

The Clinical Significance of Fecal Zonulin in Extremely Premature Babies

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Abstract Integrity and permeability of the intestinal barrier in premature babies plays a decisive role in the occurrence of digestive disorders and such a terrible complication as necrotizing enterocolitis. In order to study the dynamics of the fecal zonulin level in premature babies up to 14 days and establish the relationship between its values and other indicators characterizing the status of premature babies, we examined 108 premature babies with gestational age at birth from 22 to 32 weeks and 27 full-term newborns who made up the control group. Studies of fecal zonulin were carried out over the 3rd, 7th, and 14th days of life. The content of zonulin in feces was determined using Immunodiagnostik reagents (Germany) by enzyme-linked immunosorbent assay (ELISA). The results of the study showed that in premature babies there is a manifold increase in the values of fecal zonulin within 14 days of life compared with healthy full-term children. Indicators of fecal zonulin on the 7th day of life were significantly higher in children who developed NEC by 10 ± 4.7 days of life.

Keywords Preterm, Fecal zonulin

1. Introduction

It is well known that prematurity requires a special approach in nursing, due to the physiological immaturity of all organs and systems. A special role in the success of nursing is the problem of enteral nutrition, on the timely solution of which depends both the length of hospital stay and the occurrence of various complications [1]. In 2000 A. Fasano and employees discovered zonulin, a protein that modulates the integrity of the intestinal wall [8]. Zonulin is an analogue of the cholera vibrio toxin (Zonula occludens toxin) and is involved in the functioning of tight contacts between the epithelial cells of the intestinal mucosa. Zonulin binds to its receptor on the surface of the epithelium and enhances a cascade of reactions leading to increased cell-cell contacts and an increase in intestinal permeability. This allows various substances to overcome the intestinal cell barrier and stimulate the immune response [8-10]. It is known that Intestinal barrier maturation in preterm infants is GA and postnatal age dependent, and is influenced by feeding with a maturational effect of breast milk feeding and possibly by antibiotic exposures [14].

In the literature available to us, we have not found studies on the determination of fecal zonulin in extremely premature babies.

Taking into account the importance of the integrity of the intestinal barrier in the development of conditions associated with digestive disorders of varying severity, we set out to examine the clinical significance of fecal zonulin levels in premature infants under 14 days of age and to establish the relationship between its values and other indicators characterizing the status of extremely premature infants.

2. Materials and Methods

We examined 108 (44 boys and 64 girls; 40.7%: 59.3%) of premature infants and 27 full-term newborns, who constituted the control group. All children besides the standard examination, including laboratory blood tests (including counting leukocyte intoxication index (LII), CRP), urine, feces, chest and abdomen x-rays, ultrasound (US) of the liver, kidneys, brain. All pregnant women at admission, in addition to generally accepted laboratory tests, underwent ultrasound of the fetus with Doppler. The general characteristics of the children in the groups are given in Table 1. The majority (57.4%) of the children in the main group received adapted milk formula, 18.5% of the newborns were breastfed, the rest were mixed.

The basic diagnosis of eighty six premature babies (79.6%) within initial three days of life was respiratory disorder syndrome (RDS), in the rest of cases it was infection manifested by congenital pneumonia or sepsis.

Table 1. Clinical characteristics and features of the perinatal period of the examined newborns in the main (n=108) and control (n=27) groups

Characteristics	Basic group, M±m, (range)	Control, M±m (range)
Gestation age (weeks)	28.8±0.4* (22.4-31.5)	38.6±0.6 (37.2-39.3)
Weight at birth (gr)	1348.1±69.8* (803-2106)	3262.5±188.7 (2800.0-3960)
Length at birth (sm)	35.5±0.6* (27-40)	50.2±0.7 (47-53)
M and D ratio (abs/%)	M-40.7% / n=44 D-59.3% / n=64	M-44.4% / n=12 D-55.6% / n=15
Duration of no water period (h)	13.8±6.1* (0-192)	5.1±3.9 (0-11)
Severe preeclampsia in mother	30(27.7%)*	1(10%)
Disorder of uterine-fetal-placental blood flow II and III stages	46 (42.5%)*	1(3.7%)
Did not receive antenatal steroid prophylaxis or it was not completed	26(24.0%)	-

Note * p<0.05 compared to the values of the control group.

Associate diagnosis was perinatal damage of CNS (40.7%) and asphyxia (7.4%).

Testing of fecal zonulin in the basic and control groups was done in dynamics on the 3rd, 7th, and 14th days of life. Amount of zonulin in feces was determined using Immunodiagnostik reactants (Germany) by means of enzyme link immune sorbent assay (ELISA). According to the manufacturer instruction medium concentration of fecal zonulin in the amount of 61 ng/ml (±46 ng/ml) was considered to be normal.

For all quantitative characteristics in the compared groups we performed calculation of arithmetic means (M), mean square (standard) mistakes (m) of average confident Student's coefficient (t) with the suggested significance level

(p). Differences in all cases were considered statistically significant with p<0.05. Assessment of the research results was performed using Statistica 10 software (StatSoftInc., USA) and Excel (Microsoft, USA). For the assessment of the characteristics we used Pearson correlation analysis.

3. Results

While studying the dynamics of the fecal zonulin we determined 17 folds rise of its level on the 3rd day of life, 8 folds on the 7th day, and approximately 5 folds increase on the 14th day of life compared to the control group.

We revealed an average strength positive correlation between the level of fecal zonulin on the 3rd day of life and fibrinogen in the mother's blood (r=0.33). The values of zonulin on the 7th day had a positive correlation with amylorrhea (r=0.30) and increase of leukocytes in feces (r=0.30). On the 14th day we revealed a positive correlation with the disorder of uterine-placental circulation (0.29) and prothrombin index (PTI) of the mother (0.50). It means that increased values of zonulin at birth in premature babies could be linked with disorders of uterine-placental circulation, indigestion and inflammatory process in intestine, and that corresponds to the data provided by Murashko [11].

To reveal the prognostic importance of fecal zonulin we divided all the patients to 2 groups. The first group involved 10 children, who had development necrotizing enterocolitis on the 10.2±4.5 day of life; the second one included 81 children without necrotizing enterocolitis.

It is noticeable that in the 1st group we observed almost double reliable significant rise of zonulin amount on the 7th day of life. Taking into account that necrotizing enterocolitis developed average on the 10th day of life, we could suppose that the level of fecal zonulin on the 7th day of life is the predictor of the start of new-born necrotizing enterocolitis. On the 14th day we observed a significant decrease of the values of fecal zonulin (Table 3).

Table 2. Values of fecal zonulin (ng/ml) in the examined children

Groups	Amount of fecal zonulin					
	3 rd day		7 th day		14 th day	
	Number of studies	Value, M±m (range)	Number of studies	Values, M±m (range)	Number of studies	Value, M±m (range)
Basic	40	490.9±73.5* (81.0-1400.0)	32	507.7±77.2* (105.0-1190.0)	36	430.0±60.4* (140.0-870.0)
Control	27	28.5±3.6 (12.5-55.0)	15	64.1±5.1 (10.7-76.2)	15	89.8±5.8 (13.8-81.2)

*-reliability of differences p<0.05 compared to the values of the control group

Table 3. Zonulin values (ng/ml) in newborns with necrotizing enterocolitis (group 1) and without it (group 2)

Days of life	1 st group (n=10)	2 nd group (n=81)	P
3	339.5±78.9	373.7±65.8	> 0.05
7	618.4±89.9	343.5±76.7	<0.05
14	213.3±45.9	376.9±66.5	<0.05

Later we applied cluster analysis of the values of fecal zonulin for the definition of its clinical significance. All values of fecal zonulin on the days of its definition were classified to clusters A, B, and C. A cluster involved patients with the values of fecal zonulin up to 500 ng/ml (n=52), B cluster involved children with 500-1000 ng/ml (n=40), and C cluster included 16 children with the highest values of fecal zonulin (above 1000 ng/ml).

We determined that in all three clusters at the third day of life characteristics such as the speed of nutrition increase, meconium excretion time, weight loss per cent, fecal parameters of digestion and inflammation had no reliable significant differences. On the 7th day of life in compliance with the values of fecal zonulin we had two clusters: with fecal zonulin up to 500 ng/ml (n=20) and from 500 to 1000 ng/ml (n=12). It was noted that in the cluster of children with higher fecal zonulin (cluster B, n=12) C-reactive protein was reliably increased (the first cluster 9.6 ± 1.0 ng/ml, the second 18.0 ± 2.7 ng/ml, $t=2.94$, $p<0.001$). We also noted, that hemoglobin values in new-born children in B cluster were lower (175.1 ± 2.7 g/l and 152.5 ± 4.2 g/l, $t=2.31$, $p<0.001$).

On the 14th day of life we also had two clusters: 24 children with fecal zonulin up to 500 ng/ml (140-480 ng/ml) and 12 children with values from 500 to 870 ng/ml. In the first cluster prothrombin index of mother was equal to $82.0 \pm 1.4\%$, in the second one $96.0 \pm 1.9\%$ ($t=5.89$, $p<0.001$). The difference of the other analyzed parameters was not reliable. Analysis of clinical and laboratory values of 16 premature babies in B cluster revealed only the link to the markers of inflammatory response (CRP) ($t=3.31$, $p<0.001$).

4. Discussion

Gastrointestinal tract is an important organ which is attributed not only to digestion and absorption of nutritional compounds but also to immunologic function, modulation of inflammatory response, and triggering of autoimmune diseases.

Complexity of morphological structure of the gut is not completely revealed, and new findings show the broader meaning and significance of this organ. Particularly, in neonatal period and infancy, we are still discovering the importance of the gut in overall wellbeing. Newborns and infants are undergoing developmental changes and processes, including immunity and managing inflammatory response.

The less mature newborn the more fragile tissues of gastrointestinal track appear to be predominantly in extremely low gestational age premature infants. [13]

In references we found multiple studies on the definition of zonulin in new-born babies. For instance, A. Bein et al., who studied samples of intestine in six patients with new-born necrotizing enterocolitis, compared them to six other control samples of the patients operated with other reasons [12]. Results showed that that intestinal permeability is not linked with gestation age or weight at birth, but it is higher than in mature new-born babies and increased during

initial days of life. The data we achieved on the definition of fecal zonulin in some degree confirm these conclusions, if consider zonulin as a marker of intestinal permeability disorder. Though multiple increase of its values we determined in premature babies requires further studies.

To check the hypothesis of nutrition and antibiotic impact on the maturation of intestinal barrier in premature new-born babies B. Saleem et al. determined biomarkers of intestinal permeability in infants born before 33 weeks within initial two weeks of life [14]. The authors determined that, exclusive breast-feeding was associated with faster maturation of the function of intestinal barrier. The cluster analysis of 35 patients revealed three variants of intestinal permeability and the impact of longer application of antibiotics ($p = 0.092$) and delayed start of feeding ≥ 4 days ($p = 0.064$) in infants with abnormal types of intestinal permeability. Nevertheless, the authors note that serum zonulin correlated with intestinal permeability.

There is opinion that, zonulin driven opening of the paracellular pathway may represent a defensive mechanism, which flushes out microorganisms and contributes to the host response against bacterial colonization of the small intestine. [10]

In relation to that we suppose that high values of fecal zonulin during the first week of life in children with further realization into necrotizing enterocolitis suggests that they have a significant bacterial colonization.

Thus, dynamics of fecal zonulin in premature children revealed multiple increase of its values within initial 14 days of life compared to healthy mature babies. Values of fecal zonulin on the 7th day of life we reliably higher in new-born babies, who had development of necrotizing enterocolitis on the 10 ± 4.7 days. We revealed a direct correlation link between the values of fecal zonulin and those characterizing disorder of uterine-placental circulation. We can suppose that hypoxia conditioned by mother's disorders of microcirculation and anemia determine increase of intestinal permeability of immature baby and promotes unfavorable progression of gastro-intestinal tract maturation.

Conflict of Interest

The authors of this article confirmed the lack of conflict of interest and financial support, which should be reported.

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