

Intestinal microflora in premature newborns with infectious and inflammatory diseases

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Abstract: *The study of intestinal microflora was carried out in 113 premature newborns (gestational age at birth was from 27 to 32 weeks, body weight was from 780 to 1495 g). The main group consisted of 91 infants with infectious and inflammatory diseases (IID): pneumonia, tracheobronchitis, meningitis, omphalitis. 22 infants were conditionally healthy (without IID) and made up the control group. On the 7th - 10th day of life, all infants had disorders in the composition of the intestinal microflora. However, in infants with IID, these disorders were more pronounced than in conditionally healthy infants. Infants of the main group received the probiotic from 7-10 days of life. Positive dynamics of clinical status and in the composition of intestinal microflora was noted in all infants in this group after treatment and was approached the indicators of infants in the control group. Long-term violations of the intestinal microflora both in conditionally healthy newborns and in premature infants with IID are a basis for the use of probiotic for 1-2 months.*

Key Words: *infectious and inflammatory diseases, intestinal microflora, premature infants, microbiota probiotic*

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I. Introduction

Currently, the achievements of modern neonatology make it possible to care for premature infants with very low and extremely low body mass (from 500 to 1500 g) at birth [1, 2]. However, morpho-functional immaturity (MFI) of organs and tissues, including the organs of the gastrointestinal tract and the immune system, perinatal pathology, such as respiratory distress syndrome (SDR), hypoxic-ischemic damage to the central nervous system, conjugation jaundice, general edematous syndrome, as well as extensive instrumental intervention (hardware artificial ventilation, catheterization of blood vessels and urinary tract, etc.) are the cause of the development of infectious and inflammatory diseases (IID) in premature newborns [2, 3]. All these circumstances can lead to a violation of the formation of normal microbiocenosis (microflora) of various loci, including the gastrointestinal tract. The situation is aggravated by the use of antibacterial therapy, which at the same time is necessary for the successful treatment of infection [3, 4]. At the present time for the correction of disorders of intestinal microflora in newborn infants using various probiotics [2, 5]. Currently, the question of the peculiarities of violation of intestinal microbiocenosis in premature newborns with a birth weight of less than 1500 g is poorly studied. In addition, there is a question about the expediency of using probiotics against the background of antibacterial therapy [6, 7]. The study of the features and depth of changes in the composition of the intestinal microflora in premature newborns with IID will allow to determine the ways of prevention and treatment of disorders in the process of formation of normal microbiocenosis.

The aim of the study was to determine the dynamics of the formation of intestinal microflora in preterm infants with IID and with a birth weight of less than 1500 g.

Patients. The study of intestinal microflora was carried out in 113 newborns with a birth weight of 780 to 1495 g and a gestational age of 27 to 32 weeks. The main group consisted of 91 infants with IID (pneumonia was in 52 infants, tracheobronchitis was in 21, meningitis was in 4, omphalitis was in 14) and intestinal dysfunctions (frequent liquefied stool with mucus and undigested lumps, regurgitation, methiorism). The 22 infants were conditionally healthy (without IID, but with minor signs of non-infectious perinatal pathology) and formed a control group.

By gestational age and body weight at birth, the groups were representative: body weight at birth was 1120 ± 30 g (from 780 to 1450 g) and 1295 ± 32.25 g (from 840 to 1495 g), gestational age - 29.2 ± 0.44 weeks (from 27 to 32 weeks) and 29.4 ± 0.52 weeks (from 27 to 32 weeks) in the main and control groups, respectively ($p > 0.05$). "Ventilator-associated" pneumonia (VAP) developed on the 3rd - 8th day of life in newborns with SDR and who were on a mechanical ventilation for 3-30 days. Other forms of IID developed on the 6th-12th

day of life. All infants of the main group also had perinatal pathology (hypoxic-ischemic damage to the central nervous system, RDS, edema syndrome, conjugation jaundice, MFI, hypotrophy). The infants develop intestinal syndrome on the background of antibacterial therapy on the 7-10 day of life. It was characterized by an increase in the frequency of stool, a change in its consistency (liquid, semi-liquid with undigested lumps, "foamy" stool). There was an unpleasant smell. The color of the chair turned green or yellow-green. There was pronounced flatulence and regurgitation.

The infants received complex etiotropic, pathogenetic and symptomatic treatment. All newborns with IID were prescribed antibacterial therapy (cephalosporins of the III-IV generation, carbapenems, aminoglycosides, amoxicillin+clavulanic acid, vancomycin, zivox, metronidazole, antifungal drugs). The duration of antibacterial therapy ranged from 10 to 45 days, depending on the severity of the disease. Infants received the per os probiotic Bifidumbacterin (the drug contains: at least 10⁷ live lyophilized, strains of *Bifidumbacterium bifidum* no. 1 or 791) from 7-10 days of life, in connection with the development of intestinal syndrome. The probiotic was given to 2.5 doses 2 times a day for 2-6 weeks depending on clinical status and intestinal microflora (to eliminate intestinal dysfunction, until the withdrawal of antibiotics and the achievement of normobiocenosis or I compensated degree of dysbiosis). The infants were on parenteral and / or enteral nutrition. Enteral nutrition included maternal or donor sterilized breast milk.

II. Methods

The study of the microflora of feces was carried out by qualitative and quantitative method of microbiological seeding on a wide range of nutrient media to identify aerobic, facultative and anaerobic microflora. Microbiological examination was performed on the 7th, 14th, 19th-21st and 28-30 days of life in all newborns, as well as an additional 1-4 times in the second month of life with a long course of the disease (pneumonia) and prolonged antibacterial therapy.

Microbiological assessment of the degree of intestinal biocenosis disorders was carried out according to the modified classification of microflora changes in the intestine in newborns.

According to this classification, the first degree of dysbiosis is determined by an increase in the number of facultative opportunistic microorganisms with a high level of bifidobacteria (lg 9-10 in 1 g of feces). Grade II is characterized by an increase in the associations of opportunistic microorganisms, the content of bifidobacteria at the lower limit of the norm (lg 7-8 in 1 g of feces) and a decrease in the content of lactobacilli (less than lg6 in 1 g of feces). Grade III is a deep decompensated dysbiosis with the absence or sharp inhibition of the growth of bifidobacteria (less than lg7), a low content (lg3-4) or the absence of lactobacilli against the background of pronounced disorders of the aerobic and facultative microflora, which dominates the biocenosis in terms of quantity [2,8].

Statistical processing of the results was carried out using the Statistica 7 computer software package. The indicators M±m and percent (%) of the occurrence of the trait in the groups were calculated. The differences between the qualitative characteristics were determined out using the χ^2 criterion. A significant difference between the compared indicators was considered indicators p≤0.05.

III. Results and discussion

The degree of dysbiosis in premature infants at various stages of the neonatal period is presented in the table.

Table

The degree of dysbiosis in premature infants at different stages of the neonatal period

Groups	The survey period	Normobio-cenosis		The degree of dysbiosis					
				I		II		III	
		n	%	n	%	n	%	n	%
Control group	7th day of life	0	0	3	14	13	59	6	27
Control group	19-21 th day of life	2	9	12	55	5	23	3	14
Control group	28-30 th day of life	6	27	11	50	5	23	0	0
Main group	7 th day of life	0	0	7	8	31	34	53	58
Main group	19-21 th day of life	1	1	30	33	41	45	19	21
Main group	28-30 th day of life	16	18	43	47	26	29	6	7

As can be seen from the table, all infants of the main and control groups had no normobiocenosis at the end of the 1st week of life. In the infants of the main group at this age, the III and II degrees of dysbiosis

prevailed, and in the control group, the II and I degrees prevailed. Thus, infants with IID had more profound disorders of the biocenosis than those who were conditionally healthy. In the dynamics of observation, there was a gradual improvement in the indicators of the intestinal microflora. By the end of the neonatal period, the number of serious violations in the biocenosis was shown in infants with IID, and more than half of the examined (63%) had degree I or normobiocenosis. However, 37% of infants needed further probiotic treatment (within 2-4 weeks), because they had an imbalance in the composition of the aerobic and anaerobic components. In contrast, in the control group, all infants had a gradual improvement in the composition of the microflora during the first month of life. In 77% of healthy infants, the composition of the microflora of the intestine was normal or to I compensated for the degree of dysbiosis, have fewer infants (23%) were grade II, and violations in the biocenosis, typical for III degree, not found in one infant by the end of the first month of life.

Thus, the predominance of conditionally pathogenic microflora and the deficit of protective microorganisms in the feces of deep-premature newborns, both conditionally healthy and with IID, was observed for a long time (1-2 months). However, this microbial imbalance was more profound and more common in infants with the infection.

During the 1st month of life in the feces of preterm infants in both groups, there was a frequent change (4-8 times) of aerobic and facultative microorganisms. According to the species composition, the fecal microflora of infants in the study groups had some differences.

Thus, Gram (-) microorganisms were found in infants with infection 1.5-4 times more often than in conditionally healthy infants: *Enterobacteriaceae* (*Klebsiella pneumonia*, *Enterobacter cloacae*, *Enterobacter liquefaciens*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Serratia marcescens*, *Proteus spp.* and others), *Ps. aeruginosa*, *S. aureus*, *Candida spp.*, hemolytic (h+) *E. coli* and hemolytic (h+) *Enterococcus spp.* (mainly *Enterococcus faecalis*, less often *Enterococcus faecium*) in high titers (lg6-9).

The growth of protective microflora in infants with infection was very slow. Low titers of *Bifidobacterium spp.* (\leq lg6), *Lactobacterium spp.* (\leq lg3) and lactose-positive *E. coli* (without hemolytic properties, h-) were present in 88% of infant before the end of the disease (at the 1st-2nd month of life). At the same time, there was a high content of conditionally pathogenic microorganisms (20-90% of the total number of microorganisms). Normalization of intestinal biocenosis occurred within 5-10 days after the withdrawal of antibiotics.

However, the use of probiotics on the background of antibacterial therapy provided a slight positive trend in the composition of the microflora: increased titers of protective microflora or remained at the same level without reducing the majority of infants; and level of opportunistic flora decreased, albeit at a slower speed than in the control group. More intensive normalization of the intestinal microflora occurred after the withdrawal of antibiotics. Thus, the use of probiotic in combination with antibiotics allowed to partially protect the intestinal microflora and contributed to its stabilization.

In conditionally healthy infants, normal titers of *Bifidobacterium spp.* (lg 9-10) were in 77% by day 28 – 30 of life and in 86% by the end of week 8. In the remaining conditionally healthy infants, the titer of these microorganisms had a borderline value (lg 7-8). High titers of *Lactobacterium spp.* (lg 6-8) occurred in 31% and 50% of conditionally healthy infants at the end of the 2nd and 3rd weeks of life, respectively. *Candida spp.* in low titers, lg 3-4 was found in 59% of infants, (h+) *Enterococcus spp.* in the lg 6-8 titer and (h+) *E. coli* in the lg6-8 titer were in 19% of infants during the first two weeks of life. However, by the end of the neonatal period, (h+) *Enterococcus spp.* and (h+) *E. coli* were not detected in the feces of the control group of infants. *Clostridium spp.* They was found in 28% of conditionally healthy infants in the lg 3-4 titers and in 11% of infants with disabilities in the lg 7-9 titers. In infants with infection, titers of *Clostridium spp.* were reduced to lg 3-4, or these microorganisms were not detected at all in the feces usually at the 2nd-3rd week of treatment. Thus, the process of formation of a stable protective microbiocenosis is quite long in preterm conditionally healthy infants. In most infants, it continues throughout the neonatal period. During this time, there may be failures in this physiological process due to a change in nutrition, accelerated growth in some infants, violations of the regime, the presence of non-infectious neonatal pathology (jaundice, hypoxic-ischemic damage to the Central nervous system, edematous syndrome). These disorders can lead to gastrointestinal dysfunction and an imbalance in the intestinal microflora. In this regard, it is also advisable for conditionally healthy and preterm infants to use probiotics in the neonatal period.

IV. Conclusion

Prolonged violations of the intestinal microflora in both conditionally healthy newborns and in premature infants with IID are the basis for the use of probiotics for 1-2 months. Taking into account that dysbiosis is characterized by combined changes on the part of various groups of microorganisms, to correct the composition of the intestinal microflora, it seems advisable to prescribe complex probiotics consisting of several strains-representatives of the protective microflora.

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