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The role of antimicrobial peptides in the pathogenesis of pneumonia in preterm ınfants with perinatal encephalopathy

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Aim. In this study, the role of antimicrobial peptides (AMPs) in the pathogenesis of pneumonia in preterm infants with perinatal encephalopathy was investigated.

Materials and methods. For this aim, the concentrations of β1-defensin, lactoferrin, calprotectin, fecal calprotectin, and cathelicidin were analyzed using the enzyme-linked immunosorbent assay (ELISA) in 29 preterm infants with perinatal encephalopathy but without pneumonia or other infectious complications, and in 20 preterm infants with perinatal encephalopathy complicated by pneumonia. The comparison group consisted of 15 practically healthy preterm infants. The control group consisted of 16 full-term, practically healthy infants.

Results. The obtained results demonstrated that, in preterm infants with perinatal encephalopathy complicated by pneumonia, the serum concentration of calprotectin was 4.1-fold higher, β1-defensin – 3.2-fold higher, cathelicidin – 2.0-fold higher, lactoferrin – 4.0-fold higher, and fecal calprotectin – 3.9-fold higher compared to practically healthy preterm infants, with all differences being statistically significant. A negative correlation was found between AMP levels and gestational age, whereas positive correlations were observed with the clinical severity of the disease, the degree of intraventricular hemorrhage in the brain, and indicators of respiratory failure. In addition, positive correlations were identified among lactoferrin, cathelicidin, calprotectin, and defensin levels.

Conclusion. Thus, AMPs play a crucial role in the pathogenesis of postnatal pneumonia developing in the context of perinatal encephalopathy, with their secretion increasing in a compensatory manner to form an essential component of the primary defense line against infection.

The study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the institution's Local Ethics Committee. The informed consent was obtained from patients.

The authors declare no conflict of interest.

Keywords: preterm newborns, perinatal encephalopathy, pneumonia, antimicrobial peptides.

Роль антимікробних пептидів у патогенезі пневмонії у недоношених дітей із перинатальною енцефалопатією

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Мета – дослідити роль антимікробних пептидів (АМП) у патогенезі пневмонії в недоношених дітей із перинатальною енцефалопатією.

Матеріали та методи. Аналізували концентрації β1-дефензину, лактоферину, кальпротектину, фекального кальпротектину та кателіцидину за допомогою імуноферментного аналізу (ІФА) у 29 недоношених дітей із перинатальною енцефалопатією, але без пневмонії чи інших інфекційних ускладнень, та у 20 недоношених дітей із перинатальною енцефалопатією, ускладненою пневмонією. Групу порівняння налічували 15 недоношених, практично здорових дітей. Контрольну групу становили 16 доношених, практично здорових дітей.

Результати. Отримані результати показали, що в недоношених дітей із перинатальною енцефалопатією, ускладненою пневмонією, концентрація кальпротектину в сироватці крові була в 4,1 раза вищою, β1-дефензину – у 3,2 раза, кателіцидину – у 2,0 раза, лактоферину – в 4,0 раза та фекального кальпротектину – у 3,9 раза порівняно з практично здоровими недоношеними дітьми, причому всі відмінності були статистично значущими. Виявлено негативну кореляцію між рівнями АМП та гестаційним віком, тоді як позитивні кореляції спостерігалися з клінічною тяжкістю захворювання, ступенем внутрішньошлуночкового крововиливу в головний мозок та показниками дихальної недостатності. Крім того, позитивні кореляції були виявлені між рівнями лактоферину, кателіцидину, кальпротектину та дефензину.

Висновок. АМП відіграють вирішальну роль у патогенезі постнатальної пневмонії, що розвивається в контексті перинатальної енцефалопатії, причому їхня секреція компенсаторно збільшується, формуючи важливий компонент первинної лінії захисту від інфекції.

Дослідження було проведено відповідно до принципів Гельсінської декларації. Протокол дослідження був схвалений локальним етичним комітетом установи. Інформовану згоду було отримано від батьків пацієнтів.

Автори заявляють про відсутність конфлікту інтересів.

Ключові слова: недоношені новонароджені, перинатальна енцефалопатія, пневмонія, антимікробні пептиди.

In the neonatal period, especially in preterm infants, pneumonia remains one of the major clinical problems accompanied by high mortality rates. Since the respiratory tract structure and immune defense mechanisms in preterm infants are not fully developed, they have increased susceptibility to respiratory infections, reduced efficiency of mucociliary clearance, and a decreased number of cells that synthesize antimicrobial peptides (AMPs). At the same time, in infants with perinatal ence-

phalopathy (hypoxia-ischemia-induced neonatal encephalopathy), the risk of systemic inflammation and immune dysfunction is higher, which increases the likelihood of developing pulmonary infections and their complications [6,14,21,25]. As a result of weakened anti-inflammatory immune responses, infectious processes in the lungs can lead to chronic inflammation and chronic pulmonary pathologies, particularly pneumonia [25].

Among AMPs, human defensins, cathelicidin (LL-37), lactoferrin, and calprotectin play a role in the early immune defense of the body, providing both direct antimicrobial (bactericidal, antifungal, antiviral) and immunomodulatory effects. They strengthen the mucosal barrier, regulate inflammatory signaling, and participate in the formation of the microbiome. Changes in AMP levels can directly influence the onset and course of pneumonia in preterm infants [4,9]. The ability of these peptides to recognize receptor proteins of viral and bacterial pneumonia pathogens with high specificity provides a rationale for their use in differential diagnosis. This, in turn, may have significant scientific and practical importance in preventing the unnecessary use of antibiotics, their toxic effects, and bacterial resistance [4].

In perinatal hypoxia-ischemia-induced encephalopathy, an inflammatory cascade is activated in the central nervous system, characterized by microglial and astrocyte activation, increased production of pro-inflammatory cytokines, and disruption of the blood-brain barrier. Exacerbation of inflammation can also affect lung tissue, creating a functional link in the etiopathogenesis of pneumonia. Recent studies highlight the important role of intestinal AMP secretion and the microbiota-mediated «gut-lung» axis in lung injury and repair, emphasizing both local (oral-respiratory/intestinal) and systemic effects of AMPs. These mechanisms help to explain both the etiopathogenesis and clinical severity of pneumonia in preterm infants with perinatal encephalopathy [1,17].

In preterm infants with perinatal encephalopathy and concomitant immunodeficiency, AMPs such as calprotectin, lactoferrin, cathelicidin (LL-37), and defensins are crucial early components of the innate immune system, playing a clinically significant role in the initial defense chain. Numerous clinical studies have demonstrated the potential of AMPs both as biomarkers and as prophylactic/therapeutic agents. Several randomized controlled trials and their meta-analyses on enteral lactoferrin supplementation have shown a tendency to reduce the incidence of late-onset sepsis in preterm newborns [3,15]. Lactoferrin participates in the establishment of immune balance by regulating the synthesis and secretion of Interleukin-6 (IL-6) and other pro-inflammatory cytokines.

Defensins (particularly epithelial β-defensins) are among the main AMPs in the pulmonary mucosa of newborns. During bacterial infection, α- and β-defensins are primarily involved in the development of the immune response, and their synthesis is stimulated via the NF-κB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) signaling pathway. In addition to directly destroying microorganisms, they regulate inflammatory signaling pathways and may play an important role in the onset and progression of pneumonia in preterm infants [4,24].

Cathelicidin (human LL-37) possesses both antimicrobial and potent immunomodulatory properties. A correlation has been identified between high levels of LL-37 in epithelial cells and pulmonary secretions and the clinical course of pneumonia, while some studies have also shown that a decrease in LL-37 levels is associated with a more severe clinical presentation. Besides, the potential of LL-37 in predicting neonatal infection risk has been investigated. The expression of the LL-37 peptide is mainly regulated through the activation of Toll-like receptor (TLR) signaling pathways, a process that plays a crucial role in initiating the innate immune response. Cathelicidin LL-37 participates in endotoxin neutralization and immune modulation, reduces TNF-α levels, stimulates immune cell migration, and ultimately decreases the risk of mortality [16].

Calprotectin (S100A8/S100A9 heterodimer) is secreted primarily by neutrophils and is considered a highly sensitive biomarker of both local and systemic inflammation. During perinatal infection and inflammation, increased levels of serum and fecal/intestinal calprotectin have been observed in the amniotic fluid, respiratory tract, and gastrointestinal system (e.g., in bacterial infections and necrotizing enterocolitis) as a result of neutrophil activation. Some studies have noted the role of serum calprotectin in differentiating bacterial from viral respiratory infections [11,20].

A more in-depth investigation of these mechanisms and their implementation in clinical practice, particularly in high-risk preterm infants with perinatal encephalopathy, may hold significant scientific and practical value for the early diagnosis of pneumonia and for the development of targeted im-

munomodulatory strategies in selecting treatment approaches [2,10]. The data on the association between AMPs and disease prognosis, as well as on their functional role in the development of pneumonia in preterm infants with perinatal encephalopathy, remain insufficient, and further systematic clinical and laboratory studies are needed to clarify the specific molecular mechanisms involved.

The study ***aimed*** to investigate the role of AMPs in the pathogenesis of pneumonia in preterm infants with perinatal encephalopathy.

Materials and methods of the study

Blood and stool samples from 64 preterm infants were analyzed. The study contingent was divided into three main groups. The Group I included 15 practically healthy preterm infants (Me=35.3 weeks; Q1=31; Q3=36). The Group II included 29 preterm infants with perinatal encephalopathy but without complications such as pneumonia or other infections (Me=33.0 weeks; Q1=32.0; Q3=35.0). The Group III included 20 preterm infants with infectious and inflammatory perinatal encephalopathy complicated by pneumonia (Me=30.0 weeks; Q1=28.0; Q3=32.5). The control group consisted of 16 full-term, practically healthy infants (Me=38.1 weeks; Q1=37; Q3=40). No statistically significant differences were found between the groups in terms of weight gradient (p=0.347) or sex (p=0.799). There was a higher proportion of severely ill newborns in the Group III compared to the Group II (n=15, 75%, p=0.001). Among these newborns, congenital pneumonia was recorded in 12 (60%) cases (p<0.001), unspecified infections specific to the perinatal period in 9 (45%) cases, p<0.001) cases, and sepsis in 5 (25%) cases (p=0.007). Respiratory distress syndrome was observed in newborns with congenital pneumonia. Septic enterocolitis, necrotic enterocolitis, and asphyxia were not recorded among newborns in the Group III included in the study. Intraventricular hemorrhage in the brain (p=0.001) was recorded in 15 (51.7%) newborns in the Group II and 6 (30.0%) in the Group III, and in this group, newborns with signs of grade III hemorrhage were found (n=2; 33.3%; p=0.001) (Table 1).

The concentrations of β-defensin (hBD 2; «Cloud–Clone Corp»), lactoferrin («Cloud–Clone Corp»), LL-37 («Eastbiopharm»), and calprotectin («Eastbiopharm») in the blood serum of the newborns included in the study, were analyzed using the enzyme-linked immunosorbent assay (ELISA) method. Besides, the level of fecal calprotectin («Eastbiopharm») in the stool of the infants included in the study was also investigated. The analysis of blood and stool samples was performed during the first 3 days of the newborns' lives.

For statistical analysis, non-parametric methods, namely the Mann–Whitney U test and the Kruskal–Wallis test, were applied. The obtained results are presented as the median, first (Q1) and third (Q3) quartiles, as well as mean ± standard error (M±SE), minimum, and maximum values. Correlations (ρ) between the indicators were determined using Spearman's test. Statistical calculations were performed using the SPSS-26 software package. Differences in the indicators between groups were considered statistically significant at p<0.05.

Results of instrumental examinations

In the blood of newborns Group I, the concentration of calprotectin decreased by 13.2% compared to the Control group, p=0.049 (Me=133.8 ng/ml; Q1=110.4; Q3=157.4 versus Me=151.5; Q1=132.3; Q3=174.7), β1-defensin decreased by 11.2%, p=0.038 (Me=67.8 pg/ml; Q1=48.6; Q3=75.8 versus Me=75.4; Q1=65.6; Q3=92.6), and cathelicidin decreased by 40.4%, p<0.001 (Me=3.19 μg/ml; Q1=2.38; Q3=3.85 versus Me=4.48; Q1=4.20; Q3=5.00). In contrast, lactoferrin concentration increased by 53.2%, p=0.005 (Me=1.18 mg/ml; Q1=0.86; Q3=1.33 versus Me=0.77; Q1=0.67; Q3=0.87) compared to the control group, showing a statistically significant rise. Besides, in the Group I, fecal calprotectin concentration in the stool increased significantly by 53.4%, p<0.001 (Me=145.7 ng/ml; Q1=133.7; Q3=165.8 versus Me=95.0; Q1=88.0; Q3=109.0) compared to the Control group (Table 2).

As shown in the table, in infants of the Group II, the concentrations of β1-defensin (p=0.158) and calprotectin (p=0.271) did not change significantly compared to preterm, practically healthy infants, whereas cathelicidin increased by 40.8% (p<0.001), fecal calprotectin increased by 53.3% (p<0.001), and lactoferrin increased 2.3-fold (p<0.001).

The results showed that in infants of the Group III, the blood concentrations of calprotectin increased 4.1-fold, p<0.001 (Me=550.0; Q1=436.5; Q3=716.0), β1-defensin increased 3.2-fold, p<0.001 (Me=218.5; Q1=138.3; Q3=226.5), cathelicidin increased 2.0-fold,

Table 1

Clinical characteristics of preterm infants with perinatal encephalopathy

Indicators			Group II	Group III	P_H
Severity degree	Mild	N	9	0	0.001
		%	31.0%	0.0%	
	Moderate	N	13	5	
		%	44.8%	25.0%	
	Severe	N	7	15	
		%	24.1%	75.0%	
Sex	Male	N	16	9	0.799
		%	55.2%	45.0%	
	Female	N	13	11	
		%	44.8%	55.0%	
Weight gradient	Normal	N	2	1	0.347
		%	6.9%	5.0%	
	Low	N	16	5	
		%	55.2%	25.0%	
	Very low	N	8	7	
		%	27.6%	35.0%	
	Extremely low	N	3	7	
		%	10.3%	35.0%	
Transient tachypnea	–	N	24	20	0.227
		%	82.8%	100.0%	
	+	N	5	0	
		%	17.2%	0.0%	
Pneumothorax	–	N	28	16	0.153
		%	96.6%	80.0%	
	+	N	1	4	
		%	3.4%	20.0%	
Intraventricular hemorrhage in the brain	–	N	14	14	0.001
		%	48.3%	70.0%	
	+	N	15	6	
		%	51.7%	30.0%	
Hemorrhage degree	I degree	N	11	3	0.001
		%	73.3%	50.0%	
	II degree	N	4	1	
		%	26.7%	16.7%	
	III degree	N	0	2	
		%	0.0%	33.3%	
Respiratory failure + respiratory distress syndrome	–	N	18	8	0.234
		%	62.1%	40.0%	
	+	N	11	12	
		%	37.9%	60.0%	
Sepsis	–	N	29	15	0.007
		%	100.0%	75.0%	
	+	N	0	5	
		%	0.0%	25.0%	
Congenital pneumonia	–	N	29	8	<0.001
		%	100.0%	40.0%	
	+	N	0	12	
		%	0.0%	60.0%	
Infection unspecified but specific to the perinatal period	–	N	29	11	<0.001
		%	100.0%	55.0%	
	+	N	0	9	
		%	0.0%	45.0%	

Table 2

Levels of antimicrobial peptides in preterm infants with perinatal encephalopathy complicated by pneumonia

Indicators	Groups of newborns	N	M	m	95% confidence ınterval		Min	Max	P_H
					lower limit	upper limit			
Gestation, weeks	II	29	32.8	0.4	32.0	33.6	27	36	1.000
	III	20	30.3	0.7	28.9	31.6	25	36	
	I	15	35.3	0.4	34.4	36.1	31	36	
	Control	16	38.1	0.2	37.7	38.5	37	40	
Lactoferrin, ng/ml	II	29	2.44	0.11	2.22	2.67	1.65	3.58	<0.001
	III	20	4.71	0.31	4.06	5.36	2.18	6.98	
	I	15	1.07	0.09	0.87	1.27	0.45	1.78	
	Control	16	0.74	0.04	0.66	0.83	0.37	0.94	
Cathelicidin, ng/ml	II	29	4.38	0.19	3.99	4.78	2.89	6.94	<0.001
	III	20	6.57	0.32	5.90	7.24	3.67	9.18	
	I	15	3.11	0.20	2.69	3.54	1.95	4.38	
	Control	16	2.09	0.11	1.85	2.32	1.54	2.76	
β1 defensin, ng/ml	II	29	79.0	4.0	70.8	87.2	53.8	129.8	<0.001
	III	20	215.1	20.2	172.8	257.4	105.6	455.4	
	I	15	66.5	4.3	57.4	75.6	38.5	96.4	
	Control	16	40.5	2.9	34.2	46.8	26.8	65.4	
Serum calprotectin, ng/ml	II	29	155.0	10.4	133.6	176.4	102	334	<0.001
	III	20	576.1	33.1	506.8	645.3	294	798	
	I	15	130.7	7.2	115.2	146.2	80.6	165.4	
	Control	16	80.5	3.4	73.2	87.8	60.5	105.7	
Fecal calprotectin, μg/g	II	29	224.9	9.6	205.3	244.5	138	310	<0.001
	III	20	540.5	37.8	461.3	619.7	295	883	
	I	15	146.7	5.0	136.1	157.4	117.6	180.5	
	Control	16	101.6	4.9	91.2	112.0	79	148	

p<0.001 (Me=6.39; Q1=5.53; Q3=7.50), lactoferrin increased 4.0-fold, p<0.001 (Me=4.75; Q1=3.51; Q3=5.81), and fecal calprotectin increased 3.9-fold, p<0.001 (Me=564.0; Q1=401.0; Q3=633.0) compared to the Group I, all showing statistically significant elevations. These results are significantly higher compared to the Group II. The increase in the Group III was as follows: for calprotectin - 3.9-fold, p<0.001 (Me=140.0; Q1=116.0; Q3=165.0); for β1-defensin – 3.1-fold, p<0.001 (Me=70.3; Q1=63.8; Q3=88.1); for cathelicidin – 52.9%, p<0.001 (Me=4.18; Q1=3.78; Q3=4.68); for lactoferrin – 2.1-fold, p<0.001 (Me=2.27; Q1=1.87; Q3=2.97); and for fecal calprotectin – 2.6-fold, p<0.001 (Me=218.0; Q1=185.0; Q3=265.0).

Analysis of correlation dependencies revealed that, in the Group II, clinical severity positively correlated with lactoferrin (ρ=0.534, p<0.001), cathelicidin (ρ=0.594, p<0.001), defensin (ρ=0.575, p<0.001), serum calprotectin (ρ=0.548, p<0.001), and fecal calprotectin (ρ=0.576, p<0.001). Additionally, lactoferrin showed a negative correlation with gestational age (ρ=-0.318, p=0.002) and positive correlations with weight gradient (ρ=0.463, p<0.001), the degree of intraventricular hemorrhage (ρ=0.530, p<0.001), respiratory insufficiency (ρ=0.253, p=0.013), cathelicidin (ρ=0.779, p<0.001), calprotectin (ρ=0.784, p<0.001), defensin (ρ=0.787, p<0.001), and fecal calprotectin (ρ=0.744, p<0.001). Cathelicidin showed correlations with gestational age (ρ=-0.350, p<0.001), weight gradient (ρ=0.292, p=0.004), degree of intraventricular hemorrhage (ρ=0.494, p=0.001), calprotectin (ρ=0.784, p<0.001), defensin (ρ=0.787, p<0.001), and fecal calprotectin (ρ=0.774, p<0.001). Similarly, calprotectin exhibited a negative correlation with gestational age (ρ=-0.485, p<0.001) and positive correla-

tions with weight gradient (ρ=0.370, p<0.001), degree of intraventricular hemorrhage (ρ=0.472, p=0.001), respiratory insufficiency (ρ=0.205, p=0.043), defensin (ρ=0.921, p<0.001), and fecal calprotectin (ρ=0.846, p<0.001). A similar pattern was observed for defensin in relation to other AMPs. Defensin showed a negative correlation with gestational age (ρ=-0.370, p<0.001) and positive correlations with weight gradient (ρ=0.261, p=0.010), degree of intraventricular hemorrhage (ρ=0.455, p=0.002), respiratory insufficiency (ρ=0.263, p=0.009), and fecal calprotectin (ρ=0.859, p<0.001). Besides, fecal calprotectin exhibited a negative correlation with gestational age (ρ=-0.305, p=0.002) and positive correlations with weight gradient (ρ=0.259, p=0.010), degree of intraventricular hemorrhage (ρ=0.472, p=0.001), and respiratory insufficiency (ρ=0.230, p=0.023).

Thus, according to the obtained results, the concentrations of AMPs in the blood of preterm infants, particularly defensin, cathelicidin, and calprotectin, were reduced compared to full-term infants. This indicates that the immune system in preterm infants is not fully developed, and such children have a higher risk of infection. It is known that in neonates, AMPs at primary sites of contact with pathogens, particularly in the lungs, intestines, and skin, form an initial chain of immune defense. The reduced levels of AMPs in the lungs of preterm infants may lead to ineffective defense against pathogenic organisms and contribute to the development of pneumonia [4,13].

It is known that AMPs circulate continuously in the blood, are easily transported into plasma, and provide the organism's non-specific immune defense against potential pathogens. Compared to other components of the immune system (e.g., immunoglobulins), the advantage of AMPs is that they can be synthesized very rapidly with relatively low energy expenditure. In addition, their short amino acid chains allow them to reach the target microorganism quickly and diffuse effectively through the cell membrane. The primary antimicrobial mechanism of AMPs is damage to the microbial cell membrane. AMPs enter the bacterial cell and inhibit DNA replication, protein synthesis, enzyme activity, cell division, and metabolic processes [2,13].

Beta-defensins and cathelicidin (LL-37/hCAP-18) are effector molecules of the innate respiratory immune system. Defensins are cationic AMPs containing 18–45 amino acid residues and are expressed in neutrophils and other immune cells. In humans, there are four types of β-defensins, designated β-defensin 1 to 4 (HBDs). These defensins are secreted by keratinocytes and epithelial cells of the respiratory, gastrointestinal, and genitourinary systems in response to infectious agents. The synthesis of HBD-2 is primarily stimulated by pro-inflammatory cytokines and by lipopolysaccharides from bacteria and fungi [13].

Cathelicidins are amphipathic, positively charged peptides synthesized in many human tissues, including the epithelial cells of the skin, lungs, and gastrointestinal tract. Their synthesis is activated by infectious agents and by vitamin D [13,22].

The expression and secretion of certain AMPs, including defensins and cathelicidin, occur via Toll-like receptors (TLRs). Literature data indicate that in preterm infants, AMP levels in the blood, within immune cells, epithelial cells, in biological fluids, and stool are lower compared to full-term infants. This relative deficiency of AMPs increases the risk of invasive bacterial infections in preterm infants [4,13]. Cathelicidin (LL-37), interacting with lipopolysaccharides (LPS), reduces inflammatory load by decreasing TNF-α synthesis, and its rapid increase in serum levels during neonatal infection highlights its role both as a defense factor and as a biomarker [12].

Calprotectin (S100A8/A9) exerts a bacteriostatic effect by binding critical metal ions such as Zn and Mn, and participates in directing alveolar macrophages, thereby enhancing the initial immune defense against pulmonary infections. In preterm infants, elevated fecal calprotectin levels during the first week of life may be related to the establishment of bacterial microflora and may also reflect increased transepithelial migration of neutrophil granules and/or macrophages into the intestinal lumen. This is attributed to increased intestinal permeability associated with the immaturity of the intestinal mucosa [2].

The higher level of lactoferrin in preterm infants compared to full-term infants may be associated with the activation of the immune response against infection after birth or with postnatal activation of the immune system. This may also be attributed to the higher levels of perinatal and early postnatal stress. Preterm in-

fants are more likely to be exposed in utero and during delivery to stress factors, infectious risks, and hypoxia. These factors can lead to the activation of pro-inflammatory cytokines and the innate immune system, resulting in elevated levels of neutrophil-derived proteins such as lactoferrin. In addition, subclinical infection or intrauterine inflammation (e.g., chorioamnionitis, funisitis) in preterm infants may also contribute to increased lactoferrin levels.

After birth, synthesis of lactoferrin may increase in hepatocytes or neutrophils. Since the adaptive immune system is underdeveloped in preterm infants, components of the innate defense system (e.g., lactoferrin) may undergo compensatory activation as an initial defense response [5,13,18]. Studies have shown that lactoferrin reduces the inflammatory response in neonatal macrophages. This demonstrates not only its immunostimulatory function but also its immunomodulatory role, and the increase in its levels can be interpreted as a reactive adaptation [5]. Due to its high iron-binding capacity, lactoferrin limits the growth of bacteria such as *E. coli* and *S. pneumoniae*, neutralizes lipopolysaccharides (LPS) and viral components, thereby both attenuating inflammation and, by reducing biofilm formation by microbial polysaccharides, playing an important role in protecting the neonatal respiratory tract [23].

In preterm infants with perinatal encephalopathy not complicated by pneumonia, AMP levels did not differ significantly from those in practically healthy infants, whereas in preterm infants with perinatal encephalopathy complicated by pneumonia, AMP levels were significantly higher. The increase in AMPs in infants born with prenatal infection reflects the body's initial defense against infectious agents. However, elevated AMPs may also contribute to the development of acute inflammation. In neonates, immunosuppression in the context of encephalopathy facilitates the penetration of microorganisms into the body.

It should be noted that literature data also confirm a significant increase in AMPs (particularly defensins and LL-37) in the bronchoalveolar compartment during acute infections, including pneumonia, in preterm infants [26]. Maternal infection transmitted to the fetus via the placenta activates local or systemic immune responses, during which disruption of the blood-brain barrier allows microorganisms and pro-inflammatory mediators to penetrate brain tissue, leading to encephalitis, and subsequently causing damage to endothelial and alveolar cells in the lung tissue.

In infants born with encephalopathy (e.g., hypoxic-ischemic encephalopathy), the risk of early-onset pneumonia and sepsis is 20–40 times higher [13, 26]. In humans, LL-37, β-defensins, and other AMPs in the epidermis and mucosal linings (skin, lungs, intestine) rapidly increase following contamination or trauma, exerting antimicrobial effects [19]. In some cases, amniotic infections may lead to preterm birth and, in turn, to an increase in AMP levels in these infants. Therefore, this evidence should be taken into account when investigating the effect of gestational age on AMP levels. Since airborne infections enter the human body via the respiratory tract, the epithelial cells of lung tissue are enriched with AMPs, particularly defensins and LL-37. Therefore, during pneumonia, the secretion of AMPs in the immune cells of the mucous membranes (e.g., alveolar macrophages) and epithelial cells increases significantly. Studies have shown that in the lungs of newborns, human β-defensin-2 (HBD-2) predominates. Defensins promote the differentiation of monocytes in umbilical cord blood into mature dendritic cells and prevent their apoptosis [4, 13].

The analysis showed that, in preterm infants with pneumonia developing against the background of perinatal encephalopathy, the levels of AMPs (lactoferrin, cathelicidin, β1-defensin, and serum and fecal calprotectin) were positively correlated with the clinical severity of the disease, the degree of intraventricular hemorrhage, respiratory failure, and weight gradient, and negatively correlated with gestational age. This suggests that in cases with lower gestational age and more severe clinical course, AMPs are sharply upregulated, which may represent part of the organism's compensatory defense response against infection. The strong positive correlations observed among AMPs suggest that they act synergistically to enhance the innate immune response during the early neonatal period. The results indicate that AMPs could serve as additional biomarkers for predicting the risk of neonatal infections and the severity of the disease.

Thus, perinatal encephalopathy is not limited to neurological dysfunction but also increases susceptibility to infections in the postnatal pe-

riod. Since preterm infants do not possess fully mature immune cells after birth, AMPs are activated as a compensatory mechanism, forming the first line of defense [2]. The increase in AMPs represents a key pathogenic link in the development of neonatal infections.

Conclusion

1. In preterm infants with perinatal encephalopathy complicated by pneumonia, the serum and fecal levels of β1-defensin, lactoferrin, calprotectin, fecal calprotectin, and cathelicidin were significantly higher compared with preterm infants without infectious complications ($p<0.001$).

2. An increase in AMP concentration showed a negative correlation with gestational age, and a positive correlation with disease severity, the degree of intraventricular hemorrhage in the brain, and indicators of respiratory failure.

3. A positive correlation was observed among lactoferrin, cathelicidin, calprotectin, and β1-defensin, indicating a coordinated activation of innate immune defense mechanisms.

4. The elevation of AMP levels, against the background of perinatal encephalopathy, may play a compensatory role in the pathogenesis of postnatal pneumonia, constituting an important component of the first line of defense against infection.

The authors declare no conflict of interest.

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