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ANTIBODY TITERS TO BACTERIAL ANTIGENS IN COLON DYSBIOSIS

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Abstract : The levels of antibodies to enterobacteria of intestinal microflora in dysbiosis of the large intestine and in practically healthy individuals were determined. Cellular sensitization to antigens of intestinal microflora and its significance in extraintestinal manifestations of dysbiosis (morbidity, susceptibility to infections, allergies) were determined.

The risk groups for secondary immunodeficiency were determined by the magnitude of antibody titers to intestinal microflora antigens (antibodies to intestinal microflora as a marker of immunodeficiency).

The place of the antimicrobial immunity system in the development of immunopathology in dysbiosis of the large intestine was established.

The presence of antibodies against UPE enterotoxin was revealed in all examined children. A relationship was established between an increase in the age of children with the detection of antibodies, as well as an increase in the intensity of formation of antienterotoxic antibodies in the blood serum of children with dysbiosis of the large intestine.

A relationship was established between dysbiosis of the large intestine and the state of the antimicrobial immune response of the body of children.

Keywords: Intestinal Dysbiosis, Intestinal Microflora, IFA, diarrhea, coprofiltrate, protease.

Introduction

Currently, intestinal dysbiosis in various forms is quite common in patients not only with acute and chronic gastrointestinal diseases, but also with other diseases . Diagnosis of dysbiotic changs in the intestine presents certain difficulties, since, in addition to microbiological changes, many clinical features must be taken into account. Despite the fact that in recent years, thanks to the efforts of many scientists, intestinal dysbiosis has been described in the literature, previously unknown features of this pathology have been studied, many controversial issues remain about their clinical significance. In this regard, doctors of many specialties face difficulties not only in diagnosing this pathology, but also in providing assistance to patients with intestinal dysbiosis.

A wide range of functions allows us to consider human microflora as one of the most important factors of homeostasis. At the same time, it is known that normal microflora (mainly its transient and facultative parts) is not optimal and can acquire the significance of a pathogenic object for the body under conditions of decreased immunity. This issue is especially acute at present due to environmental problems and altered immunological reactivity of the majority of the population.

To date, there is no consensus on the role of microecological changes in the digestive tract in the etiopathogenesis of most pathological processes. Thus, some authors, when studying the

intestinal microbiocenosis in individuals with surgical pathology of the biliary tract, revealed a high (96.6%) frequency of colon dysbiosis. The issues of recognizing the microflora of the digestive tract as an etiopathogenetic factor in hospital infections and infectious complications are resolved ambiguously, although it has been established that opportunistic pathogens are most often gram-negative opportunistic bacteria and cocci. Opportunistic microorganisms become pathogenic in individuals with microecological and immune disorders, as well as those with the ability to overcome the main mechanisms of natural immunity and create conditions for the expression of virulence. The participation of indigenous microflora in the production of stimulants and activators of phagocytic and enzymatic activity, as well as the ability of muramyl dipeptides of gram-positive anaerobic and microaerophilic bacteria to stimulate immunogenesis and activate the system of mononuclear phagocytes, as well as participation in the regulation of IgA bacterial lipopolysaccharides and stimulation of the synthesis of secretory antibodies, cytokines and interferon by immunocompetent cells have been well studied. Normal microflora is a stimulator of plasma cell proliferation. When the integrity of biofilms normally present on mucous membranes, consisting of cellular mucin, fibronectin and bacterial exopolysaccharide and microcolonies of bacteria contained within this matrix - representatives of indigenous microflora, is disrupted, they are replaced by microcolonies consisting of opportunistic or pathogenic microorganisms. As a result of such a replacement, a local infectious process is formed, which can subsequently become generalized.

In addition to changes in the organs and systems of the body associated with the development of the infectious process, especially in generalized forms of dysbiosis, clinical signs of delayed-type hypersensitivity are noteworthy, which manifest themselves in the form of damage to internal organs of allergic genesis, especially the myocardium, as well as the intestines, liver, and others. It is from these positions that the origin of allergic myocarditis and pseudomembranous colitis in patients with generalized forms of staphylococcal dysbacteriosis is currently considered.

To date, the participation of endotoxins of gram-negative representatives of the microbiota in the stimulation of lymphoid tissue and effector cells of the liver, the enhancement of the mitogenic activity of B- and T-lymphocytes, the implementation of antitumor immunity (in particular, the secretion of tumor necrosis factor - TNF), the activation of the polymorphonuclear leukocyte system (neutrophils), which under physiological conditions are important elements of the antibacterial defense of the body, has been identified. However, the most important function of endotoxin for the host, apparently, is its ability to carry out antiviral protection, in particular against human immunodeficiency viruses. According to Dubo, one of the reasons why the body does not reject the microflora despite multiple interactions is its weak immunogenicity for the host. This phenomenon may be based on molecular mimicry. Fou and Lee were among the first to draw attention to this phenomenon, having identified the presence of common antigens in one of the Basteroi strains.

Materials and Methodology

For the set tasks we examined children aged from 3 to 14 years. Among the examined children, intestinal dysbiosis of III and IV degree was detected in 66 children. They were included in the main group. The control group consisted of 20 practically healthy children of the same gender and age composition. For comparison with the main group, children with various acute intestinal infections were also studied for comparison: bacterial dysentery - 20 children;

salmonellosis - 27 children; gastroenteritis caused by opportunistic flora (E. coli, S. aureus, Proteus sp., Klebsiellae sp.) - 42 children. The gender and age composition of these examined children were identical to the main and control groups. The subject of the study was the study of normal microflora of the colon, the study of antibody titers in blood serum and coprofiltrates, and antiendotoxic antibodies in blood serum. In the course of the scientific work, bacteriological, bacterioscopic, serological, immunological, IFA, and statistical methods were used.

Results

The criterion of the etiological significance of UPE should be considered a set of indicators, among which one of the most important is the detection of specific serum antibodies to the antigens of the suspected pathogen. False positive results due to cross-reacting antibodies in the agglutination reaction with the autostrain are observed in no more than 5-10% of healthy individuals and carriers. It should be especially emphasized that in healthy young children, positive and false positive reactions are not observed due to the imperfection of the immune system and a short period of antigen stimulation. Based on the above, the next stage of our research was to study the level and evaluate circulating antibodies to UPE in children with intestinal dysbiosis. To determine the intensity of antibody formation to various UPE antigens, the average geometric titers of antibodies were calculated, expressed as negative logarithms with a base of 2 (- log2). When assessing the reliability of the difference between the indicators, when the number of one compared group exceeded the number of the other by at least 25%, the error in the difference of relative indicators in percent was calculated using the formula for unequal samples

Specific antibodies to UPE antigens were found in the blood serum of most of the examined children. A positive result was obtained in 80.0±2.6%, and the immune response of the body was found with a high frequency in children with the UPE association - mixed autostrains of all the listed representatives of the colon microflora, than in children from whom mainly monocultures were isolated.

The results of determining antibodies to UPE representatives showed comparable results for 6 representatives of the Enterobacteriaceae family - seronegative results were 19.7%-30.3% of sera, respectively, seropositive 69.7-80.3%. For P.aeruginosae, the seropositive sera were slightly higher, but statistically insignificant, compared to other antigens (seronegative - 15.2%, seropositive - 84.8%).

However, for all 7 antigens, the frequency of seropositive sera was significantly higher than seronegative ($p < 0.001$). This is especially true for E. coli (80.0 ± 8.9%), C. freindii (80.0 ± 8.9%) and P.aeruginosae (85.0 ± 7.9%). The percentage of detection of which was higher than other microorganisms. Apparently, these pathogens colonized the intestine and aggravated dysbiosis of the large intestine in children.

Study of the intensity of antibody formation to the studied UPE and P.aeruginosae, all children under 2 years of age were divided into the following groups: 1 - group children under 6 months; 2 - group children aged 7-9 months, 3 - group aged 10-13 months and 4 - group aged 13-24 months.

The obtained results show that the level of specific immunity in the form of the appearance of antimicrobial antibodies increases regularly from group 1 to group 4. It is noteworthy that in groups 3 and 4 a higher antibody titer was established (- log2 7.5 to - log2 4.3) than in groups 1 and 2 - 4.0-4.2 (in - log2) - $p < 0.05$ (Fig. 1.)

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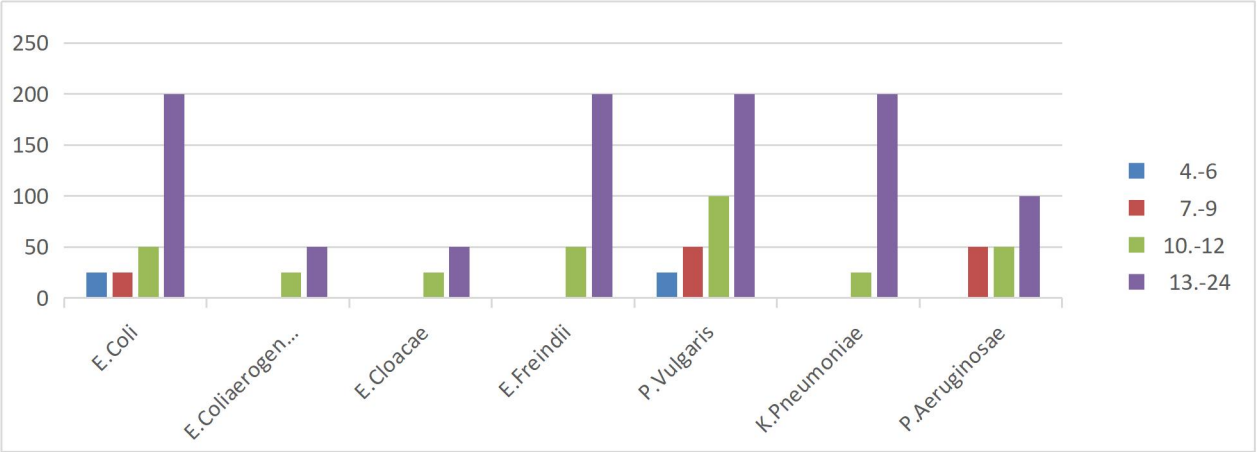

Fig. 1. Comparative indices of intensity of antibody formation against UPE antigens

Discussion

The levels of antibodies to intestinal microflora bacteria in colon dysbiosis and in healthy individuals were determined. Cellular sensitization to intestinal microflora antigens and its significance in extraintestinal manifestations of dysbiosis (morbidity, susceptibility to infections, allergies) were revealed. Risk groups for secondary immunodeficiency were determined based on the titers of antibodies to intestinal microflora antigens (antibodies to intestinal microflora as a marker of immunodeficiency). The role of antimicrobial immunity systems in the development of immunopathology in dysbiosis was established. An IFA method adapted for screening tests was developed to determine antibodies to enterobacteria antigens. A scheme for correcting intestinal dysbiosis was developed to reduce cellular sensitization to intestinal bacterial antigens. A relationship was established between intestinal dysbiosis and the state of the antimicrobial immune response.

Conclusions

1. Differences in the frequency of serum antibodies in the blood of the examined practically healthy children were established. The detected titers of antibodies to UPE antigens (E. coli, P. vulgaris, C. freundii, K. pneumoniae, E. aerogenes, E. cloacae, P. aeruginosae) had a wide range of variation, on average from 13 to 29%. The conducted division of the groups of examined subjects into 5 indicators (sharply positive, positive, weakly positive, doubtful, negative), depending on the values of the antibody titer in the blood of healthy people allows for relative normalization. 2. Specific antibodies to UPE antigens were detected in 80.3% of the studied children with grade III-IV intestinal dysbiosis, the body's immune response was found with a high frequency in children with an association with UPE. It was found that the number of seronegative indicators was 2.5-3 times lower than that of seropositive sera with all the studied UPE antigens, and for P.aeroginosa, the number of seropositive sera was slightly higher in relation to UPE antigens ($p<0.05$). With increasing age of the studied children, the level of specific immunity in the form of antimicrobial antibodies significantly increased ($p<0.05$).

3. The proposed experimental test system from antigens of collection E.coli strains for the IFA method is distinguished by sensitivity and specificity with various opportunistic enterobacteria.

4. All the examined children - with dysbiosis of the large intestine, gastroenteritis caused by UPE and practically healthy children - were found to have antibodies against UPE enterotoxin (except for healthy children under 3 years old). With increasing age of children, the detection of antibodies increases, the indicators of questionable and negative results decrease. The intensity of formation of antienterotoxic antibodies in the blood serum was significantly higher in children with colon dysbiosis ($p<0.05$) than in healthy children and children with gastroenteritis caused by UPE.

5. In children with colon dysbiosis, strains of colon microflora appear that produce proteases of immunoglobulin-destroying activity. This is especially characteristic of the total and thiol activity of proteases. The method for determining the immunoglobulin protease activity of coprofiltrates can be used as an additional diagnostic test for the diagnosis of colon dysbiosis in children. 6. Introduction of a biological preparation into the course of treatment normalizes the composition of normal microflora of the large intestine; a positive effect of biocorrection is also observed when studying sIgA in blood serum and coprofiltrates ($p<0.05$), as well as on the concentration of serum immunoglobulins - IgM, IgG and IgM ($p<0.005$).

7. When analyzing the results of IFA, it was found that the percentage of seropositive sera with antigens from the UPE decreased from 1.9 times to 2.7 times. Along with the percentage of seropositive sera, the intensity of antibody formation against antigens from the UPE also decreased.

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