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ABSTRACT

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FEATURES OF THE INTESTINAL MICROBIOME FORMATION AND THE LEVEL OF INTESTINAL INFLAMMATORY RESPONSE IN NEWBORNS

Objectives. We aim to explore the relationship between the composition of the faecal bacterial flora and the level of faecal calprotectin (FC) in sequential assessments of healthy term newborns.

Methods. The study was conducted thrice during the second, third, and fifth weeks of life. Culture method was used to identify the composition of intestinal microbiome indicators. FC levels were measured using an enzyme-linked immunosorbent assay. Mixed-effects linear modelling were applied to test for associations between calprotectin and various clinically relevant covariates/factors.

Results. Thirty-two infants were studied. Levels of Bifidobacterium was for children two weeks old (mean, standard deviation) – 8.91±1.47 CFU/g, for three weeks old – 8.44±1.58 CFU/g and for five weeks old – 8.09±1.49 CFU/g and Lactobacilli number for two weeks old – 7.69±1.15 CFU/g was higher than for three weeks old – 7.13±1.45 CFU/g and for five weeks old – 6.75±1.87 CFU/g. Level of FC for two weeks old was 280.6±123.68 mg/l, for three weeks old – 195.3±115.5 mg/l and for five weeks old – 153.5±34.6 mg/l. FC decreased with time and was higher in the presence of colonisation with Bifidobacterium, Lactobacilli, E.coli, and Opportunistic/Conditional pathogens.

Conclusions. The number of Bifidobacterium and Lactobacilli decreased during the neonatal period. FC also decreased but was higher in newborns colonised with

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Bifidobacterium, Lactobacilli, E.coli, and Opportunistic/Conditional pathogens, which apparently reflected stabilised composition of the microbiome and associated change in inflammatory processes.

Keywords. Faecal calprotectin, newborns, neonatal period.

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ОСОБЛИВОСТІ ФОРМУВАННЯ МІКРОБІОМУ КИШЕЧНИКА ТА РІВЕНЬ ЗАПАЛЬНОЇ ВІДПОВІДІ КИШЕЧНИКА У НОВОНАРОДЖЕНИХ

Мета. Дослідження взаємозв'язку між складом фекальної бактеріальної флори та рівнем фекального кальпротектину (ФК) у здорових доношених новонароджених з послідовним триразовим оцінюванням.

Методи. Дослідження проводилось тричі на другому, третьому та п'ятому тижнях життя. Визначення складу показників кишкового мікробіому здійснювалося культуральним методом. Рівні фекального кальпротектину визначалися за допомогою імуноферментного аналізу. Зв'язки між кальпротектином та різними клінічно значимими факторами перевірялися за допомогою лінійного моделювання зі змішаними ефектами.

Результати. Було обстежено тридцять двоє немовлят на другому, третьому та п'ятому тижнях життя. Встановлено кількість біфідобактерій для дітей двотижневого віку (середнє, стандартне відхилення) – 8,91±1,47 КУО/г, для тритижневого віку – 8,44±1,58 КУО/г та для п'ятитижневого віку – 8,09±1,49 КУО/г. Кількість лактобактерій для малюків на другому тижні життя становила – 7,69±1,15 КУО/г і була більшою, ніж у немовлят на третьому – 7,13±1,45 КУО/г і п'ятому тижнях – 6,75±1,87 КУО/г. Рівень ФК у дітей на другому тижні життя віку становив 280,6±123,68 мг/л, у у віці трьох тижнів – 195,3±115,5 мг/л і у п'ятитижневому віці – 153,5±34,6 мг/л. У подальшому рівень ФК знижувався і був вищим за наявності колонізації біфідобактеріями, лактобактеріями, E.coli та умовно-патогенними мікроорганізмами.

Висновки. У період новонародженості кількість біфідобактерій і лактобактерій зменшувалася. Рівень ФК також знизився але був вищим у новонароджених з колонізацією слизової оболонки кишківника біфідобактеріями, лактобактеріями, кишковою паличкою та умовно-патогенними мікроорганізмами, що, очевидно, відображало стабілізацію складу мікробіому та пов'язану з цим зміну при запальних процесах.

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Ключові слова. Фекальний кальпротектин, новонароджені, неонатальний період.

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ABBREVIATIONS

FC - Faecal calprotectin

CFU - Colony-Forming Unit

INTRODUCTION / ВСТУП

It is now recognized that the neonatal microbiome influences children's health and development in the long-term [1]. This consists not only in the formation of intestinal functions, but also in modulating work of other organ systems. The emergence and changes in the composition and functionality of the microbiome are the result of variables such as mode of birth, lactation, gestational age and the use of certain medications, including antibiotics [2, 3, 4, 5,]. The formation of the microbiome also leads to the development of inflammatory reactions, with the appearance of a number of intestinal metabolites. Inflammatory intestinal markers include a large group of heterogeneous substances that can be produced by the mucous membrane in response to the inflammatory process and are one of the methods for assessing the activity of certain diseases [6]. These markers include faecal calprotectin, which is currently considered a reliable method for assessing inflammatory processes and intestinal diseases in various age groups [7, 8]. It is used to differentiate necrotizing enterocolitis from other intestinal disease in newborns [9].

The state of the microbiome and FC levels in newborns had been studied by many authors. FC values in newborns of first days of life are characterized by higher values than in children of older age groups, with their subsequent decrease [3, 10]. Features of microbiome dynamics show its connection with FC [11]. The influence of FC on the qualitative and quantitative composition of the microbiome was noted - an increased in the dynamics of *Bifidobacterium* [12, 13] and a decreased in *Proteobacteria* [12], *Staphylococcus* [11] and *Enterococcus* [11]. In addition, gestational age [2, 4, 14], birth by cesarean section [2, 4, 14], male gender [3], low birth weight [3], postnatal age [2, 3], breastfeeding [3, 4, 14], antibiotic prescription [2, 3, 4, 5], and low 5′-Apgar score [3].

At the same time, some studies were conducted primarily in newborns of the first days of life, while other studies, although long-term, had long time intervals that could complicate the interpretation of the data [3, 11]. Not enough studies have been carried out in children in the dynamics of the neonatal period, towards its end [3].

Thus, the aim of the study was to describe FC levels in a cohort of healthy full-term newborns over time during the first month of life and the beginning of the second month of life, and to relate FC levels to the developing microbiome, nutrition, and time after birth.

MATERIALS AND METHODS

A total of thirty-two newborns with a mean age of 9.9±1.7 days were recruited with parental consent between August 2021 and December 2021. All participants were born in hospital. After birth, the babies were healthy, without concomitant pathology. The babies were discharged from hospital at an average of 3 days after birth. All babies remained at home for the duration of the study. The clinical characteristics were retrospectively reviewed from medical records.

To study the composition of the intestinal microflora, stool samples were collected three times in the second, third, and fifth weeks of life, respectively, and the culture method was used [2]. The same times were used to determine FC levels. The stool samples were examined using an enzyme-linked immunosorbent assay semi-automatic enzyme immunoassay analyzer IMark (Bio-Rad Laboratories Inc., Hercules, California, USA) to determine the level of FC in mg/l. The feces were collected at home by the parents in a disposable container with an airtight lid with a date and time stamp.

Initial statistical analyses were conducted using SPSS version 28.0 (IBM, NY, US). The continuous variables were presented as mean values ± standard deviation (M±SD).

Further analyses were undertaken in and R v4.3.2. The relationship between organism-level CFUs over time were assessed via linear mixed-effects modelling (LMM), including delivery method, gender, birthweight and feeding as fixed effects and patient ID as a random effect. Similarly, changes in FC concentration over time were associated with clinical factors during the neonatal period using LMMs. LMM models included patient as a random effect and the 4 microbiome indicators queried (*Bifidobacterium*, *Lactobacilli*, *E. coli*, Opportunistic/Conditional pathogenic microflora)

as fixed effects, alongside birthweight, time (weeks), delivery method, gender and feeding method as additional fixed effects. LMMs were fit using the lme4 (v1.1.35.1) and lmerTest (v3.1.3) R packages, with option REML=TRUE. For all statistical models, the significance threshold was defined as either $p<0.05$ as-is or divided by the number of tests performed, as per the Bonferroni method for multiple testing adjustment. All code pertaining to these analyses can be accessed from the GitHub repository https://github.com/CBFLivUni/neonatalIntestinal_fecalBacteriaInflammation.git.

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

RESULTS

General characteristics of the studied groups of newborns are presented in Table 1.

Table 1 – The general indexes of the newborns

Indexes	**Values**
Birthweight, grams, mean ± SD	3218±467
Length at birth, centimeters, mean ± SD	51±2.6
Abdominal circumference, centimeters, mean ± SD	33±1.7
Gestation age, weeks, mean ± SD	38.6±1.3
Apgar 1, mean ±SD	8.0±0.5
Apgar 2, mean ±SD	8.9±0.4
Boys, absolute value/%	19/59%
Girls, absolute value/%	13/41%
Mother's age, years, mean ± SD	29.7±6.11
Feeding by breastmilk, absolute value/%	20/62.5%
Feeding by formula, absolute value/%	8/25%
Feeding by breastmilk and formula, absolute value/%	4/12.5%
Father's age, years, mean ± SD	31.5±4.83
Child's age of analysis, days, mean ± SD	9.9±1.7
Number of pregnancies, mean ± SD	2.0±1.5
Parity, mean ± SD	1.7±1.0
Healthy women, absolute value/%	19/60.8%
Women with chronic pathology, absolute value/%	13/39.2%

Thirty-two full-term infants were studied, the number of boys outnumbered girls: 19 boys (59.4%) and 13 girls (40.6%). 5 (15.6%) of participants were born by Cesaerian section. Average values of gestational age were 38.6±1.3 weeks, weight was 3220±467 g, length was 51.2±2.6 cm. The mean values of the Apgar assessment at the 1st minute of life were 8.0±0.5 units, and 5 minutes were 8.9±0.4. Twenty children (62.5%) were fed by breastmilk, 8 children (25%) were feeding by formula and 4 (12.5%) children were fed by both breastmilk and formula.

The average levels of FC for neonates at two weeks was 280.6±123.7 mg/l (N=32), which decreased to 195.3±115.5 mg/l (N=32), and further decreased to 153.5±34.6 mg/l, (N=32) by five weeks (data not shown). Notably, a significant difference was detectable between 2 and 3 weeks, but not 3 and 5 weeks (P=0.782). This can likely be explained by observation at the patient-level, whereby while a majority of neonates show decreases between 2 and 3 weeks, between 3 and 5 weeks FC levels don't uniformly decrease but instead converge to a common value, with many patients showing increased as well as decreased FC (data not shown).

The results of feces analyses parameters in the groups are presented in Table 2.

Table 2 – Values of feces analyses

Indexes	**2 weeks**	**3 weeks**	**5 weeks**	**p value**
Intestinal microbiome:				
Bifidobacterium, CFU/g, mean ± SD	8.91±1.47	8.44±1.58	8.09±1.49	0.0042
Lactobacilli, CFU/g, mean ± SD	7.69±1.15	7.13±1.45	6.75±1.87	0.00066
Total number of *E.coli*, CFU/g, mean ± SD	6.31±1.49	6.78±1.31	6.47±1.19	
E.coli with weak enzymatic ability, CFU/g, mean ± SD	4.33±0.58	3.5±0.71	3.33±0.58	
Opportunistic pathogens, CFU/g, mean ± SD	4.72±1.28	4.41±1.04	4.28±1.17	
Level of fecal calprotectin, mg/l, mean ± SD	280.6 ±123.7	195.3±115.5	153.5±34.6	0.000068

Human intestinal microflora is a complex of microbial species that normally inhabit bowel. The most common bacteria isolated were *Bifidobacterium*. At the same time, levels of *Bifidobacterium* were detectable for children aged two weeks (8.91±1.7 CFU/g, N=32) for children aged three weeks (8.44±1.58 CFU/g, N=32) and for children aged five weeks (8.09±1.49 CFU/g, N=32), a significant change over time (p=0.004, β=-0.2, 95% CI [-0.40, -0.065]) (Figure 1). The second parameter was *Lactobacilli* number for neonates aged two weeks (7.69±1.15 CFU/g) was increased than for neonates aged three weeks (7.13±1.45 CFU/g) and neonates aged five weeks – 6.75± 1.87 (CFU/g), which was similarly significant over time (p=0.0007, β=-0.23, 95% CI [-0.37, -0.027]) (Figure 1). Total number of *E. coli* for newborns aged two weeks was 6.31±1.49 CFU/g), for newborns aged three weeks (6.78±1.31 CFU/g) and for newborns aged five weeks (6.47±1.19 CFU/g), at the same time, there was no significant difference between them (Figure 1). Levels of opportunistic pathogens for babies aged two weeks was (4.72±1.28 CFU/g), for babies aged three weeks (4.41±1.04 CFU/g) and for babies aged five weeks (4.28±1.17 CFU/g), which was similarly non-significant (p=0.11) (Figure 1).

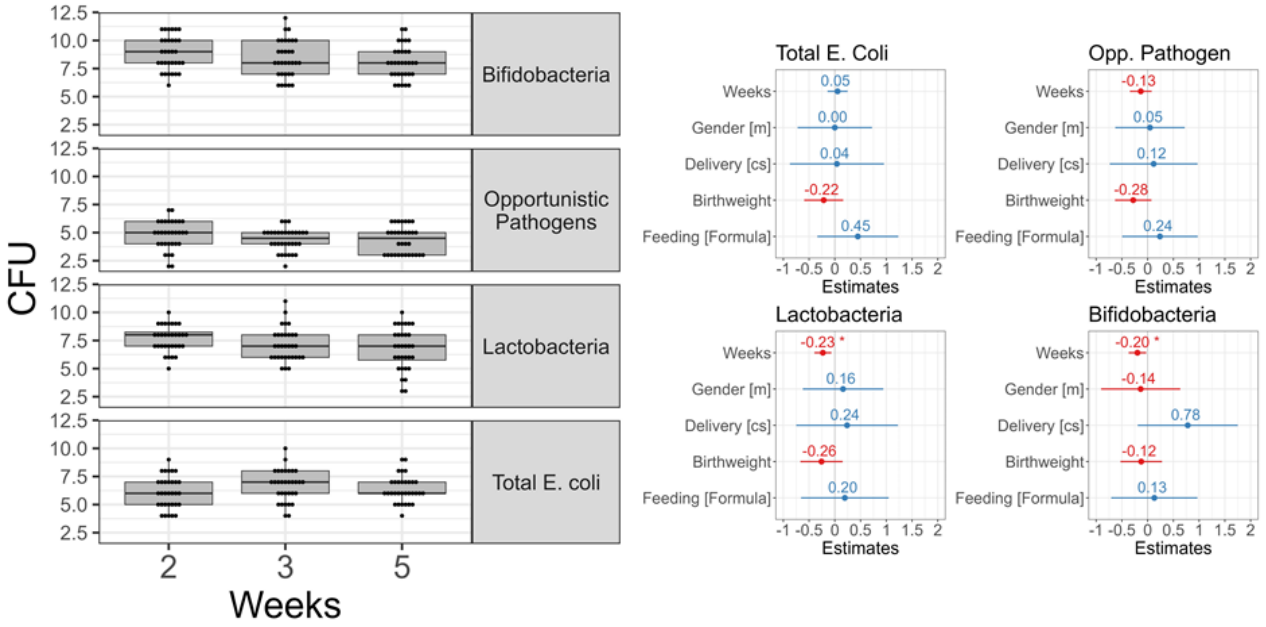

Figure 1 *– CFUs for Bifidobacteria and Lactobacteria show significant decreases in neonatal faeces. (A) CFUs were enumerated from stool samples for 4 groupings of faecal bacteria, Bifidobacteria, Lactobacteria, Total E. coli count and opportunistic pathogens. Samples were taken at 2, 3 and 5 weeks post-birth. Dots represent individual measurements. (B) Linear mixed-effects models (LMMs) were fit regressing weeks, gender, delivery method, feeding method and birthweight as fixed effects and patient as random effects onto CFUs for each grouping of bacteria, fitting one LMM per organism. Dot-and-whisker plots show coefficient estimates as well as confidence intervals and significance (*), adjusted for multiple testing by Bonferonni's method for multiple testing correction (p<0.0125). Red and blue dot-and-whiskers represent negative and positive coefficient estimates, respectively. Text in square brackets indicates the level of the relevant categorical variable that is being tested against baseline*

The associations between faecal calprotectin levels and neonatal characteristics and microbiome indicators over the follow-up period was determined using an LMM (Figure 2).

Birth weight had a negative weak non-significant (Figure 2). Age in weeks also had a moderately strong negative relationship with faecal calprotectin levels, and was significant (p=0.000068, β=-0.29, 99% CI [-0.43, -0.15]) (Figure 2). The sex of the child had no detectable effect on the value of faecal calprotectin, neither did the delivery method (Figure 2). Artificial and/or mixed feeding increased the likelihood of an increased calprotectin value, but only slightly and non-significantly (p= 0.29, β=0.18, 99% CI [-0.17,0.55]) (Figure 2). A study of the relationship between the abundance of intestinal microorganisms and the value of fecal calprotectin showed a positive relationship. This was small but significant for all microorganisms tested (p<0.05), except for Lactobacilli, which was non-significant (p= 0.05) (Figure 2). Estimated coefficients for these relationships were fairly consistent, differing only slightly in a range of 0.18-0.22 units, with similar standard errors (0.010, 0.091, 0.083, 0.078 for *Bifidobacteria*, *Lactobacteria*, Total *E. coli* and opportunistic pathogens, respectively) (Figure 2).

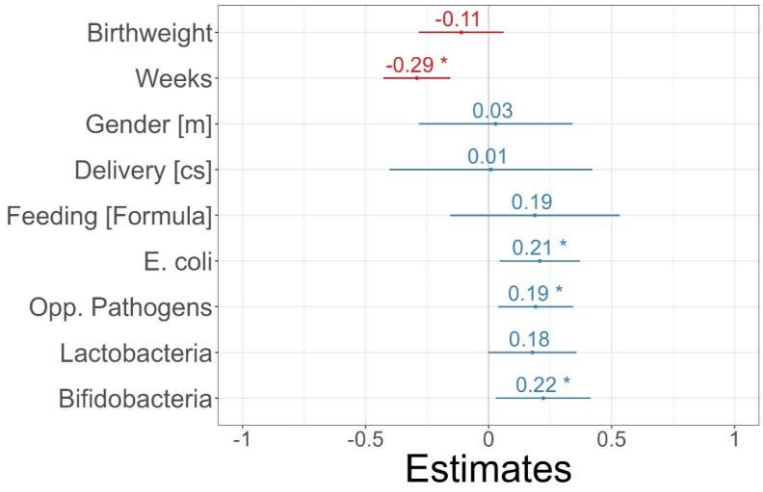

Figure 2 – *Bacteria show significant associations with calprotectin. A linear mixed-effects model (LMM) was fit for all samples, using weeks, birthweight, delivery method, feeding method, gender and all 4 bacterial groupings as fixed effects and patient ID as a random effect. Dot-and-whisker plots show coefficient estimates, alongside confidence intervals and an indicator of significance (*) (p<0.05). Red and blue dot-and-whiskers indicate negative and positive coefficient estimates, respectively. Text in square brackets indicate the level of the categorical factor that is being tested from baseline*

DISCUSSION

This is one of the first reports of the relationship between the composition of the early intestinal microflora and the level of intestinal inflammatory response by determining and comparing the composition of the intestinal microflora and the level of FC in the neonatal period of healthy term newborns. Careful follow-up generated complete time series from 32 neonates allowing detailed analysis of the longitudinal interactions between important variables. We believe this is the largest complete time series reported to date that describes the relationship between faecal colonisation, FC, and time in healthy newborns.

In common with hospitalized neonates, the level of FC is higher in neonates living at home aged two weeks compared with three and five weeks of life, which may be due to the formation of the gastrointestinal tract which includes a wide range of changes during the first few weeks of life. This trend is re-capitulated in this study, with a significant net decrease between day 2 and 3, with a subsequent convergence to a decreased value by day 5, relative to day 2. In hospitalized neonates FC levels tended to decrease with increasing age [2, 15]. FC concentrations showed a negative trend in the first 4 weeks of life (P=0.004) [3], from the neonatal period to 3 months (r=-0.490, p < 0.001) [16], from the neonatal period to 6-11 months of life (r = −0.603, P<0.01) [17]. Scientists suggest that increased secretion of FC may be involved in the innate immune system and thus may be beneficial for intestinal development in the first weeks of life, so an increase in FC may be a physiological process [2, 17]. A decrease in FC with age may indicate a maturation process, including decreased intestinal permeability, in the intestinal mucosa [16]. At the same

time, evidence was obtained that there is a positive correlation between FC and age (p=0.0004) [15], (r=0.45 p=0.0002) [16].

One of the important factors that influences FC levels is the intestinal microbiome [18]. Colonisation of the sterile intestine by microorganisms causes an inflammatory response, which is reflected by an increase in FC levels [19]. Our data show a relationship with the number of microorganisms and FC levels. The values of *Bifidobacterium*, *Lactobacilli*, and *E. coli* were high at the 2 week of life visit, after which a decrease in their level was observed, which was accompanied by a decrease in FC levels. According to this model, the relationship between FC and microbiome indicators was significant with values of 0.18 to 0.22 units. Some authors have obtained similar results. *Bifidobacterium* is associated with a significant decrease in FC levels in some studies (p=0,039) [21], but no correlation was also found in the work of other scientists (r=+0,11, p=0,19) [2]. *Lactobacillus* counts tended to be positively correlated with FC (r=+0.17, p=0.06) [2]. The number of *E. coli* had a negative correlation with FC (p = 0.039) [21] or evidence of no association was obtained (r=+0.12, p=0.15) [2]. Other studies have found no correlation between intestinal microbiome colonisation and FC levels [20, 21] in VLBW and healthy babies. It can be suggested that intestinal bacteria and specific individual components of the commensal microbiota may have different abilities to stimulate transepithelial granulocyte migration or induce the release of FC into the intestinal lumen [16]. Thus, stress caused by childbirth, adaptation to extrauterine life and the process of colonisation of the intestine by bacteria may be the cause of higher levels of FC in infants compared to older age groups. Also, these results may be associated with the immunomodulatory function of FC, which may be involved in immune formation early in life, which may be a prerequisite for proper immune tolerance to gut microbial composition in later life [21].

Using a regression model showed a negative correlation between FC levels and birth weight, significant at 3 weeks of life, as well as a negative correlation with male gender and a positive correlation with formula feeding. Some studies showed a negative significant association between birth weight and FC levels (r=−0.144, p=0.097) [3], (p=0.042) [15], but other studies have found no association between birth weight and FC levels (r=0.047, p=0.356) [17], (p=0.351) [22]. Some previous studies have shown that FC correlates with birth weight and thus may reflect the intrauterine environment, intestinal immaturity and hypoxic-ischemic damage to the intestinal mucosa [3].

Researchers had reported no association between gender and FC levels (p=0.536) [17], (p=0.087) [16] but other studies have observed negative associations of FC levels with male gender (p= 0.008) [3] or female gender in preterm infants (p=0.003) [22]. It has been suggested that prematurity and female gender may contribute to the later development of intestinal pathology or necrotizing enterocolitis with a subsequent increase in FC levels [22].

Previous studies showed a negative significant relationship in children receiving formula/mixed feeding with FC (p = 0.026) [3] levels. Some studies had reported that the average concentration of FC was significantly higher in breastfed than in formula-fed children (p<0.001) [16, 23], or there was no relationship between feeding and FC levels at all [17]. Some findings suggest that higher FC levels in exclusively breastfed infants compared to formula-fed infants <6 months old may be consistent with breast milk promoting intestinal mucosal growth and decreased intestinal permeability [16].

The limitations of the study include the small size of the sample and the use of culture rather than more specific methods. We report genus rather than species. There are no reference ranges for FC in neonates. One methodological limitation of this analysis is that the abundances of organisms correlate with each-other, meaning there may be some issues in terms of collinearity between their measurements, which may make it difficult to discern which organism is truly causative with respect to FC level. Nevertheless, such associative analysis is of merit and the robust methodology provides similar results to previous work and can be applied in a difficult field setting.

Future research could use this methodology to characterize a cohort of neonates for exposure to colonisation and a marker of intestinal inflammation shortly after birth. We provide baseline values of point estimates and the extent of variation in our setting. The cohort could be followed-up to capture long-term outcomes in order to explore relationships between colonisation, neonatal FC, and atopic diseases. It would be interesting to study the time course of colonisation and inflammation among in babies affected by perinatal asphyxia.

CONCLUSIONS

There was a change in the quantitative composition of the intestinal microflora in healthy newborns living at home, with reducing levels of decreased level of *Bifidobacterium*, *Lactobacilli* and *E. coli* during the interval from two to five weeks of life. FC also decreased during the study period, although the reduction in FC was less marked in the presence of colonisation of the preponderant bacteria. These results reflect the stabilisation of the composition of the intestinal microflora and the associated change in the level of the inflammatory process. It is feasible to study time course of multiple variables in healthy newborns.

AUTHOR CONTRIBUTIONS / ВКЛАД АВТОРІВ

All authors substantively contributed to the drafting of the initial and revised versions of this paper. They take full responsibility for the integrity of all aspects of the work.

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ARTIFICIAL INTELLIGENCE DISCLOSURE / ВИКОРИСТАННЯ ШТУЧНОГО ІНТЕЛЕКТУ

The authors confirm that no artificial intelligence-based technologies were utilized in the writing or editing of the manuscript.

REFERENCES/СПИСОК ЛІТЕРАТУРИ

1. Ronan V, Yeasin R, Claud EC. Childhood Development and the Microbiome-The Intestinal Microbiota in Maintenance of Health and Development of Disease During Childhood Development. Gastroenterology. 2021 Jan;160(2):495-506. https://www.doi.org/10.1053/j.gastro.2020.08.065
2. Rougé C, Butel M-J, Piloquet H, Ferraris L, Legrand A, Vodovar M et al. Fecal Calprotectin Excretion in Preterm Infants during the Neonatal Period. PLoS One. 2010; 5(6): e11083. https://www.doi.org/10.1371/journal.pone.0011083
3. Park J S, Cho J Y et. al. Dynamic Changes of Fecal Calprotectin and Related Clinical Factors in Neonates. Front Pediatr. 2020; 8: 326. https://www.doi.org/10.3389/fped.2020.00326
4. Łoniewska B, Adamek K, Węgrzyn D, Kaczmarczyk M, Skonieczna-Żydecka K, Clark J, Adler G, Tousty J, Uzar I, Tousty P, Łoniewski I. Analysis of Faecal Zonulin and Calprotectin Concentrations in Healthy Children During the First Two Years of Life. An Observational Prospective Cohort Study. J Clin Med. 2020 Mar 12;9(3):777. https://www.doi.org/10.3390/jcm9030777
5. Bischoff SC, Barbara G, Buurman W, Ockhuizen T, Schulzke JD, Serino M, Tilg H, Watson A, Wells JM. Intestinal permeability--a new target for disease prevention and therapy. BMC Gastroenterol. 2014 Nov 18;14:189. https://www.doi.org/10.1186/s12876-014-0189-7
6. Däbritz J, Musci J, Foell D. Diagnostic utility of faecal biomarkers in patients with irritable bowel syndrome. World J Gastroenterol. 2014 Jan 14;20(2):363-75. https://www.doi.org/10.3748/wjg.v20.i2.363
7. Tibble J, Teahon K, Thjodleifsson B, Roseth A, Sigthorsson G, Bridgeret S al. A simple method for assessing intestinal inflammation in Crohn's disease. Gut 2000; 47:506–513. https://www.doi.org/10.1136/gut.47.4.506
8. Ayling R., Kok K. Fecal Calprotectin. Adv Clin Chem 2018;87:161-190. https://www.doi.org/10.1016/bs.acc.2018.07.005
9. Farghaly MAA, Ali MAM, Ramey S, Said W, Abdelkarem A, Collin M. Characteristics of fecal calprotectin as an early marker for suspected necrotizing enterocolitis in newborns exclusively fed maternal breast milk: a case-control study. Proc (Bayl Univ Med Cent). 2023 Dec 20;37(1):43-47. https://www.doi.org/10.1080/08998280.2023.2277580
10. Yoon JM, Park JY, Ko KO, Lim JW, Cheon EJ, Kim HJ. Fecal calprotectin concentration in neonatal necrotizing enterocolitis. Korean J Pediatr. 2014 Aug;57(8):351-6. https://www.doi.org/10.3345/kjp.2014.57.8.351
11. Kaczmarczyk M, Löber U, Adamek K, Węgrzyn D, Skonieczna-Żydecka K, Malinowski D, Łoniewski I, Markó L, Ulas T, Forslund SK, Łoniewska B. The gut microbiota is associated with the small intestinal paracellular permeability and the development of the immune system in healthy children during the first two years of life. J Transl Med. 2021 Apr 28;19(1):177. https://www.doi.org/10.1186/s12967-021-02839-w
12. Shin NR, Whon TW, Bae JW. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 2015 Sep;33(9):496-503. https://www.doi.org/10.1016/j.tibtech.2015.06.011
13. Henrick BM, Chew S, Casaburi G, Brown HK, Frese SA, Zhou Y, Underwood MA, Smilowitz JT. Colonization by B. infantis EVC001 modulates enteric inflammation in exclusively breastfed infants. Pediatr Res. 2019 Dec;86(6):749-757 https://www.doi.org/10.1038/s41390-019-0533-2
14. Weström B, Arévalo Sureda E, Pierzynowska K, Pierzynowski SG, Pérez-Cano FJ. The Immature Gut Barrier and Its Importance in Establishing Immunity in Newborn Mammals. Front Immunol. 2020 Jun 9;11:1153. https://www.doi.org/10.3389/fimmu.2020.01153
15. Lisowska-Myjak B, Skarżyńska E, Żytyńska-Daniluk J. Calprotectin in Serially Collected Meconium Portions as a Biomarker for Intrauterine Fetal Environment. Fetal Diagn Ther. 2018;43(1):68-71. https://www.doi.org/10.1159/000472150

16. F, Ma J, Geng S, Wang J, Liu J, Zhang J, Sheng X. Fecal calprotectin concentrations in healthy children aged 1-18 months. PLoS One. 2015 Mar 5;10(3):e0119574. https://www.doi.org/10.1371/journal.pone.0119574
17. Velasco Rodríguez-Belvís M, Viada Bris JF, Plata Fernández C, García-Salido A, Asensio Antón J, Domínguez Ortega G, Muñoz Codoceo RA. Normal fecal calprotectin levels in healthy children are higher than in adults and decrease with age. Paediatr Child Health. 2020 Aug;25(5):286-292. https://www.doi.org/10.1093/pch/pxz070
18. M van de Guchte M, Mondot S, Doré J. Dynamic Properties of the Intestinal Ecosystem Call for Combination Therapies, Targeting Inflammation and Microbiota, in Ulcerative Colitis. Gastroenterology. 2021 Dec;161(6):1969-1981.e12. https://doi.org/10.1053/j.gastro.2021.08.057
19. Willers M, Ulas T, Völlger L, et al.. S100A8 and S100A9 are important for postnatal development of gut microbiota and immune system in mice and infants. Gastroenterology 2020;159:2130–45. https://www.doi.org/10.1053/j.gastro.2020.08.019
20. Bjorkstrom MV, Hall L, Soderlund S, Hakansson EG, Hakansson S, Domellöf M. Intestinal flora in very low-birth weight infants. Acta Paediatr.2009;98:1762–1767. https://www.doi.org/10.1111/j.1651-2227.2009.01471.x
21. Kim ES, Tarassishin L, Eisele C, Barre A, Nair N, Rendonet A al. Longitudinal Changes in Fecal Calprotectin Levels Among Pregnant Women With and Without Inflammatory Bowel Disease and Their Babies. Gastroenterology. 2021;160(4):1118-1130.e3. https://www.doi.org/10.1053/j.gastro.2020.11.050
22. Cekovic J R., Prodanovic N S., Mijailovic S S. et al. The perinatal factors that influence the excretion of fecal calprotectin in premature-born children. Open Medicine. 2022;17(1):1275-1281. https://doi.org/10.1515/med-2022-0522
23. Koivusaari K, Niinistö S, Nevalainen J, Honkanen J, Ruohtula T, Koreasalo M, Ahonen S, Åkerlund M, Tapanainen H, Siljander H, Miettinen ME, Alatossava T, Ilonen J, Vaarala O, Knip M, Virtanen SM. Infant Feeding, Gut Permeability, and Gut Inflammation Markers. J Pediatr Gastroenterol Nutr. 2023 Jun 1;76(6):822-829. https://www.doi.org/10.1097/MPG.0000000000003756

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