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ABSTRACT

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FEATURES OF THE PROGRESSION OF THE INFLAMMATORY RESPONSE IN NEWBORNS WITH NEONATAL ENCEPHALOPATHY

Introduction. Neonatal encephalopathy (NE) is one of the most common diseases of the newborn period; worldwide the incidence of NE is more than 1 million newborns, most of which are registered in developing countries. Inflammation and hypoxia-ischemia play a vital, key role in neonatal encephalopathy. A persistent inflammatory response in neonates with NE is observed during the first week of life, which correlates with the severity of brain damage, but can persist for weeks, months and even years, due to tertiary mechanisms of damage that include inflammation and epigenetic changes, decreased plasticity and decreased number of neurons.

Materials and methods. The study was conducted in 74 full-term newborns with neonatal encephalopathy. The gestational age of the children was 36 weeks or more, weight of more than 2500g. The presence and severity of neonatal encephalopathy were determined using the modified Sarnat scale. At 2 weeks of life, the examination was carried out in 74 children, and at 5 weeks of life – in 59 children, so the case monitoring was possible only for 59 newborns. The newborns were divided into subgroups 1 and 2. Subgroup 1 included children who were classified as having moderate neonatal encephalopathy. At 2 weeks of life, 55 such patients were examined, at 5 weeks – 43. Subgroup 2 included children with severe neonatal encephalopathy – 19 and 16 newborns at 2 and 5 weeks, respectively. In turn, each of the subgroups was divided into subgroups A and B, and into subgroup B newborns who received the probiotic. The biological product included bifidum bacteria; it was administered orally before the first blood draw for analysis. The levels of IL-1β and IL-10, C-reactive protein (CRP) were determined using the ELISA and the semiquantitative method.

Results. The data obtained showed an increase in the level of both the pro-inflammatory interleukin IL-1β and the anti-inflammatory interleukin IL-10 at 2 weeks of life. This was observed both in children with moderate NE and in children with severe NE. However, in the latter, the level of increase in the studied cytokines was higher. At the 5th week of life, there was a significant decrease in IL-1β and IL-10, noted in all study groups. At the same time, high values of IL-1β and IL-10 remained in children with severe neonatal encephalopathy. The results of CRP showed a higher value in children with severe NE. Over time, a decrease in CRP was noted, but it was not significant for newborns with severe NE. There were no significant differences in mean IL-1β, IL-10, and CRP values between the non-probiotic and probiotic-treated groups, although there was a trend toward lower IL-1β, IL-10, and CRP values. However, there was a higher incidence of IL-1β values within the normal range by 5 weeks of life in infants with moderate NE who received the probiotic. Also, the frequency of IL-10 values within the normal range was higher in children with severe NE who received the probiotic.

Conclusions. The levels of IL-1β, IL-10 and CRP were increased in children with neonatal encephalopathy, more significant for severe encephalopathy at both 2 and 5 weeks of life, while a decrease in IL-1β, IL-10 and CRP was determined from 2 by 5 weeks of life. Administration of the probiotic resulted in a higher incidence of IL-1β values within the normal range in the group of children with moderate neonatal encephalopathy and IL-10 in children with severe neonatal encephalopathy.

Keywords: newborns, neonatal encephalopathy, cytokines, interleukins, C-reactive protein.

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ОСОБЛИВОСТІ ДИНАМІКИ ЗАПАЛЬНОЇ ВІДПОВІДІ У НОВОНАРОДЖЕНИХ З НЕОНАТАЛЬНОЮ ЕНЦЕФАЛОПАТІЄЮ

Вступ. Неонатальна енцефалопатія (НЕ) одне з найчастіших захворювань періоду новонародженості, у всьому світі захворюваність на НЕ становить понад 1 млн новонароджених, у більшості воно реєструється в країнах, що розвиваються. Запалення та гіпоксія-ішемія відіграють найважливішу, ключову роль при неонатальній енцефалопатії. Стійка запальна реакція у новонароджених з НЕ відзначається протягом першого тижня життя, який корелюють з тяжкістю ураження головного мозку, але може зберігатися тижнями, місяцями і навіть роками, що пояснюється третинними механізмами ушкодження, що включає запалення та епігенетичні зміни, зниження пластичності та зменшення кількості нейронів.

Матеріали та методи. Дослідження було проведено у 74 доношених новонароджених дитини з неонатальною енцефалопатією. Гестаційний вік дітей був 36 тижнів і більше,

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маса понад 2500 г. Наявність та ступінь тяжкості неонатальної енцефалопатії визначалася за модифікованою шкалою Сарнату. На 2 тижні життя обстеження було проведено у 74 дітей, на 5 тижні життя у 59 дітей, таким чином динамічне спостереження було можливим лише для 59 новонароджених. Новонароджені були поділені на підгрупи 1 та 2. У підгрупу 1 входили діти, які були класифіковані як діти з неонатальною енцефалопатією середньої тяжкості. На 2 тижні життя було обстежено 55 таких пацієнтів, на 5 тижні – 43. У підгрупу 2 ввійшли діти з неонатальною енцефалопатією тяжкого ступеня – 19 та 16 новонароджених на 2 та 5 тижні відповідно. У свою чергу кожна з підгруп була поділена на підгрупи А і В, підгрупи В новонароджені, які отримували біопрепарат. Біопрепарат включав біфідобактерії, його пероральне введення відбувалося перед першим взяттям крові для аналізу. Визначалися рівні ІЛ-1β та ІЛ-10, С-реактивного білка (СРП) за методом ELISA і напівкількісним методом .

Результати. Отримані дані показали збільшення рівня прозапального інтерлейкіну ІЛ-1β, так і протизапального інтерлейкіну ІЛ-10 на 2 тижні життя. Це відзначалося як у дітей із НЕ середнього ступеня тяжкості, так і у дітей із тяжкою НЕ. Але в останніх рівень підвищення досліджуваних цитокінів був вищим. На 5-му тижні життя відбувалося достовірне зниження ІЛ-1β та ІЛ-10, що відзначається у всіх досліджуваних групах. У той же час збереглись великі значення ІЛ-1β та ІЛ-10 у дітей з тяжкою неонатальною енцефалопатією. Отримані результати СРП показали більш високе значення у дітей з важкої НЕ. У динаміці зазначено зниження СРП, проте недостовірне для новонароджених із тяжким ступенем НЕ. Не було знайдено достовірних відмінностей середніх значень IL-1β, ІЛ-10 та СРП у групах, що не отримували та отримували пробіотик, хоча тенденція до менших значень ІЛ-1β, ІЛ-10 та СРП була присутня. Однак, відзначалася велика частота значень ІЛ-1β у межах норми до 5 тижня життя у новонароджених із середнім ступенем НЕ, які отримували пробіотик. Також частота значень ІЛ-10 у межах норми була вищою у дітей з тяжкою НЕ, які отримували пробіотик.

Висновки. Рівень ІЛ-1β, ІЛ-10 та СРП був підвищений у дітей з неонатальною енцефалопатією, більш значне для енцефалопатії тяжкого ступеня як на 2, так і на 5 тижні життя, при цьому визначалася зниження ІЛ-1β, ІЛ-10 та СРП від 2 до 5-го тижня життя. Призначення пробіотика призводило до більшої частоти значень ІЛ-1β у межах норми у групі дітей з неонатальною енцефалопатією середньої тяжкості та ІЛ-10 у дітей з тяжкою неонатальною енцефалопатією.

Ключові слова: новонароджені, неонатальна енцефалопатія, цитокіни, інтерлейкіни, С-реактивний протеїн.

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INTRODUCTION / ВСТУП

Neonatal encephalopathy (NE) is one of the most common diseases of the newborn period; worldwide the incidence of NE is more than 1 million newborns, most of which are registered in developing countries [1; 2; 3]. The global incidence is 8.5 per 1000 live births, but in developed countries, it is 1–3 cases per 1000 live births. Of these, about a quarter of newborns die, a quarter have moderate or severe dysfunction of the central nervous system, and a fifth part have mild disorders [4]. Neonatal encephalopathy is characterized by changes in consciousness, tone, and reflexes [5]. At the same time, the presence of systemic inflammation and multiple organ damage is characteristic [2; 6]. The choice of therapeutic interventions is determined by improved knowledge of the pathogenesis of brain cell damage, including oxidative stress, excitotoxicity and inflammation, which lead to their death within hours and weeks after birth [7].

Inflammation and hypoxia-ischemia play a vital, key role in neonatal encephalopathy [8; 9]. A persistent inflammatory response in newborns with NE is observed during the first week of life, which correlates with the severity of brain damage [10; 11]. At the same time, the inflammatory process can persist for weeks, months and even years, which is explained by tertiary mechanisms of damage, which include inflammation and epigenetic changes, decreased plasticity and a decrease in the number of neurons [12]. Cytokines play a key role in the implementation of inflammation and damage to brain cells through excessive activation of inflammatory cells of the innate immune response, in particular neutrophils, macrophages and microglia [8; 13]. Activation of microglia is the initial stage of inflammatory responses of the central nervous system to various stimuli, including stroke. This is followed by infiltration of circulating monocytes, neutrophils and T-cells, which enhances the inflammatory response in the stimulated brain [13]. Thus, cytokines can serve as biomarkers of the severity and consequences of neonatal encephalopathy [3; 8].

The level of cytokines in newborns with neonatal encephalopathy has been studied in several studies. A high association of interleukin-6 and interleukin-16 with the electrographic degree of hypoxic-ischemic encephalopathy has been shown [14]. There was an increase in IL-6, IL-8 and MCP-1 during the first 24 hours in neonates with NE [15]. A study in 20 full-term newborns showed that elevated GFAP, IL-1, IL-6, IL-8, tumor necrosis factor, interferon, and vascular endothelial growth factor at 6–24 hours were associated with abnormal neurological outcomes [16]. A study of interleukin levels in 159 newborns with NE showed higher levels of IL-10 and IL-6 [3]. C-reactive protein (CRP), one of the biomarkers of inflammation, is widely used in the diagnosis of diseases of newborns, primarily of infectious origin, such as sepsis and necrotizing enterocolitis [17; 18]. At the same time, its increase, along with changes in the level of cytokines, may be a predictor of brain damage in newborns [19; 20].

Treatment of neonatal encephalopathy is a complex task; taking into account the significant role of inflammation, it makes sense to look for new directions in therapy and means that influence the nature and severity of inflammatory reactions. The current standard treatment for NE is hypothermia, but this does not preclude the search for treatments that can be used later and over a longer period. One of these directions may be the prescription of drugs that influence the state of the newborn's intestinal microbiome, implying the latter's influence on the level of not only local but also systemic inflammatory response within the gut-brain axis [21; 22; 23; 24].

Despite the relevance of research in this area, the results are not yet clear and complete and require further research.

The purpose of our study was to study the state of the inflammatory response of newborns with neonatal encephalopathy and the possibilities of drug influence on it. We assume that the level of the studied interleukins and CRP may be increased in children with NE, and we can also assume that the use of a biological product may affect the state of the microbiome and, accordingly, the state of the local and systemic response, which may be reflected in the studied indicators.

Material and methods

The study was conducted in 74 full-term newborns with neonatal encephalopathy. The gestational age of the children was 36 weeks of gestational age or more, weight more than 2500 g. The presence and severity of neonatal encephalopathy were determined using the modified Sarnat scale [25]. Blood sampling was carried out at 2 and 5 weeks of life. At 2 weeks of life, the examination was carried out in 74 children, and at 5 weeks of life in 59 children, so dynamic observation was possible only for 59 newborns. The newborns were divided into subgroups 1 and 2. Subgroup 1 included children who were classified as having moderate neonatal encephalopathy. At 2 weeks of life, 55 such patients were examined, at 5 weeks – 43. Subgroup 2 included children with severe neonatal

encephalopathy – 19 and 16 newborns at 2 and 5 weeks, respectively. In turn, each of the subgroups was divided into subgroups a and b. Subgroup 1a included newborns with moderate severity of NE, and subgroup 1b included newborns who received a biological drug. Accordingly, group 2a included newborns with severe NE, and subgroup 2b included newborns who received a biological drug. Separately, groups a and b were formed to assess the degree of influence of the biological product on children with NE without taking into account its severity. The biological product included bifidobacteria; it was administered orally before the first blood draw for analysis.

The level of IL-1β and IL-10 was determined by ELISA. C-reactive protein levels were determined by the semiquantitative method. For comparison with the norm, laboratory reference values were used: IL-1β – 0–21.0 pg/ml, IL-10 – 0–2.0 pg/ml.

Statistical analyses were conducted using SPSS version 28.0 (IBM, NY, US). The normality of the continuous values was tested by the Shapiro–Wilk tests. The continuous variables were presented as mean values ± standard deviation (M±SD). Univariate analysis of variance (RM-ANOVA) between microbiome value and FC value and verification by the Shapiro–Wilk test were used. P-values < 0.05 were considered statistically significant. Statistical processing also included the determination of proportions (percentages), and the assessment of the reliability of differences was carried out using the criteria (z) with the Yates correction, as well as (χ2) Pearson and (F) Fisher.

All research methods and experiments have been examined and approved by the appropriate ethics committee and have therefore been performed by the ethical standards laid down in the Declaration of Helsinki.

The project was approved by the Commission on Bioethics Meeting of the Educational and Scientific Medical Institute of Sumy State University.

Results

The dynamics of IL-1β in newborns of the study groups are presented in Table 1. The IL-1β values in the second week in all groups exceeded the upper limit of the reference values of 21 pg/ml. The maximum value of IL-1β was greater in children with severe NE (81.78±15.25 pg/ml) than in children with moderate NE (p=0.02). In the group of children receiving the biological drug, a significantly higher value was also noted in newborns with severe NE. At the same time, no significant difference in IL-1β was found between the groups that received and did not receive the biological drug. This applied to both newborns with moderate NE and children with severe NE, and for children with NE in general. The values of IL-1β in newborns who received the biological drug were lower in all groups compared to those who did not receive it, but still not significant.

Table 1– Dynamics of IL-1β in newborns of the study groups

Group	2-nd week	5-th week	P 2w:5w
1a	62,21±19,59	19,52±6,93	0,000
P between groups	1b>0,05; 2a 0,02; 2b 0,59; a>0,05; b>0,05	1b>0,05; 2a <0,001; 2b <0,001; a>0,095; b>0,05	
1b	53,38±12,35	13,09±4,47	0,000
P between groups	1a>0,05; 2a <0,001; 2b 0,049; a>0,127; b>0,05	1a>0,05; 2a <0,001; 2b <0,001; a 0,002; b>0,05	
2a	81,78±15,25	49,94±8,1	0,000
P between groups	1a 0,02; 1b <0,001; 2b >0,05; a 0,171; b 0,016	1a <0,001; 1b <0,001; 2b >0,05; a <0,001; b <0,001	
2b	77,64±10,79	40,5±4,67	0,000
P between groups	1a 0,59; 1b <0,049; 2a >0,05; a >0,05; b 0,423	1a 0,001; 1b <0,001; 2a >0,05; a >0,147; b 0,007	
A	66,9±20,33	27,32±15,23	0,000
P between groups	1a >0,05; 1b<0,127; 2a 0,171; 2b >0,05; b >0,05	1a 0,095; 1b 0,002; 2a <0,001; 2b 0,147; b 0,879	
B	60,46±16,23	21,32±13,62	0,000
P between groups	1a >0,05; 1b>0,05; 2a 0,016; 2b 0,423; a >0,05	1a >0,05; 1b >0,05; 2a <0,001; 2b 0,007; a 0,879	

At week 5 of life, IL-1β values in all groups decreased significantly. At the same time, IL-1β values below the reference range were not observed in all cases. This was found in 17 (58.62%, 41–77 95% CI) newborns of group 1a and in 14 (100%, 100 95% CI) children of group 1b, that is, 52% more in this group (p = 0.005). The average values of IL-1β were also lower in children of group 1b, but also not significant (p>0.05). In groups of children with severe NE, IL-1β levels were higher compared to those in children with moderate NE (p<0.05). But, at the same time, in groups 2a and 2b there were no patients with IL-1β values less than the upper limit of the reference values. In group A, the value of IL-1β was higher than in group B, but also not significant. The percentage of IL-1β values below the upper limit of the reference values was greater in group B – in 14 (70%, 50–90 95% CI) than in A – in 17 (43.59%, 28–59 95% CI), respectively, that is, by 27% (p=0.04).

The dynamics of IL-10 in newborns of the study groups are presented in Table 2. The average values of IL-10 in all study groups at 2 weeks of life were higher than the reference values – 2 pg/ml. The highest values were in the groups of children with severe NE, both in group 2a and group 2b compared with groups 1a and 1b, respectively (p>0.05). Lower IL-10 values were noted in the groups of newborns receiving the probiotic, but they were not significant. The number of newborns with an IL-10 value below the upper limit of the reference value in group 1a was 3 (7.89%), in group 1b – 1 (5.88%). No such newborns were found in children of groups 2a and 2b. Accordingly, in group A there were 3 (6%) children with an IL-10 value below 2 pg/ml, in group B – 1 (4.17%) (p>0.05).

Table 2 – Dynamics of IL-10 in newborns of the study groups

Group	2-nd week	5-th week	P 2w:5w
1a	4,99±2,21	1,16±0,59	0,000
P between groups	1b 0,518; 2a >0,05; 2b >0,05; a>0,05; b>0,05	1b>0,05; 2a <0,001; 2b >0,05; a>0,095; b>0,05	
1b	3,84±1,46	1,19±0,56	0,000
P between groups	1a 0,518; 2a <0,091; 2b 0,756; a 0,162; b>0,05	1a>0,05; 2a <0,003; 2b >0,05; a>0,05; b>0,05	
2a	5,79±1,21	2,2±0,56	0,000
P between groups	1a >0,05; 1b <0,091; 2b >0,05; a >0,05; b 0,399	1a <0,001; 1b 0,003; 2b 0,078; a 0,012; b 0,02	
2b	5,49±0,94	1,27±0,59	0,000
P between groups	1a >0,05; 1b 0,756; 2a >0,05; a >0,05; b >0,05	1a >0,05; 1b >0,05; 2a 0,078; a >0,05; b >0,05	
a	5,19±2,04	1,42±0,74	0,000
P between groups	1a >0,05; 1b 0,162; 2a >0,05; 2b >0,05; b 0,932	1a >0,05; 1b>0,05; 2a 0,012; 2b >0,05; b >0,05	
b	4,32±1,51	1,22±0,55	0,000
P between groups	1a >0,05; 1b>0,05; 2a 0,399; 2b >0,05; a 0,932	1a >0,05; 1b >0,05; 2a 0,002; 2b >0,05; a >0,05	

At week 5 of life, IL-10 levels in all groups had a significant decrease. In groups of newborns with severe NE – 2a and 2b – the values of IL-10 were the highest. Moreover, in group 2a they were significantly higher than in group 1a (p<0.001). But no significant differences were found between groups 1b and 2b; IL-10 values were close – 1.19±0.56 and 1.27±0.59 pg/ml, respectively. In newborns receiving the probiotic, the average values of IL-10 were lower, which was noted both for groups 1a and 1b, and groups 2a and 2b (p>0.05). The number of children with IL-10 values below the upper limit of normal in group 1a was 27 (93.1%), in group 1b – 13 (92.86%). In groups 2a and 2b – 4 (40%) and 6 (100%), respectively (p=0.003). The mean value of IL-10 in group B was also lower than in group A (p>0.05).

The dynamics of CRP in newborns of the study groups are presented in Table 3. The average CRP values at the 2nd week of life were maximum in newborns with severe NE, and it was significant for groups 2a and 1a (p = 0.012); for groups 2b and 1b there was a difference, but it was not significant

(p>0.05). In the groups receiving the biological product, lower CRP rates were noted (p>0.05). At the 5th week of life, there was a decrease in CRP values in all groups (p<0.05), with the exception of the group of newborns with severe NE who receive a probiotic. There was also a decrease here, but it was not significant. The CRP value was highest in children of group 2, but it was not significant. There were lower CRP values in children receiving the probiotic (p<0.05), this applied to groups 1, 2, and A and B.

Table 3 – Dynamics of CRP in newborns of the study groups

Group	2-nd week	5-th week	P 2w:5w
1a	55,43±28,52	25,64±28,03	0,008
P between groups	1b 0,518; 2a 0,019; 2b 0,293; a 0,341; b 0,997	1b >0,05; 2a >0,05; 2b >0,05; a>0,095; b>0,05	
1b	48±29,39	12±8,49	0,038
P between groups	1a 0,518; 2a <0,012; 2b 0,168; a 0,166; b>0,05	1a>0,05; 2a >0,05; 2b >0,05; a>0,05; b>0,05	
2a	84±22,22	39±38,42	0,026
P between groups	1a 0,019; 1b 0,012; 2b >0,05; a 0,075; b 0,572	1a >0,05; 1b >0,05; 2b >0,05; a >0,05; b >0,05	
2b	72±27,71	15±12,73	0,057
P between groups	1a >0,05; 1b 0,168; 2a >0,05; a >0,05; b >0,05	1a >0,05; 1b >0,05; 2a >0,05; a >0,05; b >0,05	
a	63,31±29,55	29,2±30,24	0,001
P between groups	1a >0,05; 1b >0,05; 2a 0,075; 2b >0,05; b >0,05	1a >0,05; 1b>0,05; 2a >0,05; 2b >0,05; b >0,05	
b	55,38±30,02	13±8,83	0,04
P between groups	1a >0,05; 1b>0,05; 2a 0,029; 2b >0,05; a >0,05	1a >0,05; 1b >0,05; 2a >0,05; 2b >0,05; a >0,05	

Discussion

The data obtained showed an increase in the level of both the pro-inflammatory interleukin IL-1β and the anti-inflammatory interleukin IL-10 at 2 weeks of life. This was observed both in children with moderate NE and in children with severe NE. In the latter, the level of increase in the studied cytokines was higher. At 5 weeks of life, there was a significant decrease in IL-1β and IL-10. A study performed on 10 newborns with hypoxic-ischemic encephalopathy (HIE) and 8 children in the control group during the first 5 days of life examined 48 cytokines, of which 17 were higher in children with HIE. These included both IL-1β and IL-10. By day 5 there was a decrease, which was associated with hypothermia [26]. When studying the level of cytokines in 27 newborns in the first 4 days of life, mild NE was detected in 7 of them, moderate in 17, and severe in 3. Elevated IL-1, IL-6, IL-8, VEGF were noted, which increased the risk of adverse neurodevelopmental outcomes. At the same time, no connection between the changes and hypothermia was found [16]. In a study conducted on full-term newborns, 159 of them with NE, and 157 without NE, the levels of cytokines were studied in the first 6 hours of life. An increase in TNFα, VEGF, and IL-10 was noted in comparison with a group of healthy newborns. IL-10 is an anti-inflammatory cytokine and plays a protective role for the central nervous system, however, its increased concentration has been associated with increased mortality and adverse neurodevelopmental outcomes [3]. In a study conducted in 50 newborns with HIE in the first 4 days of life, the concentration of cytokines was studied. It was noted that initially high IL-10 values decreased by 2–3 days of life and were independently associated with time. Reductions in IL-6, IL-8, and IL-10 have been noted to be associated with more favorable outcomes [15]. 36 newborns with HIE were studied on days 1 and 3 of life. The level of cytokines was studied and an increase in cytokines IL-1β, IL-2, IL-6, IL-8, IL-10, and IL-13 was noted in newborns with severe NE, compared with children without signs of HIE or mild HIE. There was a decrease in all studied cytokines by the 3rd day of life. At the same time, there was a pronounced association between an increase in IL-10 and IL-6 and an unfavorable outcome of the disease [27]. Thus, literature data show an increase in the levels of IL-1β and IL-10 in

the first hours of life after birth and a decrease in them after the 3rd day of life. Moreover, IL-10 is a significant factor determining the development of adverse neurodevelopmental outcomes. The results obtained in this study support an increase in both IL-1β and IL-10 in neonates with NE, more significant in severe NE. It can be assumed that an increase in IL-10, as a cytokine that characterizes to a certain extent a protective effect on the brain, may reflect both the extent of its damage and the extent of repair processes.

The results of CRP showed a higher value in children with severe NE. Over time, a decrease in CRP was noted, but it was not significant for newborns with severe NE. In a study of 74 newborns with HIE, an increase in CRP values was noted, depending on the severity of encephalopathy [28]. Another study examined the status of biochemical and hematological parameters, including CRP, in 78 newborns with HIE. It was noted that CRP values were higher in children with HIE, and multidirectional dynamics were noted. Children with HIE had an increase in CRP from birth to the 4th day of life, while children in the control group had a decrease [29]. In a study of CRP levels in 225 newborns with HIE, an increase was noted during the first 6 days after birth. It was detected in children with severe and moderate HIE, while in mild HIE no increase in CRP was observed [30]. In a study of 83 newborns with HIE, an increase in CRP was noted, which was also associated with the severity of encephalopathy [31]. An experimental study with the administration of a drug that suppresses CRP activity showed a significant decrease in apoptosis and the size of cerebral infarction in hypoxic-ischemic brain damage. This led to a decrease in adverse neurodevelopmental outcomes [32]. Thus, our studies confirm an increase in CRP in children with neonatal encephalopathy, more pronounced for severe forms of NE, which indicates the severity of systemic inflammatory reactions.

Our studies, on the one hand, confirm the negative dynamics of IL-1β and IL-10 obtained by other researchers, on the other hand, our measurement results are shifted to the right in the time scale, the studies were carried out at 2 and 5 weeks of life. It is impossible to focus on the values of IL-1β and IL-10 in other studies due to the results of different laboratories. However, negative dynamics of IL-1β and IL-10 values are present in our studies. There is also a significant difference between the values of IL-1β and IL-10 in the groups of children with severe NE and moderate NE, which shows a more pronounced inflammatory (and IL-10 reparative) reaction. The severity of systemic inflammation is confirmed by increased CRP values, also obtained in our study. The results of the influence of the prescribed biological product on the level of the inflammatory response were not clear. On the one hand, there were no significant differences in the average IL-1β values between the groups of newborns who received and did not receive the drug. On the other hand, there was a higher incidence of IL-1β values within the normal range by 5 weeks of life in neonates with moderate NE who received the probiotic. For the IL-10 value, a similar picture was revealed - the absence of significant differences in the average IL-10 values between the groups of newborns who received and did not receive the drug. At the same time, the frequency of IL-10 values within the normal range was higher in children with severe NE who received the probiotic. The average CRP values also did not differ significantly between the groups of newborns who received and did not receive the drug. Thus, some of the data obtained may indicate the effect of probiotic administration on the state of the inflammatory response. This confirms the presence of a connection and influence of the microbiome on the state of the immune response and the level of the inflammatory response, which is indicated by a number of authors [21; 22; 24]. There are, of course, study limitations to consider. The number of children in the groups with severe NE was small, especially those who received a biological drug. Research in this area should be continued to gain a greater understanding of the effectiveness of probiotic administration, selection of duration, and composition of medications.

CONCLUSIONS / ВИСНОВКИ

The levels of IL-1β, IL-10, and CRP were increased in children with neonatal encephalopathy, more significantly for severe encephalopathy at both 2 and 5 weeks of life, while a decrease in IL-1β, IL-10, and CRP was determined from 2 by 5 weeks of life.

Administration of the probiotic resulted in a higher incidence of IL-1β values within the normal range in the group of children with moderate neonatal encephalopathy and IL-10 in children with severe neonatal encephalopathy.

PROSPECTS FOR FUTURE RESEARCH / ПЕРСПЕКТИВИ ПОДАЛЬШИХ ДОСЛІДЖЕНЬ

Subsequent work is aimed at clarifying the connections between the values of cytokines, the severity of NE, and the characteristics of the relationship between the local and systemic response and the microbiome.

CONFLICT OF INTEREST / КОНФЛІКТ ІНТЕРЕСІВ

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS / ВКЛАД АВТОРІВ

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