ABSTRACT

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INTERRELATIONSHIP BETWEEN INDICATORS OF LOCAL AND SYSTEMIC INFLAMMATORY REACTION IN NEWBORNS WITH NEONATAL ENCEPHALOPATHY

Introduction. Neonatal encephalopathy is one of the most common diseases in newborns. Several factors influence the development of neonatal encephalopathy including adverse obstetric history, fetal distress, meconium-containing amniotic fluid and cesarean section. In moderate and severe neonatal encephalopathy, damage to internal organs, local and systemic inflammation may occur. Cytokines, which are activated in the central nervous system and released in response to its damage, play an important role in brain inflammation caused by neonatal encephalopathy. C-reactive protein is also a possible biomarker of neonatal encephalopathy severity, being considered a protein of the innate immune system with anti-inflammatory properties. The state of the inflammatory response can be influenced by a local inflammatory reaction, as a result of which children with neonatal encephalopathy have been shown to have increased levels of fecal calprotectin in the first weeks of life. Also, one of the results of the transferred inflammatory reaction is a change in the composition of the neonatal intestinal microbiome.

Objective. To study risk factors for neonatal encephalopathy in newborns, the features of the relationship between local and systemic inflammatory response parameters in asphyxia of varying degrees, and the features of severity and control of inflammatory response parameters.

Materials and methods. The study was conducted in 119 full-term newborns, of which 87 children had neonatal encephalopathy and 32 healthy children. To determine the features of severity and control of inflammatory response parameters, group A was identified, which included 60 newborns, 46 of them with moderate neonatal encephalopathy, 14 with severe neonatal encephalopathy. The study was conducted using a culture method to determine the composition of the intestinal microbiome in feces. Using a semi-automated Thermo Scientific Multiskan FC enzyme immunoassay analyzer, the level of fecal

calprotectin in feces and C-reactive protein and interleukins 1β and 10 in the blood serum were estimated by the enzyme immunoassay. The results were analyzed using SPSS version 28.0. The correlation between the parameters was analyzed using the Pearson correlation coefficient. The odds ratio was used to quantitatively describe the closeness of the relationship between the features in the statistical population. Binary logistic regression was used to determine the dependencies of the severity of the inflammatory reaction and create a model for calculating its severity.

Results. Risk factors for neonatal encephalopathy included mother's acute respiratory infections and fetal distress. Bifidobacterium levels were positively correlated throughout the study, and there was also a relationship with lactobacilli in the control and non-probiotic treated neonatal encephalopathy groups. E. coli values were positively associated with opportunistic pathogens in the control and probiotic treated groups. Fecal calprotectin was negatively correlated with birth weight and height, Apgar scores, and gestational age. Fecal calprotectin levels were positively correlated with E. coli and opportunistic pathogens in healthy neonates. In children with neonatal encephalopathy interleukin 1β and 10 values were positively associated with fecal calprotectin, interleukin 1β were positively correlated with interleukin 10 and C-reactive protein.

Conclusions. The most significant risk factors for the development of neonatal encephalopathy are mother's acute infectious diseases and fetal distress. The severity of hypoxia/asphyxia at birth correlated with the levels of interleukin 1β and 10 at 2 and 5 weeks of life, and Bifidobacterium at 2 weeks of life. The severity of the inflammatory response in the study population was characterized by a change in the level of interleukin 1β and Bifidobacterium at 2 weeks and a change in the level of interleukin 10 at 5 weeks.

Keywords. Neonatal encephalopathy, microbiome, fecal calprotectin, interleukin, C-reactive protein.

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РЕЗЮМЕ

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ВЗАЄМОЗВ'ЯЗОК МІЖ ПОКАЗНИКАМИ МІСЦЕВОЇ ТА СИСТЕМНОЇ ЗАПАЛЬНОЇ РЕАКЦІЇ У НОВОНАРОДЖЕНИХ З НЕОНАТАЛЬНОЮ ЕНЦЕФАЛОПАТІЄЮ

Вступ. Неонатальна енцефалопатія є одним з найпоширеніших захворювань у новонароджених. Деякі фактори впливають на розвиток неонатальної енцефалопатії, включаючи обтяжений акушерський анамнез, фетальний дистрес, меконіальні амніотичні води та кесарів розтин. При середньому та тяжкому перебігу неонатальної енцефалопатії може спостерігатися як виникнення ураження внутрішніх органів, так і локальне та системне запалення. Важливу роль у запаленні мозку, яке викликане неонатальною енцефалопатією, відіграють цитокіни. Вони активуються у центральній нервовій системі та вивільняються у відповідь на її пошкодження. Також, С-реактивний білок є можливим біомаркером ступеня тяжкості неонатальної енцефалопатії, вважаючись білком уродженої імунної системи із протизапальними властивостями. На стан запальної відповіді може впливати локальна запальна реакція, внаслідок якої у дітей із неонатальною енцефалопатією відмічено

підвищення рівнів фекального кальпротектину у перші тижні життя. Також, одним із результатів перенесеної запальної реакції, є зміна складу неонатального кишкового мікробіому.

Мета. Вивчення факторів ризику розвитку неонатальної енцефалопатії у новонароджених, особливостей взаємозв'язку показників локальної та системної запальної реакції при асфіксії різного ступеня, особливості вираженості та контролю показників запальної реакції.

Матеріали та методи. Дослідження проведено у 119 доношених новонароджених, з яких 87 дітей мали неонатальну енцефалопатію та 32 здорових дітей. Для визначення особливостей тяжкості та контролю параметрів запальної відповіді виділено групу А, до якої увійшли 60 новонароджених, з них 46 – з помірною неонатальною енцефалопатією, 14 – з тяжкою неонатальною енцефалопатією. Дослідження проводили культуральним методом для визначення складу кишкового мікробіому у фекаліях. Методом імуноферментного аналізу визначали рівень фекального кальпротектину в калі та С-реактивного білку, інтерлейкінів 1β і 10 у сироватці крові на напівавтоматичному імуноферментному аналізаторі Thermo Scientific Multiskan FC. Результати аналізували за допомогою SPSS версії 28.0. Кореляцію між параметрами аналізували за допомогою коефіцієнта кореляції Пірсона. Відношення шансів використовувалося для кількісного опису тісноти зв'язку між ознаками в статистичній сукупності. Для визначення залежностей тяжкості запальної реакції та створення моделі для розрахунку її тяжкості використовували бінарну логістичну регресію.

Результати. Фактори ризику неонатальної енцефалопатії включали гострі респіраторні інфекції матері та дистрес плода. Рівні біфідобактерій позитивно корелювали протягом усього дослідження, а також відмічено зв'язок з лактобактеріями в контрольній групі та групах, які не отримували пробіотик. Значення E. coli були позитивно пов'язані з умовно-патогенними мікроорганізмами в контрольній групі та групах, які отримували пробіотики. Фекальний кальпротектин негативно корелював із вагою та зростом при народженні, шкалою Апгар та гестаційним віком. Рівні фекального кальпротектину позитивно корелювали з E. coli та умовно-патогенними мікроорганізмами у здорових новонароджених. У дітей з неонатальною енцефалопатією значення інтерлейкінів 1β та 10 були позитивно пов'язані з фекальним кальпротектином. Інтерлейкін 1β позитивно корелював з інтерлейкіном 10 та С-реактивним білком.

Висновки. Найбільш істотними факторами ризику розвитку неонатальної енцефалопатії є гострі інфекційні захворювання матері та дистрес плода. Тяжкість гіпоксії/асфіксії при народженні корелювала зі значеннями інтерлейкіну 1β та 10 на 2 та 5 тижні життя та Bifidobacterium у віці 2 тижнів. Тяжкість запальної відповіді в досліджуваній популяції характеризувалася зміною величин інтерлейкіну 1β та Bifidobacterium на 2 тижні та зміною концентрації інтерлейкіну 10 на 5 тижні.

Ключові слова. Неонатальна енцефалопатія, мікробіом, фекальний кальпротектин, інтерлейкін, С-реактивний білок.

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INTRODUCTION / ВСТУП

Neonatal encephalopathy (NE) is one of the most common pathologies in newborns [1, 2]. The incidence is especially high in low-income countries [3]. NE is characterized by dysfunction of the nervous system, including changes in muscle tone, reflexes, and level of consciousness of the newborn [4]. In moderate and especially severe cases of NE, damage and dysfunction of internal organs can be observed, caused by both hypoxia-ischemia and inflammatory reactions [2, 5]. A number of factors can influence the development of NE, including a burdened obstetric history, fetal distress, meconium-containing amniotic fluid, and cesarean section [6, 7]. Inflammation is one of the important conditions leading to damage to the central nervous system [8]. The consequences of such a reaction can trigger tertiary mechanisms of brain damage, including a decrease in the number of neurons and epigenetic changes, with their possible persistence for a long period of time [9].

Interleukins are markers of the systemic inflammatory response. They are multifunctional immune mediators that regulate cellular immunity and the inflammatory response. They are activated in glial cells and astrocytes of the central nervous system and are released in response to brain injury. Thus, cytokines play an important role in brain inflammation caused by NE [10, 11] and are potential biomarkers of the severity and outcome of NE [12]. C-reactive protein (CRP) is an acute phase protein and a sensitive marker of inflammatory reactions [13]. It is considered a protein of the innate immune system, has anti-inflammatory properties, which may be related to its ability to increase the expression of IL-1 receptor antagonist [14], therefore CRP is a possible biomarker of the severity of NE [15].

The state of the inflammatory response can be influenced by a local inflammatory reaction, in particular at the level of the gastrointestinal tract. It is characterized by an increase in intestinal markers of inflammation, one of which is fecal calprotectin (FC) [16]. This is an acute phase protein that increases in the presence of an inflammatory reaction, is secreted by epithelial cells and shows the movement of neutrophils into the intestinal lumen [16, 17]. The study of FC indicators is usually performed to diagnose various intestinal diseases, including inflammatory, allergic and organic lesions [18]. Some studies have noted an increase in the diversity of intestinal microflora, which may be associated with the inflammatory reaction in children with NE [19]. Changes in intestinal microbiome indicators were identified, including the number of bifidobacteria [20], lactobacilli [20], E. coli [21] and opportunistic pathogens [21]. The reasons may be different, including those associated with increased contamination with microorganisms during the specific care and treatment of newborns [22, 23].

The aim of our work was to study the risk factors for the development of neonatal encephalopathy in newborns, the features of the relationship between the indicators of local and systemic inflammatory response in asphyxia of varying degrees, the features of the severity and control of the indicators of the inflammatory response.

Materials and methods

The study was conducted on 119 full-term newborns, of which 87 had neonatal encephalopathy and 32 healthy children were included in the control group. The gestational age of the patients was 36 weeks or more, weight more than 2500 g. To determine the features of the severity and control of inflammatory response indicators, group A was identified, which included 60 newborns, 46 of them with moderate NE, 14 with severe NE. The presence and severity of neonatal encephalopathy were determined using the modified Sarnat scale [24]. To assess the level of probiotic impact on inflammatory response indicators in the main group, subgroup B was identified, which included 27 children.

At 2, 3 and 5 weeks, the composition of the microbiome and the value of fecal calprotectin were determined, at 2 and 5 weeks, the levels of C-reactive protein, IL-1β and IL-10 were detected. The study was conducted using the culture method to determine the composition of the intestinal microbiome in feces. The values of microorganisms were defined as log10 colony-forming units/gram of feces. An enzyme immunoassay was also used to assess the level of fecal calprotectin (mg/ml) in feces and C-reactive protein and interleukins 1β and 10 (pg/ml) in the blood serum. Laboratory diagnostics of the above-mentioned indicators were carried out using a semi-automated enzyme immunoassay analyzer Thermo Scientific Multiskan FC. The analysis of the results was carried out using SPSS version 28.0 (IBM, New York, USA). The correlation between the parameters was analyzed using the Pearson correlation coefficient. The odds ratio (OR) was used to quantitatively describe the tightness of the relationship between features in the statistical population. Statistically significant were values of p < 0.05. Binary logistic regression was used to determine the dependencies of the

severity of the inflammatory reaction and to create a model for calculating its severity.

The design of research was endorsed by the Commission on Bioethics Meeting of the Educational and Scientific Medical Institute of Sumy State University and were performed in accordance with the ethical standards set out in the Declaration of Helsinki.

Results

The odds ratio indicator assessment showed possible risk factors for the development of NE (Table 1). Acute respiratory infections increased the likelihood of developing neonatal encephalopathy by 12 times (OR 0.07; p=0.002). Fetal distress was an 8-fold risk factor for the development of NE (OR 0.11; p=0.02). Meconium amniotic fluid increased the likelihood of NE by 5 times, but these data were not significant (OR 0.17; p=0.05). Some conditions increased the odds of developing NE by three times, including COVID-19 (OR 0.35), placental petrification (OR 0.35), gestational hypertension (OR 0.35), umbilical cord entanglement (OR 0.42), by two

times placental infarctions (OR 0.51), and anemia (OR 0.43), but the results were not significant (p>0.05). The conducted study of correlation dependencies revealed a positive association between the number of bifidobacteria at 2 week and lactobacilli at 2 week in healthy newborns (0.47, p=0.007) and newborns with NE, but a strong association was found only in the latter (Table 2). A positive correlation was found between the number of bifidobacteria at 2 week and opportunistic pathogens at 2 week in children with NE taking the probiotic (0.443, p=0.039) and healthy children (0.647, p<0.001). A weak positive correlation was observed between the number of bifidobacteria at 2 week and Apgar 1' and Apgar 5' scores in children with NE. At the age of 5 weeks, the levels of bifidobacteria weakly positively correlated with lactobacilli at 5 week in the control group (0.575, p<0.001) and in children with NE (Table 3). Also, at week 5, a positive correlation was noted with E.coli at week 5 in healthy children (0.64, p<0.001).

Table 1. Odds ratio of risk factors for neonatal encephalopathy

Pathology	OR	SE	CI	CI	p
Acute respiratory infections during pregnancy	0,071429	1,050321	0,009116	0,559659	0,002
Fetal distress	0,114583	1,055469	0,014477	0,906891	0,02
Meconium amniotic fluid	0,175	1,064208	0,021735	1,408999	0,05
COVID-19 during pregnancy	0,347619	1,09082	0,040981	2,948687	>0.05
Placental petrification	0,347619	1,09082	0,040981	2,948687	>0.05
Gestational hypertension	0,347619	1,09082	0,040981	2,948687	>0.05
Umbilical cord entanglement	0,415584	0,801404	0,086395	1,999071	>0.05
Placental infarction	0,513333	1,116387	0,057559	4,578123	>0.05
Anemia during pregnancy	0,429825	0,603909	0,131593	1,403942	>0.05
OR – odds ratio, SE – standard error, CI – confidence interval, p – p-value					

Lactobacillus values at 5 week were positively correlated with E.coli values at 5 week in children with NE and the control group (0.715, p < 0.001), although a strong association was found in the latter. E.coli values at 2 week were positively correlated with opportunistic pathogens levels at 2 week in healthy children (0.512, p=0.003). A strong positive correlation was found with opportunistic pathogens levels at 2 week in children with NE taking a probiotic (0.733, p=0.001).

Fecal calprotectin values were positively correlated between values at weeks 2 and 5 in children with NE and healthy controls, but only in the latter was a strong association found (0.83, p < 0.001). FC values at 2 weeks of life were weakly negatively correlated with birth weight and height in the control group, with Apgar 1' (-0.453, p=0.018) and Apgar 5' (-0.474, p=0.013) in

children with NE taking the probiotic. In children in the control group, a positive relationship was found between FC levels and E. coli (0.698, p<0.001) and opportunistic pathogens (0.689, p<0.001) values throughout the study.

IL-1β values were positively correlated between values at 2 and 5 weeks in children with NE (0.649, p<0.001). Also, IL-1β at 2 weeks had a positive relationship with FC, IL-10 and CRP at 2 weeks in children with NE. CRP values at 2 week had a weak negative correlation with Apgar 1' and a weak positive correlation with FC at 2 week in children with NE. IL-1β values at 5 week strongly positively correlated with Apgar 1' and Apgar 5' in children with NE. Also, IL-1β at 5 week weakly positively correlated with IL-10 at 5 week in children with NE (Tables 2, 3).

Table 2. Correlation analysis of severity of hypoxia and indicators of local and systemic inflammatory response in newborn with NE at 2 week

Indexes		A1'	A5'	FC	BIF	LAC	EC	ECW	OP	IL-1β	IL-10	CRP
A1'	PC	1	0,94	-0,102	0,458	0,498	0,05	-0,265	-0,053	-0,277	0,089	-0,375
	p		<,001	0,437	<,001	<,001	0,752	0,491	0,709	0,051	0,538	0,045
A5'	PC	0,94	1	-0,154	0,494	0,493	0,086	-0,262	-0,054	-0,224	0,085	-0,298
	p	<,001		0,24	<,001	<,001	0,585	0,496	0,702	0,118	0,559	0,116
FC	PC	-0,102	-0,154	1	0,024	0,221	0,265	0,814	0,135	0,375	0,237	0,491
	p	0,437	0,24		0,857	0,09	0,086	0,008	0,34	0,007	0,097	0,007
BIF	PC	0,458	0,494	0,024	1	0,717	0,375	-0,615	0,235	0,08	0,234	-0,024
	p	<,001	<,001	0,857		<,001	0,013	0,104	0,094	0,584	0,105	0,902
LAC	PC	0,498	0,493	0,221	0,717	1	0,667	0,094	0,152	0,227	0,18	-0,17
	p	<,001	<,001	0,09	<,001		<,001	0,809	0,281	0,112	0,21	0,378
EC	PC	0,05	0,086	0,265	0,375	0,667	1	0,35	0,248	0,523	0,197	0,151
	p	0,752	0,585	0,086	0,013	<,001		0,496	0,133	0,001	0,25	0,512
ECW	PC	-0,265	-0,262	0,814	-0,615	0,094	0,35	1	0,322	0,809	0,697	0,664
	p	0,491	0,496	0,008	0,104	0,809	0,496		0,437	0,008	0,037	0,15
OP	PC	-0,053	-0,054	0,135	0,235	0,152	0,248	0,322	1	0,472	0,399	0,061
	p	0,709	0,702	0,34	0,094	0,281	0,133	0,437		0,002	0,009	0,771
IL-1β	PC	-0,277	-0,224	0,375	0,08	0,227	0,523	0,809	0,472	1	0,342	0,462
	p	0,051	0,118	0,007	0,584	0,112	0,001	0,008	0,002		0,015	0,012
IL-10	PC	0,089	0,085	0,237	0,234	0,18	0,197	0,697	0,399	0,342	1	0,195
	p	0,538	0,559	0,097	0,105	0,21	0,25	0,037	0,009	0,015		0,312
CRP	PC	-0,375	-0,298	0,491	-0,024	-0,17	0,151	0,664	0,061	0,462	0,195	1
	p	0,045	0,116	0,007	0,902	0,378	0,512	0,15	0,771	0,012	0,312	

PC – Pearson correlation, p – p-value, A1' – Apgar score at 1 min, A5' – Apgar score at 5 min, BIF – Bifidobacterium, LAC – lactobacterium, EC – E.Coli, ECW – E.Coli with weakness ability, OP – Opportunistic pathogenes

To determine the features of the severity and control of the inflammatory response indicators in neonates with NE, classification and binary logistic regression analysis were performed. In children with NE of varying severity, the influence of the values characterizing the local and systemic inflammatory response was assessed. The indicators of the local and systemic inflammatory response at the age of 2 and 5 weeks were classified. The classification of parameters at the 2nd week of life showed that the values of the level of IL-1β, bifidobacteria, lactobacilli and fecal calprotectin have the greatest significance for the severity of the inflammatory response. The values of

IL-1β and bifidobacteria had a reliable effect on the severity of the inflammatory response, explaining 84% of cases. Thus, at the 2nd week of life, the severity of the inflammatory response largely depends on these two indicators. At the age of 5 weeks, the parameters that were classified by their impact on the level of inflammatory response included the levels of C-reactive protein, bifidobacteria, and IL-10. According to the calculations, IL-10 had a reliable impact on the severity of the inflammatory response, amounting to 63%. Models were obtained for calculating the severity of the inflammatory response in children with NE at the age of 2 and 5 weeks of life.

Table 3. Correlation analysis of severity of hypoxia and indicators of local and systemic inflammatory response in newborn with NE at 5 week

Indexes		A1'	A5'	FC	BIF	LAC	EC	ECW	OP	IL-1β	IL-10	CRP
A1'	PC	1	,940	,151	,282	-,147	-,015	,312	-,561	-,652	-,472	-,466
	p		<,001	,315	,060	,329	,935	,609	,002	<,001	,002	,093
A5'	PC	,940	1	,141	,347	-,168	-,053	-,039	-,495	-,613	-,482	-,439
	p	<,001		,350	,020	,266	,778	,950	,009	<,001	,002	,116
FC	PC	,151	,141	1	,040	,180	-,083	,449	,095	,068	,082	-,233
	p	,315	,350		,794	,230	,663	,448	,638	,721	,668	,422
BIF	PC	,282	,347	,040	1	,486	,219	,140	-,298	-,175	-,096	-,251
	p	,060	,020	,794		<,001	,245	,822	,131	,365	,619	,387
LAC	PC	-,147	-,168	,180	,486	1	,502	,774	,086	,118	,097	-,058
	p	,329	,266	,230	<,001		,005	,125	,670	,533	,609	,843
EC	PC	-,015	-,053	-,083	,219	,502	1	,629	,310	,300	-,001	-,569
	p	,935	,778	,663	,245	,005		,371	,227	,212	,996	,110
ECW	PC	,312	-,039	,449	,140	,774	,629	1	,866	,385	,000	.
	p	,609	,950	,448	,822	,125	,371		,333	,748	1,000	.
OP	PC	-,561	-,495	,095	-,298	,086	,310	,866	1	,662	,278	,280
	p	,002	,009	,638	,131	,670	,227	,333		,010	,335	,591
IL-1β	PC	-,652	-,613	,068	-,175	,118	,300	,385	,662	1	,465	,238
	p	<,001	<,001	,721	,365	,533	,212	,748	,010		,003	,608
IL-10	PC	-,472	-,482	,082	-,096	,097	-,001	,000	,278	,465	1	-,256
	p	,002	,002	,668	,619	,609	,996	1,000	,335	,003		,579
CRP	PC	-,466	-,439	-,233	-,251	-,058	-,569	.	,280	,238	-,256	1
	p	,093	,116	,422	,387	,843	,110	.	,591	,608	,579	

PC – Pearson correlation, p – p-value, A1' – Apgar score at 1 min, A5' – Apgar score at 5 min, BIF – Bifidobacterium, LAC – lactobacterium, EC – E.Coli, ECW – E.Coli with weakness ability, OP – Opportunistic pathogenes

The specified values of binary logistic regression were used to evaluate the prescribed therapy in children with NE. Given the combination of the severity of the inflammatory response and bifidobacteria deficiency, an assessment of the impact of the prescribed drugs containing bifidobacteria was carried out. An assessment of the level of inflammatory response at the age of 5 weeks in children who received and did not receive the probiotic showed that such an impact is present, but its severity was about 10%. This is explained, among other things, by the significance of the indicators at the 5th week of life, in particular the IL-10 value.

Discussion

Our study confirms that the development of neonatal encephalopathy in newborns can be influenced by a number of antenatal and prenatal factors [6, 7]. In our work, it was found that acute respiratory infections during pregnancy and fetal distress are the greatest risk factors and increase the likelihood of developing neonatal encephalopathy. Gestational hypertension [7], anemia [6], meconium amniotic fluid [6, 7], and placental pathologies [6, 7] also affect the presence and severity of NE, which is also reflected in our work, but these results were not reliable. The above aspects may be involved in the development of asphyxia during childbirth, which subsequently leads to the development of neonatal encephalopathy [6].

Our results revealed a positive correlation between the levels of bifidobacteria, lactobacilli, E. coli, opportunistic pathogens, fecal calprotectin and IL-1β throughout the study, as indicated by some researchers [16, 19, 20, 25]. Analysis of the results shows that the Apgar scores, which reflect the severity of hypoxia-

ischemia at birth, correlated with the number of bifidobacteria in children with NE at 2 weeks of age, which may reflect the level of the inflammatory process that has arisen [26]. In all groups, the levels of bifidobacteria positively correlated with the levels of lactobacilli, being common and basic types of probiotic organisms [22], although other studies have described the absence of a relationship between them [27]. E. coli levels were positively associated with opportunistic pathogens in the neonatal period, which is associated with a local inflammatory response in children who have undergone hypoxia-ischemia [21]. Fecal calprotectin levels were negatively correlated with body weight [28] and height at birth, Apgar scores [28] and gestational age [28], which reflects the presence of local inflammation and intestinal immaturity [20], although some researchers did not find a relationship with the above-mentioned values [16]. In our work, FC levels were positively correlated with E. coli and opportunistic pathogens [20], as indicated by the presence of local inflammatory changes, but the results of other studies describe a negative relationship [29] between these values.

In our study, the values of IL-1β and IL-10 were correlated with Apgar scores, which demonstrates the severity of hypoxia/asphyxia in newborns with neonatal encephalopathy [30]. The value of IL-1β positively correlated with the level of IL-10, and some scientists believe that this relationship can contribute to the early diagnosis of the severity of the systemic inflammatory process [31]. The level of IL-1β has a positive relationship with the value of CRP, regulating the latter through the production of inflammatory mediators, taking part in systemic inflammation in newborns with brain injury [25]. Also, the values of IL-1β and IL-10 had a positive relationship with fecal calprotectin

through the expression of proinflammatory cytokine by inflammatory mediators in monocytes [32] and anti-inflammatory in myeloid cells [33], showing the connection between local and systemic inflammatory process.

The results and characteristics of the binary logistic regression models showed the dynamic features of the inflammatory response in neonates with NE. The most significant indicators describing the inflammatory response at 2 weeks of life were interleukin 1β and bifidobacteria. This can be accepted as one of the justifications for the appointment/continuation of biopreparations. A number of authors also point to the significant role of interleukin 1β in the inflammatory response in NE [10]. It is also indicated that in neonates with NE, there is a change in the level of bifidobacteria [20]. However, at 5 weeks of life, the most significant indicator describing the severity of the inflammatory response is IL-10. A number of authors also indicate that IL-10 can be a prognostic factor indicating the severity of central nervous system lesions in NE [12]. At the same time, the IL-10 value can characterize the severity of reparative processes [34]. The use of mathematical models to assess the severity of the inflammatory response when prescribing a probiotic showed that such a reaction exists, but its effect is small. The effect of probiotic therapy on the inflammatory response in children with NE was also noted, which is also reflected in other studies [29]. This can be explained by additional colonization of the intestine with bifidobacteria and a possible trophic effect on the intestinal mucosa, additionally supporting the mucous barrier [26]. Our previous studies also showed the possibility of the influence of these agents. It is necessary to continue research in this direction with a set of material, using various options of drugs.

CONCLUSIONS / ВИСНОВКИ

The most significant risk factors for the development of neonatal encephalopathy in our study are acute infectious diseases suffered by the mother and fetal distress.

The severity of hypoxia/asphyxia at birth correlated with the levels of interleukin 1β and 10 at 2 and 5

weeks of life, and Bifidobacterium at 2 weeks of life.

The severity of the inflammatory reaction in the study population was characterized by changes in the levels of interleukin 1β and Bifidobacterium at 2 weeks of life and changes in the level of interleukin 10 at 5 weeks of life.

PROSPECTS FOR FUTURE RESEARCH / ПЕРСПЕКТИВИ ПОДАЛЬШИХ ДОСЛІДЖЕНЬ

We would like to study the effect of other drugs on the local and systemic inflammatory response in newborns with neonatal encephalopathy.

AUTHOR CONTRIBUTIONS / ВКЛАД АВТОРІВ

Concept and design of research – SP, writing the first version – AP, final approval of the version for publication – SP, agree to be responsible for all aspects of the work – AP.

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The authors declare no conflict of interest.

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