IMMUNE STATUS IN PRESCHOOL CHILDREN WITH CELIAC DISEASE IN UZBEK POPULATION

Abdujabarova Z.M.

The Republican Specialized Scientific-Practical Medical Center of Pediatrics, Tashkent,
e-mail: khurshidakhon@gmail.com

In 16 children with typical (n = 11) and atypical form (n = 5) of celiac disease aged between 4–6 years old, there were studied parameters of cellular immunity, phenotype of immune-competent cells (CD8, CD25, CD95), cytokines, serum and secretory immunoglobulin A (IgA). Studies showed that in children with celiac disease is marked T-cell immune deficiency against the background of hyperactivation of B-lymphocytes. More pronounced this manifests itself in children with atypical form of celiac disease, causing an increase in both serum and, in particular, secretory immunoglobulins. At the same time, subpopulations of T-lymphocytes are changed: reduction of helpers, increase of cytotoxic lymphocytes and killer cells. Contents of CD markers of apoptosis and, in particular, IL-2 receptor increases, which coincides with high levels of cytokines, especially IL-4.

Keywords: pediatric gastroenterology, celiac disease, immunity, serum and secretory immunoglobulin A, cytokines, interleukin, tumor necrosis factor-α

Celiac disease is a chronic, genetically determined disease which is characterized by persistent intolerance to gluten with the development of hyperregeneratory atrophy of small intestinal mucosa and associated malabsorption syndrome. Progress in the immunological diagnosis has led to increased detection rate of this disease in 10–20 times in patients with asymptomatic or atypical course, and at risk groups – hundreds of times more likely than in general population. The exact frequency of this pathology in the population can be established, apparently, only due to large-scale screening studies. The disease is triggered by eating gluten-containing products from wheat, rye, barley and oats. According to recent studies conducted by Revnova et al. (2000), these peptides play a key role in the pathogenesis of celiac disease [4].

Studying the variety of manifestations of the disease, we can conclude that celiac disease is a systemic illness that involves many organs and exceeds a border of isolated food intolerance to gluten.

Among the unresolved problems for celiac disease so far a priority remains the identification of immunological status, determination of phenotypic characteristics of children with celiac disease.

The purpose of study was to study the basic parameters of immunity in preschool children with celiac disease in Uzbek population, which determine the processes of proliferation and autoimmunization.

Material and methods

We examined 16 children with celiac disease aged from 4 to 6 years who were hospitalized in the department of gastroenterology. The diagnosis was verified based on the criteria of the European Association of Pediatric Gastroenterologists (1999). The diagnosis was made in the presence of communication of manifestation of the disease with the introduction into nutrition of gluten-containing products, based on the results of histological examination of biopsy samples of the mucous of duodenum bulb-out part, high levels of antigliadin antibodies IgG and IgA, clinical effect of gluten-free diet, improving of absorption and morphology of the small intestine in exclusion of gluten from the diet. Stage of gluten enteropathy established in accordance with the classification of M. Marsh (1995). The severity of the pathological process and the period of disease were assessed comprehensively including all major clinical symptoms of bowel impairment, frequency and expressiveness of syndromes of extraintestinal manifestations of disease.

16 patients with celiac disease were divided into 2 groups depending on the disease phenotype. 11 (68,7%) children were making a diagnosis with typical form of celiac disease, the main symptoms of which were abundant, offensive, light or colored, soft, foam or clay, poorly laundered stool three or more times per day, chronic diarrhea, increased abdominal circumference, abdominal pain, decreased appetite, retarded body weight, impaired emotional status (irritability, aggressive behavior, restless sleep). Atypical form of celiac disease diagnosed in 5 (31,3%) patients. They had severe secondary metabolic disorders that masked the symptoms of main disease. Most often, these were disorders of phosphorus-calcium metabolism with the development of severe rachitis-like syndrome, bone deformities, pain in legs, short height, and anemia.

Prolonged treatment with a gluten-free diet leads to restoration of normal structure of the mucosa, improving absorption and recovery of most patients. In other part of patients, the piles remain partly atrophied, but the epithelium becomes highly differentiated, malabsorption symptoms become less pronounced. Among the patients examined were children who kept a gluten-free diet, but clinical improvement was not observed. We reviewed the clinical symptoms of the disease by dividing the children into 2 groups: with refractory and non-refractory.

Immunological studies were performed at the Institute of Immunology, Academy of Sciences of the Republic of Uzbekistan. As a control, we used data from the Institute of Immunology obtained by Aripova et al. (2004) [2]. We studied parameters of cellular immunity: the contents of leukocytes, lymphocytes, total amount of T-lymphocytes (CD3), T-helpers/inductors (CD4) and T-suppressors/cytotoxic lymphocytes (CD8), ratio of CD4/CD8 (immune-regulatory index – IRI), B-lymphocytes (CD20), natural killer cells (CD16), and activation markers of lymphocytes bearing a receptor for

IL-2 (CD25) and apoptotic factor (CD95). Phenotype of immune-competent cells (CD8, SD25, SD95) was determined using monoclonal antibodies to differentiation markers (product of the Institute of Immunology of Russian Academy of Medical Sciences, Moscow, Russia) in the reaction of indirect rosette-forming by Zalyalieva-Prokhorova's method [8].

In blood serum of patients we also determined the contents of cytokines: interleukin-4 (IL-4), tumor necrosis factor-α (TNF-α) by immune-fermentative method. Test-systems for the determination of the cytokines (made by «Cytokine» Ltd., St. Petersburg, Russia) based on the «sandwich» method of hard-phase immune-fermentative analysis with the usage of horseradish peroxidase as an indicator enzyme. Also, we determined the contents of serum and secretory immunoglobulin A (IgA). Secretory one (sIgA) was identified in the saliva used dilution 1/2000. For his purpose we used set of A-8668 IgA-secretory test-system (IFA-BEST «Vector-Best» Ltd).

Digital material is processed by variational statistics.

Results and discussion

The data are shown in the table. The total number of leukocytes in children with typical and atypical celiac disease remained within normal limits, so no significant differences between groups were revealed. Study of total lymphocytes amount showed their decrease in non-refractory form and a tendency to increase in other forms of celiac disease. If the level of B-lymphocytes tended to increase with non-refractory form of celiac disease, then in refractory form and, especially, in atypical one their level significantly exceeded the values of control group of children in 1,58 and 1,72 times, indicating that the expression of the development of autoimmune processes in children. This is confirmed also by studies of Aruin (2000), Allahverdiyeva (2004) [1,3]. The level of T-lymphocytes decreased, more pronounced in atypical form of the disease.

The study of the contents of subpopulation of T-lymphocytes showed a progressive decrease in the levels of T-helpers/inductors in 1,18, 1,14 and 1,28 times in groups with non-refractory and refractory forms of typical celiac disease, as well as in atypical one, respectively, in comparison with the normative values in children at the similar age. At the same time, the content of T-cytotoxic lymphocytes had a tendency to increase with non-refractory form of celiac disease and to decrease – in other forms. This led to reduce of immunoregulatory index in all groups of children, especially in non-refractory form of typical celiac disease (in 1,46 times, $P < 0,05$). The content of natural killer cells significantly increased in 1,84, 1,64 and 1,31 times, respectively, compared to normative values, more pronounced in children with non-refractory from of typical celiac disease.

The level of CD25+ in blood serum of children with atypical form of celiac disease was significantly reduced in 1,3 times, to a lesser extent – in typical refractory form, and was not changed in non-refractory typical form of celiac disease.

From previous studies is known the role of APO-I/Fas (CD95+) receptor in the process of apoptosis, and the degree of its expression reflects the level of lymphocyte apoptosis [5, 6, 7]. On the other hand, the processes of programmed cell death can also be implemented through specific receptors, whose main function is to induce apoptosis. From this it follows that the determination of the number of cells, expressing CD95+, may also reflect the state of cells and their willingness to apoptosis. In children with celiac disease this index tended to decrease, more pronounced in atypical form of celiac disease. Apparently, the reduction of CD95+ level conditioned deceleration of apoptosis and, as a consequence, an increase in their content (autosensitization of organism) [5, 6].

Indeed, the study of humoral protection factors revealed a significant decrease of serum IgA (in 1,12 times) in children with non-refractory typical form of celiac disease and increase in groups with refractory typical and, especially, atypical forms of the disease in 1,39 and 1,51 times, respectively. At the same time, the level of secretory IgA in all groups of children was increased: in 2,42, 2,44 and 2,2 times in non-refractory and refractory forms of typical celiac disease, as well as in atypical form, respectively.

Probably, a decrease in the level of T-lymphocytes is realized through specific receptors. The study of serum cytokine IL-4 in children with celiac disease showed an increase in its content in 1,37; 1,77 and 1,85 times in groups with non-refractory and refractory forms of typical celiac disease, as well as in atypical form, respectively, in comparison with the values of control group. The amount of TNF-α increased to a lesser extent, more pronounced in refractory form of typical celiac disease.

Thus, the analysis of immunological parameters in children with celiac disease revealed more profound immune disorders in refractory typical and, especially, atypical forms of the disease.

Conclusions

1. In children with celiac disease is marked T-cell immune deficiency against the background of hyperactivation of B-lymphocytes. More pronounced this manifests itself in children with atypical form of celiac disease, causing an increase in both serum and, in particular, secretory immunoglobulins.
2. In children with celiac disease are changed subpopulations of T-lymphocytes: reduction of helpers, increase of cytotoxic lymphocytes and killer cells.

3. Contents of CD markers of apoptosis and, in particular, IL-2 receptor increases, which coincides with high levels of cytokines, especially IL-4.

References

1. Allahverdiyeva L.I. State of immune status in children and adolescents with allergic rhinitis // J. Immunology. – 2004. – №5. – P. 284–286.

2. Aripova T.U., Umarova A.A. et al. Normal indexes of basic parameters of the immune system in children in the age aspect // Methodical recommendations. – Tashkent, 2004. – 16 p.

3. Aruin L.I. Apoptosis in pathological processes in digestive organs // Clinical medicine. – 2000. – № 1. – P. 5–10.

4. Revnova O.M., Lyle H.B. Clinical aspects of celiac disease in children // J. Pediatrics. – 2000. – №5. – P. 107–109.

5. Oleynik E.K., Shibaev M.I., Oleynik V.M. Immunological status of patients with tumors of the gastrointestinal tract in Karelia // J. Immunology. – 2004. – №2. – P. 100–103.

6. Nikonova M.F., Litvina M.M., Varfolomeeva M.I. et al. The immune system in children // J. Immunology. – 1999. – №2. – P. 20–23.

7. Tribole E., Kupper C., Pietzak M. Celiac sprue // N Engl. J. Med. – 2002. – Vol. 347 (6). – P. 446–48.

8. Zalyalieva M.V., Prokhorova R.S. Methods for determining lymphocyte subpopulations // №1 DP 20000774 D/P MPK 6601 №33/48 from 26.02.2001.