

Metabolism of the intestinal microbiota in children of the first year of life with intestinal pathology and its surgical correction as a potential marker of biological age

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Abstract. The article presents data on the study of the metabolic activity of the intestinal microbiota in children of the first year of life. The study was performed by gas-liquid chromatographic analysis with the determination of short-chain fatty acids (SCFA) in fecal matter, which reflects the microbiota metabolic activity. The content of fecal matter was determined, reflecting the activity of aerobic microbiota-acetic acid (C2); anaerobic microbiota - propionic (C3), butyric (C4) acids; the sum of acids (E); anaerobic index (AI), which is an integral indicator of the intestinal environment. It was found that despite surgical intervention in the intestine in the first months of life, the metabolic activity of microbiota in children with intestinal resection remains stable throughout the first year of life.

1 Introduction

In recent decades, the development of perinatal medicine and pediatric surgery has improved the survival rate of children who underwent operative therapy during the neonate period. An urgent problem in the treatment of patients after abdominal surgery is the restoration of intestinal microbiota, which serves as an important indicator of digestive tract functioning. In recent years, scientific interest in the metabolic function of intestinal microbiota and particularly products of short-chain fatty acids (SCFA) has increased significantly. This is due to the identification of their diverse biological functions that support human health at the systemic level and the potential to influence their concentrations with therapeutic and preventive purposes [1–5]. However, the age characteristics of SCFA production are normally not considered in both clinical studies and practical use of SCFA determination method, which can lead to erroneous conclusions regarding the effectiveness of therapeutic interventions and inadequate choice of optimal therapies [6]. Currently there are no major clinical studies that would focus on determining dynamic changes in the content and ratios of SCFA in fecal matter depending on the age. The practice of scientific research has shown that the method of SCFA determination in fecal matter is an available, non-invasive, non-expensive method which indirectly reflects the bacterial growth intensity and the state of the entire intestinal microbial and tissual

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complex and can also be used to diagnose various pathological conditions [7, 8]. Research of short-chain fatty acids (SCFA) by gas-liquid chromatographic analysis (GLCA) has high sensitivity and specificity, ease of reproduction, possibility of quick results obtaining [9, 10].

The aim of the study was to assess the metabolic activity of the gut microbiota, which is one of the potential markers of biological age; assessment was performed in children of the first year of life who have undergone intestinal resection.

2 Materials and Methods

The study included 37 children who underwent intestinal resection in their first year of life. For the analysis, the children were divided into groups according to age: the first group contained children between 1 and 3 months old in total of 9 persons; the second group was between 3 and 6 months of age (n=22), the third group - children of 6-12 months of life (n=37). All children were observed during the first year of life over a period of time. After surgical treatment, children received long courses of antibacterial therapy, specialized therapeutic nutrition and probiotic agents. The study inclusion criteria were: prompt treatment and resection of a part of the intestine in the first months of life, age from 1 to 12 months of life, consent of the patient's parents to participate in the study. Evaluation of antenatal and perinatal anamnesis was carried out based on analyzing the maternity hospital discharges, epicrisis of the surgical inpatient hospital and the history of the child's development. The rate of physical development (PD) was analyzed in each age group. The PD evaluation was conducted using regional centile tables. The level of biological maturity and morpho functional status (MFS) was determined [11]. The study of the SCFA in fecal matter was carried out using the GLC according to the method proposed by M.D. Ardatskaya, N.S. Ikonnikov, O.N. Minushkin [12]. The collection of biological material (fecal matter) for conducting the GLC analysis was carried out in the conditions of outpatient observation. The SCFA (C₂-C₆with isomers) determination method in bio substrates consisted of two steps: the sampling process and the gas-liquid chromatographic analysis itself. All samples prepared according to the described method were analyzed on model 6890 gas chromatograph by Hewlett Packard (USA). The content of individual acids was determined automatically as a processing result of the obtained chromatograms using the program "Chromos" (CJSC "Chemanalitservis", Russia). The following products of microbial metabolism (markers) were identified: C₂ - acetic acid; C₃ - propionic acid; iC₄ - isobutyric acid; C₄ — butyric acid; iC₅ — isovalerianic acid; C₅ — valerianic acid; iC₆ — isocaproic acid; C₆ — caproic acid. The identification of peaks on the obtained chromatograms was carried out by comparing the time of holding the analytical standard and the studied samples using the "Chromos" software.

Statistical processing of actual material was carried out with the help of the application programs Statistica 10.0. Descriptive statistics methods were used to analyze variables (mean values - M, standard deviations - σ , standard errors - m, median - Me). Comparative analysis for parametric indicators was carried out using the Student criterion, in case of non-parametric indicators - the Mann—Whitney criterion, the χ^2 criterion. The differences were considered reliable in the validity criterion value $p \leq 0.05$.

Results and Discussion

Antenatal period analysis revealed a high gestosis incidence in mothers during pregnancy — 97.3% (n=36). During pregnancy, almost half of mothers underwent ARVI (48.6%, n=18), a third of pregnancies occurred against the background of CFPI - 37.8% (n=14). 43.5% (n=16) of mothers were diagnosed with anemia, 45.9% (n=17) were observed with urogenital pathology; 13.5% (n=5) of pregnant women were diagnosed with gestational diabetes. 72.9% (n=27) of children were born by caesarean section. Almost half

of the children - 48.7% (n=18) were born at the 1st birth, as the 2nd birth - 37.8% (n=14), as the 3rd birth and more - 13.5% (n=5). The average gestation period in children who underwent intestinal resection was 35.9 ± 0.6 (min - 25, max - 40) weeks. Average weight at birth was 2606.59 ± 156.39 (min - 820, max 4100) g, average body length - 47.13 ± 1.05 (min - 32, max - 57) cm. Analysis of the surgical intervention level revealed that the resection of the small intestine part was carried out in half of children — 59.5% (n=22), resection of the large intestine section - in 40,5% (n=15). The cause of surgical treatment on the small intestine in 68.2% (n=15) was a congenital malformation of the gastrointestinal tract (CMF GIT), in 31.8% (n=7) - necrotizing enterocolitis (NEC). The following defects were identified in the CMF structure: jejunum atresia — 26.7% (n=4), abdominal fissure — 13.3% (n=2), ileum section volvulus — 20% (n=3), embryonal hernia— 6.7% (n=1), ileum atresia — 13.3% (n=2), multiple small intestine atresia — 26.7% (n=4). In 86.6% (n=13) of cases CMF GIT was detected prenatally. The cause of colon resection in 100% of cases was Hirschsprung disease. Overall, the average term of operative treatment was 17.7 ± 4.4 days. At small intestine resection - 4.0 ± 1.5 (min - 1, max - 30) days, in particular: at CMF - 1.1 ± 0.1 (min - 1, max - 2) days, at NEC - 8.6 ± 3.9 (min - 1, max - 30) days. The average term of surgical treatment for large intestine resection was 31.9 ± 8.8 (min - 2, max - 120) days. The PD assessment was conducted in each age group. By 3 months of life according to the level of biological maturity, PD corresponded to the real age of almost half of the children (44.4%, n=4), by 6 months this indicator decreased to 27.3% (n=6), by 12 months again almost half of the children (45.9%, n=17) corresponded to the real age in terms of biological maturity. In terms of biological maturity, the number of children with lag behind real age throughout the first year was observed in half of the children: 3 months of age — 44.4% (n=4), 6 months — 50% (n=11), 12 months — 45.9% (n=17). The distribution of children exceeding the real age was as follows: 3 months of age — 11.2% (n=1), 6 months — 22.7% (n=5), 12 months — 8.2% (n=3). Harmonious morpho functional status was determined in 55.6% (n=5) of children 3 months of age, in the second half of the year this figure was 40.9% (n=9). Due to weight deficiency, disharmonious morpho functional status persisted throughout the first year of life in half of the observed children: aged 3 months — 44.4% (n=4), 6 months — 51.9% (n=13), 12 months 40.5% (n =40.5). Attention should be paid to that 31.9% (n=7) and 8.1% (n=3) of children aged 6 and 12 months respectively had disharmonious status due to excess body weight. A study of SCFA levels in fecal matter was also conducted depending on age. SCFA fecal matter content in children after intestine resection did not have significant differences in separate age periods throughout the first year of life. The level of acetic acid (C2) as a obligate flora activity marker amounted to 0.792 ± 0.017 mg/g in all children: in children aged 1-3 months — 0.828 ± 0.049 mg/g, in children aged 3-6 months - 0.826 ± 0.024 mg /g, aged 6-12 months — 0.764 ± 0.025 mg/g. Both being indicators of anaerobic processes in the gut, propionic acid (C3) and butyric acid (C4) in all children amounted to 0.13 ± 0.014 and 0.075 ± 0.008 mg/g respectively. The highest C3 level was observed in children aged 6-12 months and amounted to 0.167 ± 0.022 mg/g, the lowest values were observed in children from 3 to 6 months - 0.079 ± 0.015 mg/g. The average value of fecal matter C4 in children aged 1-3 months was 0.047 ± 0.018 mg/g, in children from 6 to 12 months - 0.069 ± 0.009 mg/g. The maximum fecal matter C4 value was also observed in children 6-12 months and amounted to 0.095 ± 0.015 mg/g. Total acids content in fecal matter amounted to 38.451 ± 9.961 mg/g. The lowest total acid content was seen in children 1-3 months of age - 22.583 ± 7.077 mg/g, the maximum value was in 3-6 months - 57.611 ± 29.065 mg/g. In total, the anaerobic index amounted to 0.308 ± 0.032 mg/g, the maximum value was seen in children 6-12 months of age (0.367 ± 0.049 mg/g), the lowest value - in children 3-6 months of age (0.232 ± 0.034 mg/g). Analysis of intestinal microbiota metabolic activity in children depending on the resection level revealed a

reliable predominance of fecal matter C3 level in children who have undergone large intestine resection (Table 1, 2).

Table 1. SCFA content in fecal matter in children from 1 to 12 months of life who have undergone resection of the small (I) and large (II) intestines

Children of the 1st year of life who have experienced resection of the small (I) and large (II) intestines									
	1-3 months, n=9		3-6 months, n=22		6-12 months, n=37		1-12 months, n=68		p≤
	I	II	I	II	I	II	I	II	
	1	2	3	4	5	6	7	8	
C2, mg/g									
M	0.89	0.74	0.82	0.85	0.79	0.71	0.816	0.753	1.2=0.134
m	0.03	0.09	0.03	0.05	0.03	0.04	0.02	0.03	3.4=0.572
Me	0.85	0.77	0.78	0.86	0.84	0.67	0.84	0.752	5.6=0.099
σ	0.08	0.18	0.11	0.12	0.15	0.15	0.13	0.154	7.8=0.076
min	0.81	0.50	0.68	0.67	0.48	0.47	0.48	0.467	
max	0.99	0.92	0.99	0.99	0.99	0.99	0.99	0.992	
Total acid content, mg/g									
M	15.78	31.09	26.66	123.94	24.48	40.36	24.22	61.43	1.2=0.313
m	11.32	6.59	10.37	87.63	5.84	11.48	4.90	24.44	3.4=0.121
Me	3.04	33.34	12.73	28.98	16.98	19.31	15.35	26.80	5.6=0.186
σ	25.32	13.18	40.12	231.84	27.40	44.46	31.76	124.64	7.8=0.069
min	0.41	15.35	0.22	4.39	0.400	9.65	0.222	4.39	
max	59.72	42.22	160.67	640.05	117.33	158.45	160.67	640.05	

Table 2. Markers of intestinal microbiota anaerobic activity in children 1 to 12 months of life who have undergone resection of the small (I) and large (II) intestines

Children of the 1st year of life who have experienced resection of the small (I) and large (II) intestines									
	1-3 months, n=9		3-6 months, n=22		6-12 months, n=37		1-12 months, n=68		p≤
	I	II	I	II	I	II	I	II	
	1	2	3	4	5	6	7	8	
C3, mg/g									
M	0.07	0.19	0.08	0.08	0.137	0.21	0.109	0.171	1.2=0.103
m	0.03	0.06	0.02	0.03	0.03	0.04	0.02	0.03	3.4=0.883
Me	0.08	0.19	0.05	0.07	0.10	0.25	0.08	0.148	5.6=0.097
σ	0.07	0.12	0.07	0.08	0.12	0.14	0.10	0.135	7.8≤0.034
min	0.004	0.06	0.003	0.001	0.001	0.0001	0.0001	0.0001	
max	0.14	0.33	0.21	0.21	0.42	0.405	0.42	0.405	
C4, mg/g									
M	0.03	0.07	0.10	0.08	0.07	0.08	0.074	0.074	1.2=0.395
m	0.02	0.04	0.02	0.03	0.01	0.008	0.010	0.010	3.4=0.460
Me	0.01	0.04	0.09	0.07	0.05	0.06	0.063	0.065	5.6=0.565
σ	0.03	0.08	0.07	0.08	0.07	0.03	0.067	0.05	7.8=0.979
min	0.005	0.003	0.002	0.004	0.0001	0.015	0.0001	0.003	
max	0.066	0.17	0.24	0.242	0.26	0.13	0.259	0.242	

Anaerobic index (-)									
M	0.13	0.42	0.24	0.21	0.300	0.46	0.259	0.386	1.2=0.313
m	0.05	0.20	0.04	0.07	0.06	0.08	0.035	0.06	3.4=0.121
Me	0.18	0.29	0.29	0.162	0.19	0.49	0.196	0.329	5.6=0.186
σ	0.10	0.40	0.16	0.18	0.28	0.31	0.226	0.309	7.8=0.069
min	0.009	0.08	0.01	0.007	0.001	0.014	0.001	0.007	
max	0.23	0.99	0.46	0.487	1.08	1.14	1.082	1.139	

3 Conclusion

Most children who underwent intestine resection had a complicated course of the antenatal period. Throughout the first year of life, these children experienced reduced growth rates, which resulted in 45.5% real age lagging and 40.5% body weight deficiency determined by the end of the first year of life. In the analysis of intestinal microbiota metabolic activity, there were no significant differences in the content of the studied acids and anaerobic index during the individual age periods of the first year of life, which indicates a stable state of intestinal microbiota in children who have undergone surgical treatment with intestinal resection. It should be noted that the higher values of C3 levels in children who have suffered large intestine resection indicate the anaerobic flora activity, which is an indication for further observation and the subject of clinical understanding of “anaerobization” of the intestinal environment in this category of patients. On the one hand, the results obtained on the SCFA content in fecal matter may characterize the functional state of intestinal microbiota in children who underwent surgical intervention on the intestine in the first months of life; on the other hand, they “claim” to be potential biological age markers in a period of early ontogenesis.

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