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FEATURES OF THE INTESTINAL MICROBIOME AND THE LEVEL OF LOCAL INFLAMMATORY RESPONSE IN NEWBORNS WITH NEONATAL ENCEPHALOPATHY

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FEATURES OF THE INTESTINAL MICROBIOME AND THE LEVEL OF LOCAL INFLAMMATORY RESPONSE IN NEWBORNS WITH NEONATAL ENCEPHALOPATHY

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Introduction. Hypoxic lesions caused by neonatal encephalopathy (NE) can contribute to disruption of the microbiome formation due to both hypoxia itself and inflammatory reactions. One of the markers for determining the local inflammatory response is fecal calprotectin (FC). It correlates with the level of inflammation and may influence the microbiome composition.

Goal. To study the features of microbiome formation and level of local inflammatory response of newborns with NE by determining the composition of intestinal microbiome and level of FC.

Materials and methods. The study was conducted at 2, 3 and 5 weeks and included 32 controls and 87 newborns with NE. A culture and enzyme immunoassay methods were used to examine the composition of the intestinal microbiome and FC levels. Statistical analyses were performed using SPSS version 28.0.

Results. Newborns with NE had a decrease of levels of Bifidobacterium ($p<0.001$) and Lactobacilli ($p<0.001$) at 2 weeks, lower results were noted in severe form of NE. Values of E.coli, Opportunistic pathogens had tendency to increase with higher levels in severe form of NE. FC levels at 2 weeks were increased in children with NE.

Conclusion. Disturbances in the microbiome composition in neonates with NE consisted of a decrease in Bifidobacterium at 2 and 3 weeks, Lactobacilli at 2 weeks and an increase in Opportunistic pathogens at 2 and 3 weeks. In newborns with NE, the level of FC was higher than in controls – for moderate form at 3 and 5 weeks and severe form at 2 and 3 weeks. In newborns with NE received a probiotic, Bifidobacterium values recovered earlier and FC levels were lower.

Key words: neonatal encephalopathy, intestinal microbiome, fecal calprotectin.

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ОСОБЛИВОСТІ КИШКОВОГО МІКРОБІОМУ ТА РІВНЯ ЛОКАЛЬНОЇ ЗАПАЛЬНОЇ ВІДПОВІДІ НОВОНАРОДЖЕНИХ ІЗ НЕОНАТАЛЬНОЮ ЕНЦЕФАЛОПАТІЄЮ

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У дослідженні вивчались особливості становлення мікробіому та рівня запальної відповіді кишечника новонароджених із неонатальною енцефалопатією (НЕ) шляхом визначення складу кишкового мікробіому та рівня фекального кальпротектину (ФК) на 2-му, 3-му та 5-му тижнях. У дітей з НЕ відбувалося зниження рівнів біфідобактерій на 2-му і 3-му тижні та лактобактерій на 2-му тижні; підвищувалася кількість умовно-патогенних мікроорганізмів на 2-му і 3-му тижнях. У дітей із НЕ рівень ФК відзначався вищими значеннями, ніж у контрольній групі – у дітей із середньою формою на 3-му та 5-му тижнях та з тяжкою на 2-му та 3-му тижнях. У малюків із НЕ, які отримували пробіотик, показники біфідобактерій відновлювалися раніше, а рівні ФК були нижчими.

Ключові слова: неонатальна енцефалопатія, кишковий мікробіом, фекальний кальпротектин.

Introduction. Neonatal encephalopathy (NE) is one of the leading causes of neonatal mortality and disability [1]. The incidence of NE ranges from 1 to 8 per 1000 live births in developing countries and reaches 26 per 1000 live births in underdeveloped countries [2]. The most important pathophysiological role in NE is inflammation in combination with hypoxia-ischemia. Damage processes caused by inflammation can continue for months due to its duration and the presence of epigenetic changes [1]. Hypoxia can lead to damage not only to the brain, but also to other organs and systems, one of which is the intestine. Also, hypoxic lesions can contribute to disruption of the formation of the microbiome due to both hypoxia itself and concomitant inflammatory processes [3]. Interrelation between the gut and the brain can be expressed in the current paradigm of the brain-gut axis. This is a set of various neurohumoral factors that are interconnected and

have an impact on intestinal functions and the composition of the microbiota, influencing on intestinal permeability due to the activation of intestinal neurotransmitters and the immune response of the mucous membrane [4].

There are a number of studies of the development and establishment of healthy newborns microbiota, but there is not enough research on the intestinal microbiome of newborns with NE. Being a stressful condition, hypoxia-ischemia leads to significant changes in hormone and glucose levels, which is an additional risk factor for increasing the richness and diversity of the intestinal microbiota [5]. It is important to add that neonates suffering from hypoxia-ischemia were at increased risk of contamination due to close contact with hospital staff, exposure to more medications and long-term hospital treatment [6, 7].

Fecal calprotectin (FC) correlates with the severity of intestinal inflammation as it reflects the movement of neutrophils into the small intestine [8]. FC is an important protein in the acute phase of inflammation – it has an antibacterial effect, is secreted by epithelial cells, and induces apoptosis and chemotaxis [9, 10]. Researchers had examined FC as a non-invasive marker of intestinal inflammation in conditions such as inflammatory bowel disease, organic lesions and allergic disorders [11]. We understand that FC is determined to diagnose inflammatory reactions in infectious and organic diseases [12] in the neonatal period, so we decided to note the effect of NE on the level of intestinal inflammatory reaction of newborns after hypoxia-ischemia.

Problem statement. Currently, there is insufficient data on the study of children with neonatal encephalopathy, so we decided to identify the presence of a connection between hypoxia and the possible occurrence of changes in the composition of the microbiome and the intestinal inflammatory response.

The aim of our study was to investigate the peculiarities of the formation of the intestinal microbiome and the local inflammatory response of newborns with NE and the possibilities of drug influence on them. We imply that the studied parameters of FC and the intestinal microbiome may be altered in children with NE, and that the use of a probiotic may have an impact on the composition of the intestinal microbiome and the level of FC.

Materials and methods. The main group included 87 children with NE and the control group (C) included 32 healthy newborns. The children were born full term, with a gestational age in the range of 37–41 weeks. The presence and severity of NE was determined according to the modified Sarnat scale [13]. Depending on the degree of NE and prescribed medications, children were divided into 2 groups and 4 subgroups. The first (1) group (N=66) was divided into subgroups: 1a – children with moderate NE (N=40) and 1b – children with moderate NE, in addition received a probiotic (N=26). The second (2) group (N=21) was divided into subgroups: 2a – children with severe NE (N=14) and 2b – children with severe NE received a probiotic (N=7). To assess the effect of the prescribed probiotic in children with NE, subgroups were additionally identified A (N=60) – children who did not receive a probiotic and subgroup B (N=27) – newborns who received a probiotic in addition. To study the composition of the intestinal microbiome, stool samples were assembled three times at the age of 2, 3, and 5 weeks old, respectively, and the culture method was used [14]. The same times were used to define fecal calprotectin levels (FC) [8]. Bacterial counts expressed as log10 CFU/g (colony-forming units/grams) of feces. The stool samples were examined using an enzyme-linked immunosorbent assay by semi-automatic enzyme immunoassay analyzer Thermo Scientific Multiskan FC to determine the level of FC in mg/l. Stool samples were assembled at home in a container with an airtight lid with a date and time stamp. Statistical analyses were performed using SPSS version 28.0 (IBM, NY, US). Normality of the continuous values was tested by Shapiro–Wilk method. The continuous variables were presented as mean values ± standard deviation (M±SD). The P-value was set as <0.05. The reliability of differences was determined by the Student's test with Bonferroni correction and Fisher's Z-test. The project was approved by the Commission on Bioethics Meeting of the Educational and Scientific Medical Institute of Sumy State University (Protocol № 1/12, December 12, 2023).

Results. The number of boys and girls was the same in all groups. Gestation age and birthweight were not significantly different. Apgar scores at 1 minute were lower in children with moderate NE than in healthy ones (p=0.001), but the minimum values were recorded in newborns with severe NE compared to controls (p=0.001) and patients with moderate NE (p=0.001). Apgar scores at 5 minutes were lower in the severe group compared to the controls (p=0.001) and children with moderate NE (p=0.001); in newborns of group 1, the values were low compared to healthy ones, but no difference was noted (p>0.05). Breastfeeding was more often observed in healthy children compared to neonates with severe NE (p=0.02). Also, breastfeeding was observed more often in children of group 1 than in group 2 (p=0.048). Mixed feeding was more often observed in newborns with moderate NE than in healthy (p=0.03). Formula feeding was observed more often in children of group 2 than of group 1 (p=0.007). The frequency of C-section ranged from 14.28 to 16.66% and showed no differences. The mother's age was in the range of 18–39 years and the father's age was in the range of 21–52 years, but there was no difference. Mother`s pathology of the cardiovascular and endocrine systems was found more often in children with severe form compared to control group. The above disorders and previous infectious diseases more often were observed in group 1 compared to controls (Table 1).

When studying the level of Bifidobacterium at the age of 2 weeks, significant lower rates were noted in all subgroups compared to the controls. In children of subgroup 2a, the indicators (4.21±1.37) were lower (p<0.001) than in newborns of 1a (6.24±1.32). At the same time, there was no significant difference in the subgroups of children with moderate and severe forms of NE who received probiotics. Also, no differences were found in subgroups A and B (Table 2).

At 3 weeks, the level of Bifidobacterium had a positive dynamic in subgroups 1a (p=0.025) and A (p=0.015) compared to 2 weeks. At the same time, lower rates were noted in children 1a (p=0.001), 1b (p<0.001), 2a (p<0.001), 2b (p=0.013), A (p<0.001), B (p<0.001) in relation to the healthy group. In children of subgroup 2a (5.15±1.14) the values were lower (p=0.002) than in 1a (7.02±1.42). In the groups of moderate and severe forms who received probiotics, no differences were found (p>0.05). Also, no significant difference was found in subgroups A and B, but there was a tendency to higher values of the level of Bifidobacterium in subgroup B.

Table 1

The general indexes of the newborns

Indexes	Controls	Group 1	Group 2	p value <0.05
Boys, absolute value/%	19/59.37	37/56.06	11/52.38	–
Gestation age, weeks, mean ±SD	38.6±1.3	39.17±0.15	38.76 ±0.25	–
Birthweight, grams, mean ± SD	3218±467	3488.33 ±64.01	3291.95 ±109.45	–
Apgar 1, mean ±SD	8.0±0.5	7.15±0.13	3.38±0.49	c:1 0.001 c:2 0.001 1:2 0.001
Apgar 5, mean ±SD	8.9±0.4	8.42±0.1	5.04±0.48	c:2 0.001 1:2 0.001
Feeding by breast milk, absolute value/%	20/62.5	36/54.55	7/33.33	c:2 0.02 1:2 0.048
Mixed feeding, absolute value/%	4/12.5	20/30.3	5/23.81	c:1 0.03
Feeding by formula, absolute value/%	8/25	10/15.15	9/19.05	1:2 0.007
Caesarean section, absolute value/%	5/15.62	11/16.66	3/14.28	–
Endocrine pathology, absolute value/%	0	11/16.67	7/33.33	c:1 0.009 c:2 0.0007 1:2 0.006
Cardiovascular pathology, absolute value/%	0	8/12.12	8/38.1	c:1 0.04 c:2 0.0002 1:2 0.02
Transferred infectious pathology, absolute value/%	1/3.13	17/25.76	1/4.76	c:1 0.004 1:2 0.027

Note: c:1 – p between controls and group 1; c:2 – p between controls and group 2; 1:2 – p between groups 1 and 2.

Table 2

Dynamics of Bifidobacterium

Group	2nd week	3rd week	5th week	p value <0.05
C	8.91±1.47	8.44±1.58	8.09±1.49	–
p between groups <0.05	1a <0.001; 1b <0.001; 2a <0.001; 2b <0.001; A <0.001; B <0.001	1a 0.001; 1b <0.001; 2a <0.001; 2b 0.013; A <0.001; B <0.001	2a <0.001; A 0.008	
1a	6.24±1.32	7.02±1.42	7.23±1.31	2:3 0.025 2:5 0.005
p between groups <0.05	C<0.001; 1b>0.05; 2a <0.001	C 0.001; 2a 0.002	–	
1b	6±1.41	5.94±1.34	7.87±1.13	2:5 <0.001 3:5 <0.001
p between groups <0.05	C <0.001; 2a 0.012	C <0.001	–	
2a	4.21±1.37	5.15±1.14	6.09±1.3	2:5 0.003
p between groups <0.05	C <0.001; 1a <0.001; 1b 0.012; A 0.01	C 0.001; 1a 0.002; A 0.039	C <0.001; 1b 0.025	
2b	4.43±1.27	6.17±1.33	7.33±1.51	2:5 0.005
p between groups <0.05	C <0.001	C 0.013	–	
A	5.77±1.59	6.58±1.58	6.96±1.4	2:3 0.015 2:5 <0.001
p between groups <0.05	C <0.001; 2a 0.01	C <0.001; 2a 0.039	C 0.008	
B	5.59±1.53	6±1.31	7.71±1.23	2:5 <0.001 3:5 <0.001
p between groups <0.05	C <0.001	C<0.001	–	

Note: c:1 – p between controls and group 1; c:2 – p between controls and group 2; 1:2 – p between groups 1 and 2.

At the age of 5 weeks, positive dynamics in the level of Bifidobacterium was observed in comparison to 2 weeks in the main group, excluding controls. Also, at 5 weeks, an increase in indicators were detected relative to 3 weeks in children 1b (p<0.001) and B (p<0.001). Reduced levels were noted in subgroups 2a (p<0.001) and

A (p=0.008) in relation to the control group. The number of Bifidobacterium in subgroups 1a and 2a did not differ. No changes were found in moderate to severe forms received the probiotic (p >0.05). Also, no differences were noted in subgroups A and B, but there was a tendency to higher values in subgroup B.

When studying the level of Lactobacillus at the age of 2 weeks, lower rates were found in subgroups 1a (p<0.001), 2a (p<0.001), 2b (p<0.001), A (p<0.001) and B (p<0.001) in relation to the controls. In newborns of subgroup 2a, the indicators were lower than in 1a (p=0.003). Children in 2b had lower levels of Lactobacillus than children in 1b (p=0.009) despite both subgroups were receiving probiotics. No differences were found between subgroups A and B (Table 3).

At 3 weeks compared to 2 weeks, the level of Lactobacillus increased, but significantly only in children of subgroups 2a (p=0.036) and 2b (p=0.027). Reduced indicators were noted in 2a (p=0.002) and A (p=0.004) in comparison to the controls. No differences were found in subgroups 1a and 2a (p>0.05). No changes were found in the moderate and severe subgroups that received probiotic (p>0.05). There were no significant differences in subgroups A and B.

At the age of 5 weeks, there was positive dynamics in the level of Lactobacillus in comparison with 2 weeks of controls (p=0.046), 2a (p<0.001), 2b (p<0.001) and A (p=0.021). Also, at 5 weeks, an increase in indicators was detected relative to 3 weeks in subgroup 2a (p=0.035). A trend towards an increase of Lactobacillus in the subgroups was noted, but no significant changes were found.

The level of total number of E.coli tended to increase with more intensive growth in neonates with NE were not received a probiotic, but no significant difference was found in relation to all subgroups, which is explained by the small number of determinations in the studied samples (p>0.05) (Table 4).

E.coli with weak enzymatic ability at the age of 2 weeks higher values were noted in subgroup 2a compared to healthy ones (p=0.049). No significant differences were found in the remaining subgroups. At 3 weeks, higher values were found in subgroups 1a (p=0.012), 2a (p=0.01), 2b (p=0.022), A (p=0.004), B (p=0.048) compared to the controls. No differences were found in subgroups 1a and 2a. No changes were found in the moderate to severe grade received probiotic (p >0.05). Also, no significant differences were noted in subgroups A and B. At 5 weeks, no significant differences were found in the subgroups (Table 5).

Levels of Opportunistic pathogens at the age of 2 weeks were higher in children 1a (p=0.002), 2a (p=0.041) and A (p<0.001) compared to the healthy group. At week 3, the indicators were elevated in children 1a (p<0.001), 2a (p=0.006) and A (p<0.001) compared to the controls. At the age of 2 and 3 weeks, no significant changes were noted in the remaining subgroups. At the age of 5 weeks, the rates of Opportunistic pathogens were lower compared to 2 weeks in children 1a (p<0.001), 1b (p=0.004), A (p<0.001) and B (p=0.014). Also, at 5 weeks, negative dynamics was revealed in relation to 3 weeks in 1a (p<0.001), 1b (p=0.047), A (p<0.001). Increased values were noted in children of 2a compared to 1a subgroup (p=0.02). There was no difference in moderate and severe grades in children who received probiotics (p=0.287). There were also no differences noted in subgroups A and B (Table 6).

Table 3

Dynamics of Lactobacilli

Group	2nd week	3rd week	5th week	p value <0.05
C	7.69±1.15	7.13±1.45	6.75±1.87	–
p between groups <0.05	1a <0.001; 2a <0.001; 2b <0.001; A <0.001; B <0.001	2a 0.002; A 0.004	–	
1a	5.96±1.49	6.17±1.36	6.2±1.17	–
p between groups <0.05	C <0.001; 2a 0.003	–	–	
1b	6.45±1.28	6.18±1.24	6±1.25	–
p between groups <0.05	2a <0.001; 2b 0.009	–	–	
2a	4.21±1.37	5.38±1.19	6.64±0.67	2:3 0.036 2:5<0.001 3:5 0.035
p between groups <0.05	C <0.001; 1a 0.003; 1b <0.001; B 0.017	C 0.002	–	
2b	4.14±0.9	6±1.26	7.33±1.21	2:3 0.027 2:5<0.001
p between groups <0.05	C <0.001; 1b 0.009	–	–	
A	5.55±1.64	5.98±1.37	6.3±1.09	2:5 0.021
p between groups <0.05	C <0.001	C 0.004	–	
B	5.85±1.56	6.13±1.22	6.38±1.36	–
p between groups <0.05	C <0.001; 0.017	–	–	

Note: c:1 – p between controls and group 1; c:2 – p between controls and group 2; 1:2 – p between groups 1 and 2.

The values of fecal calprotectin at 2 weeks were higher in subgroups 2a (p<0.001) and A (p=0.008) compared to the controls. No differences were found in subgroups 1a and 2a (p=0.155), although FC values in newborns with severe NE were high (474.14±151.89). In children who received probiotics, the FC value had not a significant difference

Table 4

Dynamics of total number of E.coli

Group	2nd week	3rd week	5th week
C	6.31±1.49	6.78±1.31	6.31±1.49
1a	7.03±1.7	6.95±1.43	7.09±1.28
1b	6.2±1.26	6.67±1.3	7.1±1.37
2a	7.25±1.42	8.17±1.47	7.75±1.49
2b	6.17±1.47	7.2±1.48	7.25±1.26
A	7.09±1.64	7.41±1.54	7.26±1.34
B	6.19±1.29	6.82±1.33	7.14±1.29

Note: p between groups and weeks <0.05 not detected.

Table 5

Dynamics of E.coli with weak enzymatic ability

Group	2nd week	3d week	5th week
C	4.33±0.58	3.5±0.71	3.33±0.58
p between groups <0.05	2a 0.049	1a 0.012; 2a 0.01; 2b 0.022; A 0.004; B 0.048	–
1a	6.2±1.3	6.75±0.96	5.5±0.71
p between groups <0.05	–	C 0.012	–
1b	5.67±1.15	5.5±0.71	–
p between groups <0.05	–	–	–
2a	7.25±1.26	7±1	6.67±1.53
p between groups <0.05	C 0.049	C 0.01	–
2b	6.8±0.84	7±0	5.75±1.26
p between groups <0.05	–	C 0.022	–
A	6.67±1.32	6.86±0.9	6.2±1.3
p between groups <0.05	–	C 0.004	–
B	6.38±1.06	6.25±0.96	5.6±1.14
p between groups <0.05	–	C 0.048	–

Note: p between weeks <0.05 not detected.

Table 6

Dynamics of opportunistic pathogens

Group	2nd week	3rd week	5th week	p value <0.05
C	4.72±1.28	4.41±1.04	4.28±1.17	–
p between groups <0.05	1a 0.002; 2a 0.041; A <0.001	1a<0.001; 2a 0.006; A <0.001	–	
1a	6.05±1.43	6.03±1.21	3.9±1.25	2:5 <0.001 3:5 <0.001
p between groups <0.05	C 0.002	C <0.001	2a 0.02	
1b	5.8±1.52	5.29±1.27	3.89±0.78	2:5 0.004 3:5 0.047
p between groups <0.05	–	–	–	
2a	6.15±1.34	6.13±1.25	5.83±0.98	–
p between groups <0.05	C 0.041	C 0.006	1a 0.02	
2b	5.71±1.11	5.67±0.82	5.5±1.05	–
p between groups <0.05	–	–	–	
A	6.08±1.41	6.05±1.22	4.35±1.44	2:5 <0.001 3:5 <0.001
p between groups <0.05	C <0.001	C <0.001	–	
B	6.08±1.41	6.05±1.22	4.35±1.44	2:5 0.014
p between groups <0.05	–	–	–	

Note: c:1 – p between controls and group 1; c:2 – p between controls and group 2; 1:2 – p between groups 1 and 2.

compared with similar values in the control group, which could be due to the intake of the probiotic. Similarly, FC indicators had not a significant difference in subgroups B and controls. At the same time, no differences in FC values were found in subgroups A and B (Table 7).

At the age of 3 weeks, negative dynamics of FC values occurred in all subgroups, but significant changes were noted in healthy (p=0.003), 1a (p=0.039) and A (p=0.014). Higher FC levels were observed in children 1a (p=0.004), 2a (p<0.001), A (p<0.001) and B (p=0.004) compared to the controls. No differences were found in subgroups 1a and 2a (p=0.257). FC showed lower levels in children who received probiotics, but no significant difference was found.

At 5 weeks, there was a further decrease in the level of FC in the control group, but only significantly in relation to 2 weeks. In children of the remaining subgroups, negative dynamics of FC values were also observed, which was significant both in 2 and 3 weeks relative to all studied groups. Higher FC levels were observed in subgroups 1a (p=0.03) and A (p=0.011) compared to the controls. There were no differences between moderate and severe subgroups. At the same time, the average FC values in newborns who received probiotics were lower than in the subgroups that were not, but no significant difference was noted. There were also no differences in subgroups A and B.

Discussion. Our studies confirm in newborns with neonatal encephalopathy the positive dynamics of the levels of Bifidobacterium and Lactobacillus and the negative of fecal calprotectin, which other researchers indicated [5, 8, 15]. At the same time, according to our data, in children with NE the level of Bifidobacterium was lower compared to the controls. In children of subgroup 2a, the levels were lower than in controls and the 1a subgroup throughout the entire study, but for the latter it was significant only at weeks 2 and 3. At the age of 5 weeks, positive dynamics of Bifidobacterium was observed in comparison to 2 weeks in the main group, excluding healthy. In children with severe NE, values remained low at 5 weeks. This may be due to a number of factors, including formula feeding [5, 6, 16], C-section [16, 17] and the presence of possible inflammatory changes in the intestine after hypoxia [6]. Although the results of other authors indicate that there is no effect of feeding and delivery on the levels of Bifidobacterium after hypoxia-ischemia [15].

Indicators of Lactobacillus in children with NE were lower in relation to controls, but significant only at 2 and 3 weeks. Lower values were observed in newborns with severe NE. At 2 weeks children in 2a had lower levels than in the 1a subgroup. Also, children in 2b had lower levels of Lactobacillus than children in 1b despite both subgroups receiving a probiotic. At the age of 5 weeks, there was a positive dynamics of indicators in relation to 2 weeks in controls, 2a, 2b and A subgroups, however, low values of Lactobacillus persisted in children with severe NE. Researchers suggest that formula feeding [5], C-section [16] and environmental factors [16] may influence the reduction of Lactobacillus levels in children with NE.

E.coli with weak enzymatic ability at the age of 2 weeks, higher rates were noted in subgroup 2a compared to healthy ones. At 3 weeks, increased values were detected in subgroups 1a, 2a, 2b, A, and B relative to the controls. At 5 weeks, there was a trend toward lower E. coli values in neonates with NE, although higher levels remained in children with severe grades. Factors influencing the increase in E.coli levels are formula feeding [5], delivery [16, 18] and environment [16].

The level of Opportunistic pathogens was higher in children receiving a probiotic compared to controls at 2 and

Table 7

Dynamics of fecal calprotectin

Group	2nd week	3rd week	5th week	p value <0.05
C	280.59 ±123.68	195.31± 115.52	153.53±34.64	
p between groups <0.05	2a<0.001; A 0.008	1a 0.004; 2a <0.001; 2b 0.017; A <0.001; B 0.004	1a 0.03; A 0.011	2:3 0.003 2:5<0.001 3:5 0.291
1a	361.85±137.4	299.86±115.1	206.94±82.9	2:3 0.039 2:5<0.001 3:5 0.02
p between groups <0.05	–	C 0.004	C 0.03	
1b	354.7±117.68	298.94±100.96	156.13±62.27	2:5 <0.001 3:5 <0.001
p between groups <0.05	–	–	–	
2a	474.14±151.89	394.31±131.67	213.27±62.09	2:5<0.001 3:5 0.003
p between groups <0.05	C<0.001	C <0.001	–	
2b	415.57±136.69	373.5±121.27	179.83±53.26	2:5 0.005 3:5 0.025
p between groups <0.05	–	C 0.017	–	
A	388.05±147.57	322.18±124.7	208.46±77.82	2:3 0.014 2:5 <0.001 3:5 <0.001
p between groups <0.05	C 0.008	C <0.001	C 0.011	
B	370.48±123.17	318.39±108.98	162.9±59.53	2:5 <0.001 3:5 <0.001
p between groups <0.05	–	C 0.004	–	

Note: c:1 – p between controls and group 1; c:2 – p between controls and group 2; 1:2 – p between groups 1 and 2.

3 weeks. At 5 weeks of age, there was a downward trend in Opportunistic pathogens. Increased values of Opportunistic pathogens in children with severe NE persisted at 5 weeks. This may be due to a number of factors, including formula feeding [5] and the presence of a possible inflammatory response in the intestine [15, 18].

FC values were higher in children with NE compared to the controls throughout the study. Higher FC levels were observed in children with severe NE. At 5 weeks there was a further decrease in FC values in all groups compared to 2 weeks, but elevated values persisted with a severe form of NE. Some authors believe that aspects that could influence the increase in FC levels include gestational age [14], C-section [14], male gender [8], postnatal age [8, 14] and breastfeeding [8].

Children with NE had lower levels of Bifidobacterium and Lactobacillus than controls, and some scientists confirm the data obtained [14, 15, 17]. Higher values of E.coli and Opportunistic pathogens have been recorded in newborns with NE, as also suggested by some studies [5,15,18]. After hypoxia-ischemia, the richness and diversity of intestinal flora increased [19]. The findings are explained by the unstable composition and function of the neonatal gut microbiome, followed by its improvement with age [16]. In our study, children with NE FC levels were higher at 2 weeks, with a gradual decrease in the subsequent postnatal week, which is also reflected in the works of some scientists [8, 14]. An increase in the FC value may reflect the intrauterine environment, intestinal immaturity and ischemic damage to the intestinal mucosa in children who suffered from NE [14]. Also, a decrease in FC with the subsequent postnatal week may be associated with changes in the microbial composition of stool and normalization of the gut [14].

Conclusions. 1. Disturbances in the microbiome composition in children with neonatal encephalopathy were noted throughout the study period and consisted of a decrease in Bifidobacterium levels at 2 and 3 weeks, and Lactobacillus at 2 weeks and an increase in Opportunistic pathogens at 2 and 3 weeks.

2. In newborns with neonatal encephalopathy, the level of fecal calprotectin was characterized by higher values, which were higher than in controls – for children with a moderate form at 3 and 5 weeks and with a severe form at 2 and 3 weeks.

3. In newborns with neonatal encephalopathy received a probiotic, Bifidobacterium values recovered earlier and fecal calprotectin levels were lower.

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