial layers of the lungs [14]. Breast milk prevents these pathological changes because it contains a well-developed anti-inflammatory system, including several bioactive factors, including IL-10, oligosaccharides, milk fat epidermal growth factor globule 8 (MFG-E8), *etc.* The factors inhibit leukocyte adhesion to the endothelium, which is a critical stage in the

spread of inflammation, suppress leukocyte infiltration, and reduce activity of Th1 effector cells, NK cells, and macrophages, as well as the production of a number of proinflammatory cytokines, especially IL-8, which increases significantly in severe COVID-19 [15]. It is also known that children are less susceptible to the SARS-CoV-2 impact because of immaturity of the immune system due to insufficient activity of angiotensin-converting enzyme II (ACE2), the main cellular receptor for SARS-CoV-2 [2,4].

We also revealed insufficient activity of GPX, the main glutathione-dependent enzyme involved in the inactivation of lipid hydroperoxides. Enzymes, as redox regulators with antioxidant properties, are associated with active mediators and cell organelles, and, accordingly, are direct participants in the pathogenesis of diseases associated with redox imbalance [7]. Thus, activity of the enzymatic component of the AOD system is reduced in patients of the main group.

Thus, contrary to popular opinion, newborns can also be a sensitive age group to COVID-19 and need more attention to the course of metabolic reactions during the neonatal adaptation period, which is an aggravating background during infection. It is necessary to monitor the pro- and antioxidant status in newborns with a new coronavirus infection, which should be an important component in the prevention of further complications.

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