УДК 616-053.3-056.3-06:616.34-002:616.39-07

CORRESPONDENCE BETWEEN FEECAL ZONULIN AND CALPROTECTIN IN FOOD ALLERGY IN EARLY AGE CHILDREN

Ch.G. Yakubova, I.M. Akhmedova
Center for the Development of Professional Qualifications of Medical Workers, Republic of Uzbekistan
Scientific supervisor - Doctor of Medical Sciences, Associate Professor I.M. Akhmedova

Abstract. The clinical significance of fecal zonulin and calprotectin levels in diagnosing food allergies in young children was assessed, and the relationship between their levels and with severity of intestinal inflammation was studied. The results showed that elevated zonulin and calprotectin concentrations above threshold levels confirm the presence of intestinal inflammation. Elevated fecal zonulin levels manifest earlier than calprotectin, allowing them to be used as a predictor of intestinal barrier integrity and for early prediction of food allergy risk. Calprotectin dynamics correlate with the severity of neutrophilic inflammation in the intestine.

Keywords: food allergy, intestinal inflammation, zonulin in feces, feecal calprotectin.

ВЗАИМОСВЯЗЬ ПОКАЗАТЕЛЕЙ ФЕКАЛЬНОГО ЗОНУЛИНА И КАЛЬПРОТЕКТИНА ПРИ ПИЩЕВОЙ АЛЛЕРГИИ У ДЕТЕЙ РАННЕГО ВОЗРАСТА

Ч.Г. Якубова, И.М. Ахмедова
Центр развития профессиональной квалификации медицинских работников МЗ РУз, Республика Узбекистан
Научный руководитель - д.м.н., доцент И.М. Ахмедова

Резюме. Произведена оценка клинического значения уровней фекального зонулина и кальпротектина при диагностике пищевой аллергии у детей раннего возраста, а также изучена связь их уровней со степенью тяжести воспалительного процесса в кишечнике. Результаты показали, что превышение концентраций зонулина и кальпротектина пороговых уровней подтверждает наличие воспалительных процессов в кишечнике. Превышение уровня зонулина в кале проявляется раньше, чем кальпротектина, что позволяет использовать его в качестве предвестника нарушения целостности барьера кишечника, а также раннего прогнозирования риска пищевой аллергии. Динамика уровня кальпротектина коррелирует с тяжестью нейтрофильного воспаления в кишечнике.

Ключевые слова: пищевая аллергия, воспаление кишечника, зонулин в кале, фекальный кальпротектин.

Introduction. The increasing prevalence of food allergy (FA), including in children, makes this form of pathology an increasingly pressing problem in pediatrics. To improve the early diagnosis and monitoring of gastrointestinal forms of food allergies in children, diagnostic methods using various biomarkers have been developed in recent years. One of them is fecal calprotectin - a calcium-binding leukocyte protein consisting of a complex of two monomers - S100A8 and S100A9. The level of its concentration is directly proportional to the number of leukocytes migrating into the intestinal wall, which allows the use of calprotectin as an accurate, non-invasive, and sensitive indicator reflecting the level of inflammation in the intestine [1].

The permeability of the intestinal barrier [2] dynamically responds to exogenous and endogenous factors [3] and participates in the pathogenesis of many diseases. In this regard, clinical researchers have shown increased interest in the non-immune component of this process, including proteins that affect the functioning of intercellular tight junctions (TJ). One such protein is zonulin, a human homologue of the Vibrio cholerae-produced toxin Zot (zonula occludens toxin) [4, 5], the study of which has made an important contribution to understanding the mechanisms of connection between intestinal barrier permeability disruption and the development of several diseases.

Purpose of the study: to assess the clinical significance and correlation of fecal zonulin and calprotectin levels with the severity of food allergies in young children.

Materials and methods. 75 children aged 3 months to 3 years with a diagnosis of food allergy, undergoing inpatient treatment in the gastroenterology department and the consultative-diagnostic polyclinic of the Republican Specialized Scientific and Practical Medical Center of Pediatrics of the Republic of Uzbekistan, were examined. Of these, 44 (58.66%) were boys and 31 (41.33%) were girls. The average age of the children was 12.5±1.1 months. The control group included 25 healthy children of the same age.

The diagnosis of "food allergy" was established according to the following criteria:

- presence of a positive allergic and food history;
- corresponding data from the food diary (the connection between the consumption of certain food products and clinical manifestations);
- presence of general and specific IgE to food allergens in blood serum;
- positive effect of elimination diets.

The assessment of children's physical development was carried out in accordance with WHO's international standards for child growth and development. The "WHO Anthro" program was used to calculate the Z-SCORES scale values [6].

To assess the symptoms of allergies to cow's milk proteins, the CoMiSS clinical scale was used [7]. In patients with gastrointestinal manifestations of FA, the severity was determined by the severity of clinical symptoms. [8]. To assess the severity of FA skin manifestations, standardized SCORAD severity indices were used [9].

The concentrations of zonulin and calprotectin in the stool were determined by enzyme-linked immunosorbent assay using the "IDK Zonulin ELISA" and "IDK Calprotectin ELISA" reagent kits from Immunodiagnostik (Germany).

Statistical analysis used methods of variational and descriptive statistics: a quantitative assessment of statistical parameters was carried out - arithmetic mean (M), standard deviation (σ), standard error (m), Student's t-test (t), confidence interval. Differences in mean values were considered significant at a significance level of $P < 0.05$. Calculations were performed using a program developed in the Microsoft Office Excel 2010 package.

Results. When studying the anamnesis, it was established that in 46 (61.81%) children of the main group, the heredity was complicated by allergic anamnesis. Of these, 40 (88.2%) patients had hereditary food allergies, 4 (8.82%) had drug allergies, and only one case (2.94%) had insect bites allergies. In relatives of children in the control group, allergic history was recorded three times less frequently - only in 2 (8%). The most frequent manifestations of food allergies in our study were skin and digestive system lesions. All children in the main group had clinical manifestations of food allergy to cow's milk proteins: 48 (68.5%) children had IgE-mediated, 27 (31.5%) children had non-IgE-mediated.

In 49 children (65.3%) of the main group, a skin syndrome was observed; they were diagnosed with atopic dermatitis. To quantitatively assess the severity of skin manifestations, the SCORAD scale was used, according to which the course of the disease corresponded to: in 15 children (30.4%) - mild severity (15.0±2.064 points), in 10 children (21%) - moderate severity (34.0±3.6 points), in 6 children (12%) - severe atopic dermatitis (58.0±8.2 points).

Gastrointestinal food allergy phenotypes manifested in all children of the main group. Their quantitative assessment according to the CoMiSS scale showed the following: 33 children (44%) had mild gastrointestinal symptoms (4.2±0.5 points); 26 children (35%) had moderate gastrointestinal symptoms (10±0.7 points); 16 children (21%) had severe gastrointestinal symptoms (14.9±2.6 points).

Zonulin concentration was assessed as normal at values < 83.15 ng/ml, elevated at values 83.15÷110.0 ng/ml, and elevated at >110 ng/ml. The threshold values of calprotectin concentration were: for the 0-3 month age group - 195-621 mcg/g, for the 3-6 month age group - 85-988 mcg/g, for the 6-12 month age group - 109-418 mcg/g, and for the 1-4 year age group - 53-119 mcg/g. The zonulin concentration value averaged for the main group had a sufficiently high level (184.16±11.61 ng/ml). In children aged 3-6 months (24.13%), the concentration was 193.71±28 ng/ml, in children aged 6-12 months (41.37%) - 198.50±16.16 ng/ml, and in children aged 1-3 years (34.48%) - 153.8±17.44 ng/ml. In children of the control group, the zonulin concentration value did not exceed 71.20±1.56 ng/ml.

As for the calprotectin concentration, its average value for the main group was 581.32±48.54 mcg/g. In children aged 3-6 months (5.26%), the concentration was 1008.50±11.50 mcg/g, in children aged 6-12 months (47.36%) - 643.44±56.48 mcg/g, in children aged 1-3 years (47.36%) - 473± 60.45 mcg/g. In children of the control group, the calprotectin concentration value did not exceed 64.86±5.07 mcg/g.

Discussion. The results showed that an increase in the concentration of zonulin and calprotectin confirms the presence of inflammatory processes in the intestines. In most cases (65.5%), the increase in their levels occurs sequentially. Initially, the concentration of zonulin increases: its level can rise as early as the first months of a child's life. Consequently, zonulin manifests as a precursor to the integrity of the intestinal barrier - it reacts to intestinal inflammation earlier than calprotectin. Increased calprotectin concentration is observed in most cases in children from 6 months of age. In such cases, more pronounced clinical manifestations of food allergy occur.

Conclusions. Clinical studies have shown that an elevated level of zonulin in stool manifests earlier than calprotectin, which allows it to be used as a precursor to intestinal barrier integrity disorder, as well as to predict the risk of food allergies. The dynamics of calprotectin levels correlate with the severity of neutrophilic inflammation in the intestines.

References

1. Zakharova IN et al. Difficulties in diagnosing non-IgE-dependent gastrointestinal forms of food allergy: How informative is fecal calprotectin? Meditsinskiy Sovet. 2024;18(11):138–44. (In Russ.) https://doi.org/10.21518/ms2024-314;

2. Allaire JM et al. The intestinal epithelium: central Coordinator of Mucosal Immunity. Trends in Immunology. 2018;39(9):677-96. https://doi.org/10.1016/j.it.2018.04.002;

3. Serek P, Oleksy-Wawrzyniak M. The Effect of Bacterial Infections, Probiotics and Zonulin on Intestinal Barrier Integrity. International Journal of Molecular Sciences. 2021;22(21):11359. https://doi.org/10.3390/ijms222111359;

4. Fasano A. Zonulin and its regulation of intestinal barrier function:the biological door to inflammation, autoimmunity and cancer. Physiological Reviews. 2011;91(1):151-75. https://doi.org/10.1152/physrev.00003.2008;

5. Khavkin AI, Bogdanova NM, Novikova VP. Biological role of zonulin: a biomarker of increased intestinal permeability syndrome. Rossijskij vestnik perinatologii i pediatrii. 2021;66:(1):31-8. (In Russ.). https://doi.org/10.21508/1027-4065-2021-66-1-31-38;

6. World Health Organization. (n.d.). Child growth standards. Retrieved June 15, 2025, from https://www.who.int/tools/child-growth-standards/standards;

7. Comiss Brochure. Retrieved June 15, 2025, from http://longread.1medinfo.ru/comiss/ComissBrochure.pdf;

8. Deev IA et al. sIgG4 and other predictors of tolerance formation in early childhood food allergy. Pediatric Pharmacology. 12(3), 283–9. https://doi.org/10.15690/pf.v12i3.1352;

9. PubMed. (1993). [Article]. PubMed. https://pubmed.ncbi.nlm.nih.gov/8435513/.