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Noninvasive Marker Of The Intestinal Epithelial Barrier State In Infants With Food Protein-Induced Enteropathy.

Prikhodchenko Nelly G*, Shumatova Tatyana A, Nee Antonina N, Katenkova Elina U, Zernova Ekaterina S, and Grigoryan Lamara A.

Pacific State Medical University, Vladivostok, Russia (690002, Vladivostok, prospect Ostryakova2)

ABSTRACT

18infants with food protein-induced enteropathy were examined for the studies the level of fecal calprotectin depending on the state of the intestinal epithelial barrier. It was defined fecal calprotectin, and all patients were organized gastroenteroscopy with biopsies. The own plate study of the mucous shell was discovered growth to cellular density to lymphocytes account and plasmatic cells, it were revealed enterocytes with big amount of mitochondrial. The breach structured-functional intestinal condition was accompanied essential increase the fecal calprotectin. That was indicative of direct participation this squirrel in mechanism of the damage of the gastrointestinal tract. The High fecal calprotectin content was saved in speaker of the disease and has a well-marked trend to reduction at normalizations clinical picture of the disease. It is promising for monitoring the disease course and evaluating the therapy effectiveness.

Keywords: fecal calprotectin, food protein-induced enteropathy,intestinal epithelial barrier, cow milk allergy, children

Corresponding author

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INTRODUCTION

Food allergy is a common disease affecting approximately 5-8% of children and 2-5% of adults [1,2,3]. The prevalence has increased over the last two decades, suggesting an important environmental contribution to susceptibility. According to modern data, enteropathy induced by food proteins (food protein-induced enteropathy, FPE) is one of the frequent manifestations of gastrointestinal food hypersensitivity in young children [4,5,6]. The food-protein enteropathywas first described in the 1960s after the identification of a group of infants with malabsorption associated with cow's milk. Such infants develop chronic diarrhoea, steatorrhoea and poor weight gain within the first months of life. There may be associated anaemia and hypoalbuminaemia [6]. The most common triggers are cow's milk and soy, but similar reactions have also been reported to chicken, rice and fish. Such foods trigger a T-cell mediated immune response within the small intestine, with nonspecific villous atrophy and lymphocytic infiltration [6,7]. The diagnosis is based on clinical symptoms and their resolution with allergen avoidance. Endoscopic small bowel biopsy may be helpful [5]. The pathophysiology of these non-IgE-mediated allergic disorders is poorly understood, and useful in vitro markers are lacking. To improve the diagnosis of FPE, dynamic control and the definition of treatment effectivenessis necessary to use more effective markers [3, 4, 5, 6], one of which is fecal calprotectin (FC) [7, 8]. The FC objectively indicates the presence or absence of inflammation, and one can judge the severity of the inflammatory process. As a potential marker of local inflammation in the intestine, FC has a number of advantages: proteolytic enzymes do not affect it and its concentration does not change even when storing the stool for 7 days at room temperature [9].There are studies devoted to the study of the role of FC in the pathogenesis of a number of digestive tract diseases in children [7,8], its role in the diagnosis of inflammatory bowel diseases is known, but studies of this marker for protein-induced enteropathy in children are lacking.

The purpose was to study the dynamics of fecal calprotectin level depending on the structural and functional state of the intestinal epithelial barrierin children with food protein-induced enteropathy.

MATERIAL AND METHODS

18 children with cow milk protein-induced enteropathy aged 1 to 12 months (Group I) were monitored. All children underwent complex clinical and immunological, biochemical and functional examination in dynamics.Determination of calprotectin in fecal stoolswas performed with BÜHLMANN Calprotectin ELISA reagents, BSM (USA), using the sandwich ELISA enzyme-linked immunosorbent assay.Calprotectin was determined in the course of the disease: at 1 day, at 5-7 days in the course of the disease and at the stage of clinical and laboratory remission for 21-28 days. 20 healthy children, comparable in sex and age, were the comparison group (Group II, control).

In the period of pronounced clinical manifestations,all FPE patients underwent esophagogastrojunoscopy with enterobiopsy. The biopsy material was obtained through the working channel of the fibrogastroscope PENTAX FG 24V by means of a fetal goiter from the jejunum to the Treits ligament. To obtain optimal information, a step biopsy was performed in the small intestine - 2-3 pieces were taken up to the Trace ligament. The material for the morphological analysis was fixed in 10% neutral formalin. For the histological examination of biopsy specimens, the method of staining with hematoxylin and eosin was used. Morphometry of structures and the state of architectonics of the muscular mucosa was carried out with the help of a microscope of the firm "Carl Zeis" and a screw eyepiece micrometer MOU 1-16. In the preparations of the small intestine, the height of the villi, the length of the crypts, and the thickness of the small intestine epithelium were measured. The status of the mucosa of the small intestine was judged from the ratio of the length of villi to the depth of crypt (B / K), which normally approaches 3. Also, interepithelial lymphocytes counted (in terms of 1000 nuclei), as well as the density of the cellular infiltrate of layer of the mucosa in 1mm and the percentage ratio of the cellular composition.

All patients gave written consent to participate in the study. The study was carried out taking into account the requirements of the Helsinki Declaration of the World Association "Ethical Principles of Scientific Medical Research with Human Participation" as amended in 2000 and the "Rules of Clinical Practice in the Russian Federation" approved by Order No. 266 of the Ministry of the Russian Federation of June 19, 2003.

Statistical processing of the material was carried out using specialized software packages for research ("Excel-2010" and "Statstica10.0" for Windows)

RESULTS

The study showed that all patients in Group I had gastrointestinal manifestations of food allergy of varying severity. All patients of Group I experienced a prolonged disorder of stool consistency and frequency, abdominal distension, regurgitation, decreased appetite, lack of sufficient weight gain, deficient conditions (anaemia, hypoalbuminaemia) developed. In some patients, the clinic of persistent diarrhea was combined with skin syndrome, manifested by local lesions (up to 5% of the body surface), hyperemia, edema, peeling, excoriation. The children were recorded: frequent watery stools, frothy, with an acidic odor (100%), regurgitation in 16 (88.9%), vomiting in 4 (22.28%). Patients were characterized by flatulence (in 94.5% of cases), intestinal colic (83.4%).

Mean concentrations of calprotectin in stool samples in children with FPE were 384.41 ± 46.05 μg / g, in health children from the comparison group - 58.38 ± 8.05 μg / g ($p < 0.001$). The preservation of high figures with a fuzzy tendency to increase of this index ($p = 0.06$) was registered on the 5th-7th day of in-hospital stay (396.41 ± 96.75 μg / g), at the 21-28th day, when the state was stabilized, there was a significant decrease in the production of calprotectin (186.29 ± 14.16 μg / g, $p < 0.001$).

Endoscopic examination of the small intestine in the period of pronounced clinical manifestations showed the presence of focal hyperemia and edema in all (100%) patients. They had additional changes described as a symptom of "semolina", the manifestation of which was amplified in the distal direction, in 6 (33, 3%) of children resembled the character of a "cobblestone pavement". Histological study of the small intestine mucosa showed that 12 (66.7%) patients had a decrease in the length of the villi to 487.8 + 2.0 μm, without deepening the crypts (171.8 + 6.6 μm). Specific changes in the structure of intestinal villi and enterocytes were not detected. In the lamina propria of the mucosa, a histological study showed an increase in cell density due to lymphocytic infiltration and plasma cells. Investigation of structural features of the small intestine mucosa revealed enterocytes, a large number of mitochondria, ribosomes, endoplasmic reticulum was determined in the cytoplasm. A repeated study of biopsy specimens in 5 children in the period of normalization of intestinal absorption showed complete restoration of the structure of the small intestine.

Direct correlation of moderate strength between the presence of structural lesions of the intestinal mucosa in children with FPE and the level of fecal calprotectin was revealed ($r=0,67$).

DISCUSSION

The human gastrointestinal tract is composed of several organs with a complex cellular and functional structure. The intestinal tract not only is responsible for the breakdown and absorption of essential nutrients and the uptake of electrolytes and water, but also represents a crucial component of the body's defense system against the external environment [10,11]. Participation in the implementation of the basic metabolic and barrier functions, maintenance of interorganic and intersystem connections determines the important role of the intestine in the adaptive reactions of the body. One of the most important conditions for the development of food allergy is the disruption of the intestinal barrier,

which selectively protects the body from antigenic substancesv[11]. In this protection, the structural and functional consistency of the epithelial layer and the intensity of the local immune response plays an important role [12]. Our study showed that some membrane, lysosomal and mitochondrial enzymes localized in enterocytes take part in the mechanisms of compensation for the occurring disorders. The histological examination of the lamina propria of the mucosa results in an increase in cell density due to interepithelial lymphocytes and plasma cells, the detection of enterocytes with a large number of mitochondria, ribosomes, endoplasmic reticulum indicates a compensatory response and a large physiological load of the cells. Disruption of the structural and functional state of the intestine in FPEis accompanied by a significant calprotectinincrease. It indicates the direct participation of this protein in the mechanisms of gastrointestinal tract damage. The increase in the volume of intestinal lesions in children with FPE and growth of inflammatory infiltration (including polymorphonuclear leukocytes and lymphocytes) inevitably leads to neutrophil migration into the intestinal lumen, destruction and subsequent release of cytosolic calcium-dependent proteins of the S100A8 / A9 family, especially such as calprotektin [13]. The high content of calprotectin in fecal stools persists in the dynamics of the disease and has a distinct tendency to decrease with the normalization of state. The

remaining elevated levels of fecal calprotectin may indicate ineffectiveness of therapy, an increase in the content of calprotectinis noted with an approaching exacerbation of the disease.

CONCLUSIONS

Thus, the fecal calprotectin is the marker of intestine inflammation in protein-induced enteropathy. The determination of fecal calprotectin concentrations is an informative criterion of the extent of lesion and massiveinflammatory infiltration in children. The absence of contraindications makes it possible to use the calprotectinanalysis both in the acute period and in the remission. The use of fecal calprotektinmay to improve the diagnosis of FPE. It is promising for monitoring the disease course and evaluating the therapy effectiveness.

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