

PEDIATRICS

LONGITUDINAL CHANGES IN SERUM INTESTINAL FATTY ACID-BINDING PROTEIN AND CLAUDIN-2 LEVELS IN CHILDREN DURING THE FIRST 24 MONTHS OF LIFE: A PROSPECTIVE OBSERVATIONAL COHORT STUDY

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ABSTRACT

Background. Maturation of the intestinal epithelial barrier in early life is accompanied by variability in biomarkers of enterocyte injury and permeability. However, longitudinal serum profiles of I-FABP and claudin-2 in seemingly healthy children, including those born preterm, remain insufficiently studied.

Aim. To assess the dynamics of serum I-FABP and claudin-2 concentrations in children born at 34–41 weeks' gestation during the first 24 months of life, with consideration of gestational age.

Methods. This prospective observational cohort study included three time points: up to 7 days; 1–1.5 months/postmenstrual age (PMA) 40–41 weeks; and 18–24 months. At time points 1–2, 98 newborns were enrolled; by time point 3, follow-up was completed in 88 children: preterm (34–36 weeks, $n = 30$) and term (37–41 weeks, $n = 58$). Loss to follow-up was 10.2 % ($n = 10$). I-FABP and claudin-2 were measured by ELISA.

Results. I-FABP remained stable during the first 1–1.5 months and increased by the second year of life: in preterm children 1.12 [1.07; 1.30] – 1.30 [0.97; 1.87] – 6.09 [3.18; 6.65] ng/mL ($p < 0.001$), and in term children 1.10 [0.80; 1.95] – 1.21 [0.92; 1.73] – 3.89 [2.33; 6.10] ng/mL ($p < 0.001$); no between-group differences were observed at the respective time points ($p = 0.738$; 0.610; 0.131). In preterm children, claudin-2 did not change significantly: 6.76 [3.00; 9.14] – 7.59 [5.20; 10.96] – 7.77 [6.33; 9.20] ng/mL ($p = 0.497$). In term children, claudin-2 increased: 1.85 [1.02; 5.22] – 5.10 [3.47; 8.31] – 7.95 [6.32; 11.28] ng/mL ($p < 0.001$). At time points 1–2, claudin-2 values were higher in preterm than in term children ($p = 0.006$; $p = 0.009$), whereas at time point 3 they were comparable ($p = 0.326$).

Conclusions. Within the 34–41-week gestational range, gestational age does not determine I-FABP levels, whereas claudin-2 is gestational age-dependent. Interpretation of these biomarkers requires consideration of both chronological age and gestational age.

Keywords: intestinal permeability, preterm infants, I-FABP, claudin-2, intestinal epithelial barrier, early childhood

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ДИНАМИКА ИНТЕСТИНАЛЬНОГО БЕЛКА, СВЯЗЫВАЮЩЕГО ЖИРНЫЕ КИСЛОТЫ И КЛАУДИН-2 В СЫВОРОТКЕ КРОВИ У ДЕТЕЙ В ПЕРВЫЕ 24 МЕСЯЦА ЖИЗНИ: ПРОСПЕКТИВНОЕ ОБСЕРВАЦИОННОЕ КОГОРТНОЕ ИССЛЕДОВАНИЕ

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РЕЗЮМЕ

Обоснование. Созревание кишечного эпителиального барьера в раннем возрасте сопровождается вариабельностью маркеров повреждения и проницаемости энтероцитов; продольные профили сывороточных I-FABP (Intestinal fatty acid-binding protein) и клаудин-2 у условно здоровых детей, включая поздних недоношенных, изучены недостаточно.

Цель. Оценить динамику концентраций I-FABP и клаудин-2 в сыворотке крови у детей, рождённых на сроке 34–41 недели гестации, в первые 24 месяца жизни с учётом гестационного возраста.

Методы. Проспективное обсервационное когортное исследование (3 этапа: до 7 суток; 1–1,5 месяца/ПМВ 40–41 нед.; 18–24 месяца). На 1–2 этапах включено 98 новорождённых; к 3 этапу завершили наблюдение 88 детей: недоношенные 34–36 нед. ($n = 30$) и доношенные 37–41 нед. ($n = 58$); потери 10,2 % ($n = 10$). I-FABP и клаудин-2 определяли методом ИФА.

Результаты. I-FABP был стабильным в первые 1–1,5 месяца и увеличивался ко 2-му году жизни: у недоношенных 1,12 [1,07;1,30] – 1,30 [0,97;1,87] – 6,09 [3,18;6,65] нг/мл ($p < 0,001$), у доношенных 1,10 [0,80;1,95] – 1,21 [0,92;1,73] – 3,89 [2,33;6,10] нг/мл ($p < 0,001$); межгрупповых различий не выявлено ($p = 0,738$; 0,610; 0,131). Для клаудин-2 у недоношенных изменений не отмечено: 6,76 [3,00;9,14] – 7,59 [5,20;10,96] – 7,77 [6,33;9,20] нг/мл ($p = 0,497$). У доношенных клаудин-2 возрастал: 1,85 [1,02;5,22] – 5,10 [3,47;8,31] – 7,95 [6,32;11,28] нг/мл ($p < 0,001$); на 1–2 этапах значения были выше у недоношенных ($p = 0,006$; $p = 0,0009$), на 3-й — сопоставимы ($p = 0,326$).

Заключение. В диапазоне 34–41 недели гестации гестационный возраст не определяет уровень I-FABP, тогда как клаудин-2 зависит от гестационного возраста; интерпретация маркеров требует учёта возраста и срока гестации.

Ключевые слова: кишечная проницаемость, недоношенные, I-FABP, клаудин-2, кишечный эпителиальный барьер, ранний возраст

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BACKGROUND

The maturation of the intestinal wall determines the formation of a barrier function and intestinal permeability, which are key factors for efficient nutrient absorption, immune defense, and, consequently, resistance to infections in children during the first years of life. Results from modern cohort studies provide compelling evidence for an association between impaired intestinal barrier function in infancy and reduced growth rates [1], systemic inflammatory response [2, 3], and subsequent health outcomes in later childhood, with the severity of pathological processes depending on age, feeding type, antigenic load, and disease frequency [2–4].

Intestinal fatty acid-binding protein (I-FABP) is a cytosolic protein of small intestinal epithelial cells that is detected in biological fluids (blood and urine) only upon enterocyte damage [5, 6]. Consequently, in the current literature, I-FABP is considered primarily as a marker of intestinal epithelial cell injury rather than a direct indicator of intestinal permeability [5, 7]. In contrast, Claudin-2, a tight junction protein responsible for paracellular transport of water and cations, is regarded as a non-invasive marker of intestinal permeability [8, 9].

The high diagnostic and prognostic value of I-FABP for early detection and prediction of conditions associated with enterocyte death in neonatal and pediatric practice has been convincingly demonstrated [5, 8, 10]. For example, Prikhodchenko N.G. et al. demonstrated the diagnostic significance of I-FABP in children with protein-induced enteropathy [11] and gastrointestinal forms of food allergy [12]. The prognostic and diagnostic potential of I-FABP in necrotizing enterocolitis has been confirmed not only in original studies [7], but also in a meta-analysis [13]. The specificity of I-FABP as a marker of intestinal injury is clearly illustrated by the results of a study assessing intestinal permeability via serum biomarkers upon early introduction of allergens, particularly peanuts, into the diet: no changes in I-FABP concentration were detected in cases of predominant skin involvement without clinically significant gastrointestinal symptoms [14].

The diagnostic potential of Claudin-2 is currently less well studied. However, publications are emerging that demonstrate alterations in tight junction protein expression, particularly Claudin-2, in children with conditions associated with increased intestinal permeability [15, 16].

Although the first studies on intestinal permeability in children were published more than 30 years ago [17], a significant knowledge gap remains regarding age-related variability of biomarkers of intestinal injury and permeability in apparently healthy infants. Most studies on I-FABP and Claudin-2 concentrations in biological fluids have been conducted in high-risk groups, such as NEC [7, 13], are experimental in nature [16], or are limited to specific disease entities [11, 12, 14].

At the same time, it has been established that intestinal barrier function indices in early life undergo dynamic changes depending on nutrition, infectious diseases, and social factors [2–4, 18, 19]. However, longitudinal profiles of I-FABP and Claudin-2 concentrations during the first 24 months of life in apparently healthy children, including preterm infants, remain insufficiently characterized.

The gestational age range of 34–41 weeks includes late preterm, early term, and full-term infants. These groups exhibit significant differences in morphofunctional maturity of the intestinal barrier, postnatal adaptation characteristics, and nutritional requirements. The absence of age-specific reference values and longitudinal profiles of I-FABP and Claudin-2 concentrations complicates the differentiation of physiological variability from changes in permeability and enterocyte death in pathological conditions accompanied by biomarker elevation, thereby limiting the clinical interpretation of results.

AIM

To determine the levels of I-FABP and Claudin-2 in apparently healthy children during the first 2 years of life in a longitudinal manner.

METHODS

Study Design

To achieve the aim, a prospective observational cohort study with longitudinal follow-up of patients was conducted. A flowchart of the study design is presented in Figure 1.

Eligibility Criteria: gestational age 34–41 weeks; postnatal age less than 7 days at the first assessment; at the second assessment: for Group 1 patients – postmenstrual age (PMA) 40–41 weeks; for Group 2 – postnatal age 1–1.5 months; at the third assessment – less than 24 months; written informed consent for participation in the study provided by the child's legal representative.

Exclusion Criteria: surgical pathology and/or congenital gastrointestinal malformations; clinical manifestations of perinatal infection; concomitant genetic syndromes, chromosomal abnormalities, metabolic disorders; severe congenital malformations of other organs and systems requiring surgical intervention in the neonatal period; established diagnosis of disaccharidase deficiency or other enteropathies; need for total parenteral nutrition; loss of contact with the family or refusal of the legal representative to continue participation; development during follow-up of a severe disease or condition potentially affecting gastrointestinal function and/or prescription of long-term specific therapy potentially affecting intestinal permeability markers.

Study Setting

Patient enrollment and subsequent follow-up were conducted at the Voronezh Regional Children's Clinical Hospital No. 1. Laboratory analyses were performed at the Research Institute of Experimental Biology and Medicine of the Burdenko Voronezh State Medical University, Voronezh, Russia.

Study Duration

The initial study cohort was formed from March 2023 to May 2024. Children were examined at three time points: within the first 7 days of life; upon reaching postmenstrual age 40–41 weeks in Group 1, and at age

1–1.5 months in Group 2; and at age 18–24 months. The third follow-up was conducted from March 2025 to January 2026.

Description of Medical Intervention

Blood sampling was performed by peripheral venipuncture by a trained nurse using standard technique. Blood was collected in the morning, on an empty stomach.

Study Outcomes

Primary Outcome: dynamics of serum I-FABP and Claudin-2 levels during the first 24 months of life in children depending on gestational age.

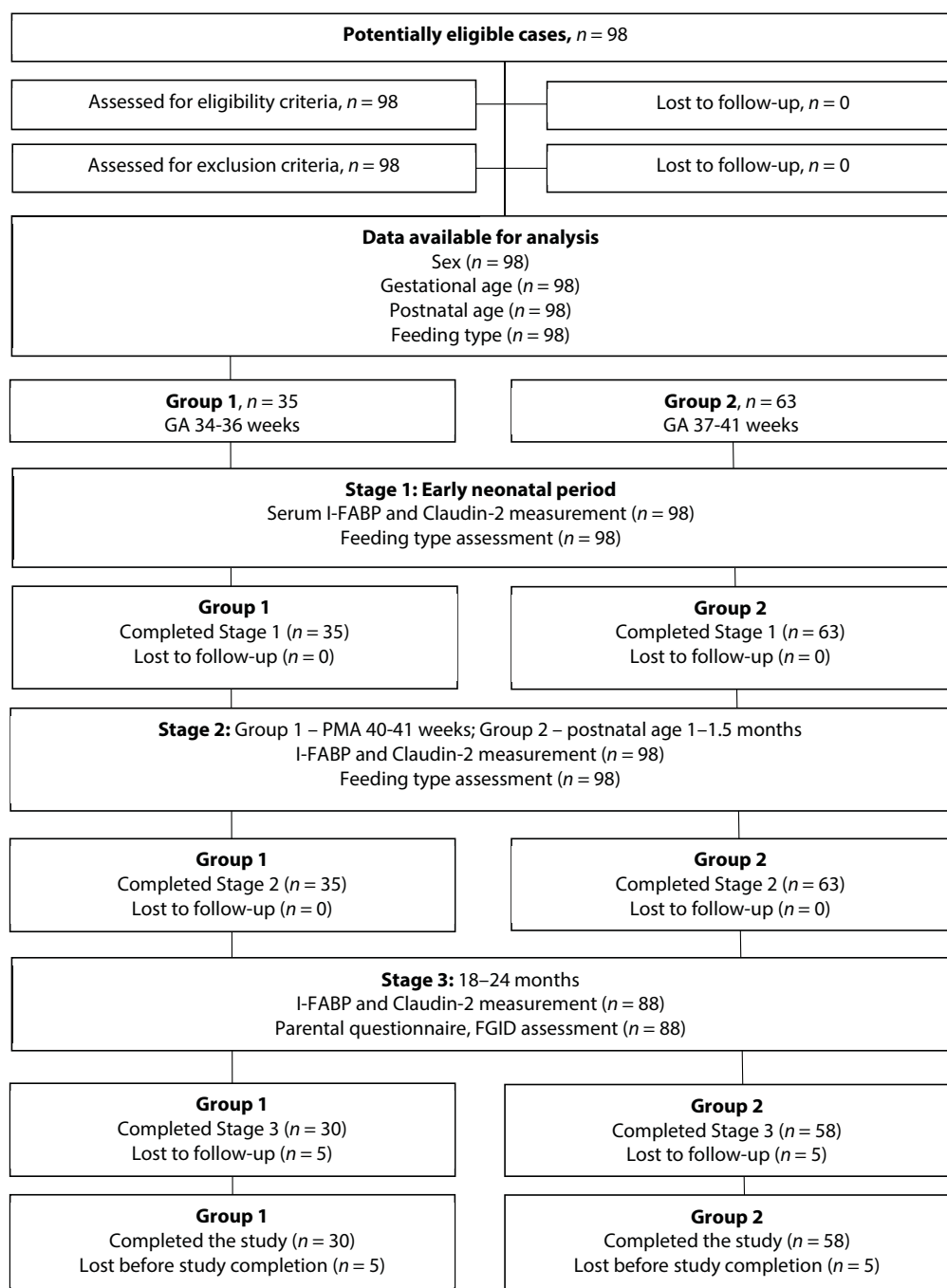


FIG. 1.
Study design

Secondary Outcomes: type of feeding, frequency of functional gastrointestinal disorders in infants.

Subgroup Analysis

Subgroups were formed based on gestational age and feeding type.

Outcome Registration Methods

I-FABP levels were measured using the Human IFABP/FABP2 (Intestinal Fatty Acid Binding Protein) ELISA Kit, and Claudin-2 levels using the Human CLDN2 (Claudin-2) ELISA Kit on a Multiskan Go microplate reader (Thermo Fisher Scientific, Finland), with a Wellwash microplate washer (Thermo Fisher Scientific, Finland) and a PST-60HL-4 thermoshaker (Biosan, Latvia). Serum was obtained by standard centrifugation (3000 rpm, 10 minutes).

To identify children who developed functional gastrointestinal disorders during the first 24 months of life, parents completed a questionnaire for detecting functional gastrointestinal disorders in children aged 0 months to 3 years 11 months prior to the third assessment [20, 21]. The questionnaire consists of two sections: the first for parents of children under one year, and the second for parents of children over one year, and enables diagnosis of the following functional gastrointestinal disorders: regurgitation, dyschezia, infantile colic, constipation and diarrhea, cyclic vomiting syndrome, and rumination. The questionnaire items are presented as questions with multiple-choice answers. Interpretation is performed using a pre-established key. For each functional gastrointestinal disorder, diagnostic criteria are considered met if all key-specified answers are obtained, except for functional constipation, which requires the presence of 2 or more criteria. "Red flags" requiring additional examination are separately indicated.

Stool consistency was assessed using the Amsterdam Stool Scale (Bekkali scale) for infants. The scale evaluates stool frequency, consistency, and color.

Feeding type and dietary characteristics at each follow-up stage were ascertained from parents during history taking.

Ethical Approval

The study was approved by the local ethics committee of the Burdenko Voronezh State Medical University (Protocol No. 4, dated September 21, 2020).

Statistical Analysis

Sample Size Calculation Principles. Sample size was not calculated a priori.

Statistical analysis was performed using Stat-Tech v. 4.11.1 (Stattech LLC, Russia). Effect size calculations were performed using the Python programming language in the Jupyter Notebooks environment with pandas and numpy libraries. Normality of quantitative variable distributions was assessed using appropriate tests (Kolmogorov – Smirnov for $n > 50$, Shapiro – Wilk for $n < 50$). For normally distributed data, quantitative variables are presented as arithmetic means (M) and standard deviations (SD), with 95 % confidence intervals (95% CI). For non-normally distributed data,

medians (Me) with lower and upper quartiles (Q1–Q3) were used. Categorical data are presented as absolute numbers and percentages. Comparison of two independent groups for quantitative variables with normal distribution and equal variances was performed using Student's *t*-test; with non-normal distribution and equal variances – the Mann – Whitney U test; with unequal variances – the Brunner – Munzel test. Analysis of 2×2 contingency tables was performed using Pearson's chi-square test (when expected frequency > 10) or Fisher's exact test (when expected frequency < 10); for larger tables – Pearson's chi-square test.

Comparison of three or more dependent groups with non-normal distribution was performed using the Friedman test, with post-hoc comparisons using the Conover – Iman test with Holm correction. Differences were considered statistically significant at $p < 0.05$.

To assess the practical and clinical significance of identified differences, effect sizes were additionally calculated. For non-parametric tests of independent groups, the effect size was used. For the Friedman test (multiple related comparisons), Kendall's coefficient of concordance *W* was calculated. Interpretation of *r* and *W* values followed Cohen's criteria: 0.1 – small, 0.3 – medium, ≥ 0.5 – large effect.

RESULTS

Study Participants

The initial cohort included 98 newborns with gestational age (GA) 34–41 weeks, median GA 38.0 [36.0–39.0] weeks, of whom 62 (63.3 %) were male and 36 (36.7 %) female. All children completed the second follow-up. The third study stage represented continued follow-up of the formed cohort up to 24 months of age (median 21.0 [20.0–23.0]). The final stage was completed by 88 children, including 30 preterm and 58 full-term infants. Loss to follow-up was 10.2 % ($n = 10$), due to withdrawal of consent by legal representatives or loss of contact with the family. Thus, the final study sample comprised 88 children, divided into two clinical groups: Group 1 – newborns with gestational age 34–36 weeks ($n = 30$), Group 2 – newborns with gestational age 37–41 weeks ($n = 58$). Age at enrollment was 3 [3; 6] days in Group 1 and 4 [2; 6] days in Group 2 ($p = 0.157$), and at the end of follow-up – 20 [19; 22] months and 21 [20; 23] months, respectively ($p = 0.268$).

To assess the risk of selection bias due to loss to long-term follow-up, a comparative analysis of patients who completed the study and those lost before the third follow-up was performed. Due to significant heterogeneity of the overall cohort, the analysis was stratified by preterm and full-term groups. Results are presented in Tables 1 and 2.

In Group 1, the proportion of girls among those lost before the third stage was higher compared to those who completed the study. Notably, sex is not

a primary exposure in this study and has a limited influence on the main outcome – serum I-FABP and Claudin-2 concentrations.

In the full-term group, no statistically significant differences in baseline characteristics were found between children who completed follow-up and those lost to follow-up. Thus, differences between children who completed the study and those lost before the end of follow-up have limited impact on the results and are unlikely to introduce selection bias.

Primary Outcomes

Dynamics of I-FABP in the study groups are presented in Table 3. Notably, serum I-FABP concentration increased significantly at the third time point, while remaining relatively stable during the first 1–1.5 months of life (first and second time points) (Table 3). At the third follow-up, median serum I-FABP levels in preterm infants increased 4.7-fold compared to levels at PMA 40–41 weeks, and in full-term infants – 3.2-fold compared to the median concentration at 1–1.5 months of age. Notably, the longitudinal profile of I-FABP in preterm infants during the first 24 months corresponds to that of full-term infants: no statistically significant differences in biomarker concentration between groups were observed at any follow-up stage.

Dynamics of Claudin-2 in the study groups are presented in Table 4. Notable differences in longitudinal

profiles of Claudin-2 between groups were observed: for preterm infants, no significant fluctuations in biomarker concentration were noted during the first 24 months. In contrast, for full-term infants, Claudin-2 increased with growth – median levels at the second time point increased 2.7-fold and at the third time point 4.3-fold compared to the early neonatal period.

Comparative analysis of biomarker concentrations between groups showed that at the first two time points, serum Claudin-2 concentration was significantly higher in preterm infants compared to full-term infants; at the third time point, biomarker levels were comparable between groups.

Secondary Outcomes

All children included in the study tolerated enteral feeding. None required total parenteral nutrition. During the follow-up period, no child developed a severe disease or condition potentially affecting gastrointestinal function and/or requiring long-term specific therapy potentially affecting intestinal permeability markers. Stool consistency, frequency, and color assessed by the Bekkali scale were within normative values for each age group.

At the first I-FABP and Claudin-2 assessment, the proportion of exclusively breastfed children in Group 1 was 16.7 % ($n = 5$) vs. 20.7 % ($n = 12$) in Group 2 ($p = 0.881$); at the second assessment – 26.7 % ($n = 8$)

TABLE 1

COMPARISON OF BASELINE CHARACTERISTICS BETWEEN PRETERM CHILDREN WHO COMPLETED THE THIRD FOLLOW-UP VISIT AND THOSE LOST TO FOLLOW-UP

Parameter	Lost to follow-up, $n=5$	Completed the study, $n=30$	p
Female, abs. (%)	4 (80.0)	6 (20.0)	0.017*
Male, abs. (%)	1 (20.0)	24 (80.0)	
Neonatal antibiotic exposure, abs. (%)	1 (20.0)	19 (63.3)	0.141
No neonatal antibiotics, abs. (%)	4 (80.0)	11 (36.7)	
Vaginal delivery, abs. (%)	4 (80.0)	17 (56.7)	0.627
Cesarean delivery, abs. (%)	1 (20.0)	13 (43.3)	
Gestational age, weeks, Me [IQR]	35.0 [35; 35]	35.0 [34; 36]	0.689
Apgar score at 1 min, points, Me [IQR]	7.0 [7.0; 7.0]	7.0 [6.0; 7.0]	0.575
Apgar score at 5 min, points, Me [IQR]	8.0 [8.0; 8.0]	8.0 [8.0; 8.0]	0.533
Birth weight, g, $M \pm SD$ 95% CI	2528.0 $\pm$ 343.3 2102.1–2953.9	2595.8 $\pm$ 584.2 2377.7–2813.9	0.804
Birth length, cm, $M \pm SD$ 95% CI	48.8 $\pm$ 1.6 46.8–50.8	49.1 $\pm$ 3.5 47.7–50.4	0.869
Head circumference at birth, cm, Me [IQR]	33.0 [33.0; 34.0]	32.0 [30.25; 33.0]	0.050
Maternal age, years, $M \pm SD$ 95% CI	25.8 $\pm$ 5.6 18.9–32.7	30.4 $\pm$ 5.7 28.3–32.5	0.103

Note. * – differences between groups are statistically significant ($p < 0.05$).

and 39.7 % ($n = 23$), respectively ($p = 0.289$). Median duration of breastfeeding in Group 1 was 8 [1; 18] months, in Group 2 – 4 [1; 15] months ($p = 0.465$). At the third biomarker assessment, all children were receiving solid foods. No statistically significant differences in serum I-FABP and Claudin-2 concentrations were found in groups stratified by feeding type during the first months of life.

According to parental questionnaire results, no child was diagnosed with functional gastrointestinal disorders. No respondent reported complaints of recurrent

vomiting, regurgitation, straining during defecation, hard stools, or pathological impurities in stool. Most respondents reported regular bowel movements: 1–3 times/day in 63.6 % ($n = 56$), 3–6 times/week in 36.4 % ($n = 32$). Regarding stool consistency, most parents chose “not very hard, but not soft” – 96.6 % ($n = 85$); 3.4 % ($n = 3$) reported inconsistent stool consistency (“different each time”). Diagnostic criteria for functional gastrointestinal disorders were met in none of the questionnaires.

Adverse Events

No adverse events were recorded during follow-up.

TABLE 2

COMPARISON OF BASELINE CHARACTERISTICS BETWEEN TERM CHILDREN WHO COMPLETED THE THIRD FOLLOW-UP VISIT AND THOSE LOST TO FOLLOW-UP

Parameter	Lost to follow-up, $n=5$	Completed the study, $n=58$	p
Female, abs. (%)	2 (40.0)	24 (41.4)	1.00
Male, abs. (%)	3 (60.0)	34 (58.6)	
Neonatal antibiotic exposure, abs. (%)	1 (20.0)	17 (29.3)	1.00
No neonatal antibiotics, abs. (%)	4 (80.0)	41 (70.7)	
Vaginal delivery, abs. (%)	2 (40.0)	41 (70.7)	0.315
Cesarean delivery, abs. (%)	3 (60.0)	17 (29.3)	
Gestational age, weeks, Me [IQR]	39.0 [38; 40]	39.0 [38; 40]	0.831
Apgar score at 1 min, points, Me [IQR]	7.0 [7.0; 7.0]	7.0 [7.0; 8.0]	0.308
Apgar score at 5 min, points, Me [IQR]	8.0 [8.0; 9.0]	9.0 [8.0; 9.0]	0.084
Birth weight, g, $M \pm SD$ 95% CI	3150.0 $\pm$ 540.1 2479.4–3820.6	3374.7 $\pm$ 484.0 3247.4 – 3501.9	0.327
Birth length, cm, $M \pm SD$ 95% CI	53.0 [53.0; 54.0]	53.0 [52.0; 55.0]	0.969
Head circumference at birth, cm, Me [IQR]	34.0 [33.5; 36.0]	35.0 [34.0; 36.0]	0.633
Maternal age, years, $M \pm SD$ 95% CI	33.6 $\pm$ 2.8 30.1–37.1	28.56 $\pm$ 5.92 26.99–30.13	0.066

TABLE 3

I-FABP DYNAMICS IN CHILDREN BORN AT 34–41 WEEKS OF GESTATION DURING THE FIRST 24 MONTHS OF LIFE

Group / Gestational age	I-FABP, 1 st measurement, ng/mL	I-FABP, 2 nd measurement, ng/mL	I-FABP, 3 rd measurement, ng/mL	p	W
	Me [Q ₁ ; Q ₃]	Me [Q ₁ ; Q ₃]	Me [Q ₁ ; Q ₃]		
Group 1 (34–36 weeks)	1.12 [1.07; 1.30] ($n=30$)	1.30 [0.97; 1.87] ($n=30$)	6.09 [3.18; 6.65] ($n=30$)	<0.001* $p_{(1-3)} < 0.001$ $p_{(2-3)} < 0.001$	0.573
Group 2 (37–41 weeks)	1.10 [0.80; 1.95] ($n=58$)	1.21 [0.92; 1.73] ($n=58$)	3.89 [2.33; 6.10] ($n=58$)	<0.001* $p_{(1-3)} < 0.001$ $p_{(2-3)} < 0.001$	0.544
p	0.738	0.610	0.131	–	
r	0.036	0.055	0.161	–	

Note. * – differences between groups are statistically significant ($p < 0.05$).

TABLE 4

CLAUDIN-2 DYNAMICS IN CHILDREN BORN AT 34–41 WEEKS OF GESTATION DURING THE FIRST 24 MONTHS OF LIFE

Group / Gestational age	Claudin-2 1 st measurement, ng/mL	Claudin-2 2 nd measurement, ng/mL	Claudin-2 3 rd measurement, ng/mL	<i>p</i>	<i>W</i>
	Me [Q ₁ ; Q ₃]	Me [Q ₁ ; Q ₃]	Me [Q ₁ ; Q ₃]		
Group 1 (34–36 weeks)	6.76 [3.00–9.14] (<i>n</i> =30)	7.59 [5.20–10.96] (<i>n</i> =30)	7.77 [6.33–9.20] (<i>n</i> =30)	0.497	0.023
Group 2 (37–41 weeks)	1.85 [1.02–5.22] (<i>n</i> =58)	5.10 [3.47–8.31] (<i>n</i> =58)	7.95 [6.32–11.28] (<i>n</i> =58)	<0.001* <i>p</i> ₍₁₋₂₎ = 0.007 <i>p</i> ₍₁₋₃₎ < 0.001 <i>p</i> ₍₂₋₃₎ = 0.017	0.251
<i>p</i>	0.006*	0.009*	0.326	–	
<i>r</i>	0.296	0.277	0.105	–	–

Note. * – differences between groups are statistically significant (*p* < 0.05).

DISCUSSION

Summary of Primary Outcomes

This study examined the dynamics of serum I-FABP and Claudin-2 levels in children with gestational age 34–41 weeks during the first 24 months of life. The results demonstrate that I-FABP concentration remains stable during the first 1–1.5 months of life, is independent of gestational age, and increases by 24 months of age in both full-term and preterm infants.

Dynamics of serum Claudin-2 levels differ between children with varying gestational age within the 34–41 week range: late preterm infants exhibit stable biomarker concentrations during the first 24 months, while full-term infants show an increase in serum Claudin-2 with postnatal growth. Thus, gestational age within the 34–41 week range is not a determinant of I-FABP levels during the first 24 months of life. For Claudin-2, variability depending on the degree of gestational maturity is characteristic.

Discussion of Primary Outcomes

Our data complement the findings of international studies emphasizing the need for age-specific interpretation of I-FABP and longitudinal monitoring to enhance diagnostic value [10]. Shores D.R. et al. proposed normative I-FABP values considering gestational age, noting significant variability even in clinically stable children and an increase in I-FABP with postnatal growth, which the authors attribute to changes in intestinal epithelial load and enterocyte vulnerability to external agents [10].

An age-dependent trend in intestinal barrier and permeability markers was also demonstrated in another cohort study [4] and is generally consistent with our findings: from 12 to 24 months of age, a trend toward increased markers of intestinal epithelial injury

and permeability was observed, which may be related to active dietary changes, microbiome shifts, and increased antigenic load in children of this age [4]. Thus, the increase in I-FABP concentration we observed may reflect these processes. Hasan M.M. et al. showed that I-FABP levels in young children are associated with markers of environmental enteropathy and stunting. The authors emphasize that I-FABP elevation is not always accompanied by clinically significant gastrointestinal symptoms and may reflect chronic and/or recurrent subclinical enterocyte injury, suggesting that this marker may indicate increased intestinal epithelial load rather than overt injury [1].

Therefore, elevation of intestinal wall markers, including I-FABP, may be interpreted as reflecting the load on the intestinal epithelium during periods of active dietary and microbiota changes occurring during the first 24 months of life.

It is important to emphasize that in the present study, I-FABP elevation at 24 months of age was not accompanied by clinical signs of gastrointestinal pathology. Thus, it is reasonable to assume that the observed dynamics reflect a physiological response of the intestinal epithelium to changes in diet, feeding patterns, and increased antigenic load occurring during the second year of life.

Several studies have demonstrated I-FABP elevation in enteropathies and gastrointestinal forms of food allergy [11, 12], whereas in allergy cohorts without clinically significant gastrointestinal involvement, no differences in I-FABP levels were detected [14]. The results of the present study complement the knowledge on I-FABP variability across different clinical groups with data on age-related variability of the marker. Thus, I-FABP levels should be interpreted considering the patient's clinical status and age.

Claudin-2 dynamics differ significantly between groups and likely reflect morphofunctional differences in the intestinal wall of preterm versus full-term infants. Claudin-2, a tight junction protein capable of increasing intestinal permeability to water and cations, may be considered an adaptive mechanism in the context of increased nutritional demands in preterm infants. On the other hand, this characteristic, in the presence of risk factors such as intestinal hypoxia/ischemia, ICU hospitalization, hemodynamic instability, and colonization with hospital flora, may serve as a substrate for progression of necrotizing enterocolitis or reduced enteral feeding tolerance. Based on the obtained results, it can be concluded that interpretation of Claudin-2 concentrations during the first 24 months should consider the child's gestational and postnatal age.

The changes in biomarkers observed during the first 24 months of life should not be interpreted as evidence of pathological intestinal permeability or impaired intestinal barrier integrity due to enterocyte damage. In our view, this represents age-related variability of these markers.

Study Limitations

The limitations of this study are, on the one hand, related to the absence of direct intestinal permeability assessment using gold-standard methods (e.g., lactulose/mannitol test), which precludes comparison of I-FABP and Claudin-2 levels with direct permeability measurements. Additionally, data on the intestinal microbiome of the children, which may have influenced I-FABP and Claudin-2 levels, were not available. Investigation of these parameters was not within the scope of the study, but is noted as a limitation and potential direction for future research.

On the other hand, the study was subject to loss to follow-up and involved a relatively small number of patients, which may have affected the statistical power of the analysis.

CONCLUSION

In children born at 34–41 weeks of gestation, the age-related dynamics of I-FABP during the first 24 months of life are characterized by an increase by the second year of life, with comparable longitudinal I-FABP concentration profiles in preterm and full-term children. Gestational age within the 34–41 week range does not independently influence I-FABP levels.

In preterm infants born at late gestational ages, longitudinal Claudin-2 profiles are characterized by the absence of significant fluctuations in concentration during the first 24 months. In full-term infants, conversely, Claudin-2 increases with postnatal growth.

Accumulation of data on I-FABP and Claudin-2 dynamics allows for more accurate interpretation of biomarker values and facilitates differentiation between physiological age-related changes and pathological intestinal epithelial injury in early childhood.

Conflict of interest

The authors declare no conflict of interest.

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