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TO STUDY AND EVALUATE THE SIGNIFICANCE OF THE LEVEL OF SERUM ANTIBODIES TO BACTERIAL ANTIGENS IN INTESTINAL DYSBACTERIOSIS

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Objective. To study and evaluate the significance of the level of serum antibodies to antigens of opportunistic enterobacteria, the phagocytic activity of neutrophils and the level of sensitization to antigens of opportunistic enterobacteria in individuals with intestinal dysbiosis.

Materials and methods of the study The object was 88 children with III-IV degree colon dysbiosis, 79 children with diarrheal diseases, 30 practically healthy children. The subject of the research was the study of the normal microflora of the colon, the study of the antibody titer in the blood serum and coprofiltrates, antiendotoxic antibodies in the blood serum.In the process of carrying out scientific work, bacteriological, bacterioscopic, serological, immunological, ELISA and statistical methods were used.

Results of the study. To achieve the set objectives, we examined children aged from 4 months to 14 years. Among the examined children, grade III-IV colon dysbiosis was detected in 79 children. They were included in the main group. The first control group consisted of 30 practically healthy children. To compare all indicators of the main group, children with various diarrheal diseases were studied (2nd control group): bacterial dysentery - 30 children; salmonellosis - 27 children; gastroenteritis caused by opportunistic enterobacteria (E. coli, Proteus sp., Klebsiellae sp.) - 22 children. The age and sex composition of all examined children in the main and both control groups were identical. The clinical diagnosis of diarrheal diseases was established based on the life and disease history, clinical symptoms and laboratory tests. The etiologic diagnosis was confirmed bacteriologically.

The material for the study was the blood serum of sick and healthy children. For studies with coprofiltrates, feces were taken according to the generally accepted method, then diluted with saline. The feces diluted in a test tube were filtered, and the coprofiltrates were used to determine antibodies against UPE in ELISA.

ELISA was used to indicate antibodies to UPE antigens. The method is based on the principle of an indirect solid-phase enzyme-linked immunosorbent assay on polystyrene (indirect ELISA). The results were taken into account spectrophotometrically at a wavelength of 492 nm. The prepared complex bacterial antigens were brought to a concentration of 40 μg/ml. This concentration was used to sensitize the solid phase - polystyrene plates manufactured by Medpolymer, Russian Federation. After washing the immunological plates sensitized with antigens with a washing solution and drying, the studied blood sera, preliminarily triturated in a buffered physiological solution from 1:25 to 1:6400, were added to the wells. The mixture was incubated for 60 minutes, washed, a commercial reagent of antibodies against human IgG labeled with horseradish peroxidase (conjugate) was added, thoroughly washed and developing solutions - commercial OFD and perhydrol - were added to the wells. The counting was carried out visually by the coloring of the solution in the wells. The last dilution of the serum, which gave a more intense coloring of the solution in the well than in the control wells of the panel (negative control), was taken as the titer.

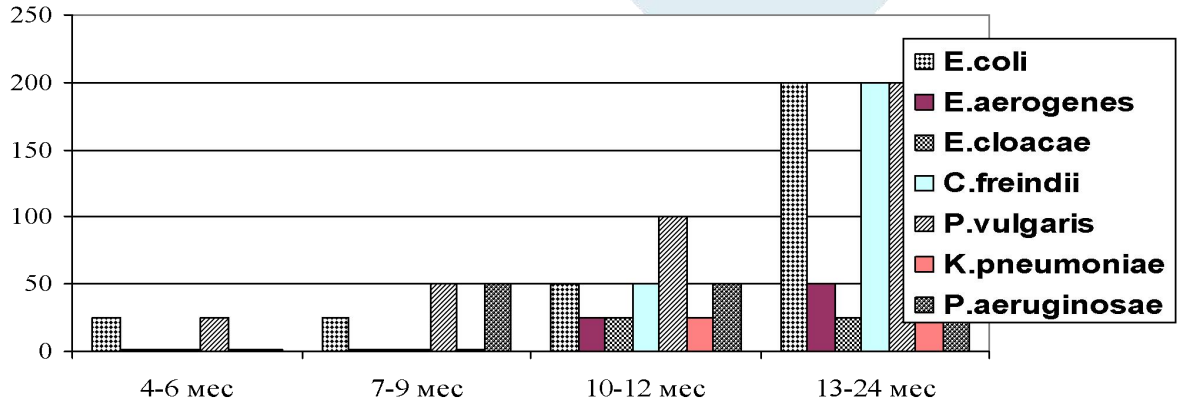

Fig. 1. Comparative indices of intensity of antibody formation against UPE antigens

The reaction results were taken into account, conditionally dividing them into the following groups proposed by us: strongly positive (titer from 1:1600 and more); positive (titer from 1:400 to 1:800); weakly positive (titer from 1:100 to 1:200); doubtful (titer from 1:25 to 1:50) and negative (titer 0). Conclusions: 1. Differences in the frequency of occurrence of serum antibodies in the blood of the examined practically healthy children were established. The revealed 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 titer of antibodies 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 seronegative indices were 2.5-3 times less than the seropositive sera with all the studied UPE antigens, and for P.aeroginosa the seropositive sera were slightly higher in relation to the UPE antigens ($p<0.05$). With increasing age of the studied children, the level of specific immunity in the form of antimicrobial antibodies significantly increases ($p<0.05$).

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

4. All examined children - with colon dysbiosis, 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 doubtful 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 practically healthy children and children sick with gastroenteritis caused by UPE.

5. In children with colon dysbiosis, strains of colon microflora appear that produce immunoglobulin-destroying proteases. This is especially true for 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 diagnosing colon dysbiosis in children.

6. Introduction of a biological preparation into the course of treatment normalizes the composition of the normal colon microflora, 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 elisa results, it was found that the percentage of seropositive sera with antigens from the upe decreased from 1.9 to 2.7 times. Along with the percentage of seropositive sera, the intensity of antibody formation against antigens from upe also decreased.

REFERENCES

1. Bagryantsev v. I., shubin f. N., tsvetkov v. S. Experience in using enzyme immunoassay for diagnosing pseudotuberculosis // issues of microbiology, pathogenesis and laboratory diagnostics of yersiniosis: Coll. Sci. Tr. — novosibirsk, 2018. — p. 53-56.
2. Belenkiy b. G., baltabaeva m. A., malov i. V. Evaluation of the specificity of some antigen preparations in enzyme immunoassay // yersiniosis (microbiology, epidemiology, clinical picture, pathogenesis, laboratory diagnostics): Abstract of reports of the all-union scientific and practical conf. — vladivostok, 2017. — p. 83-84.
3. Belyakov i. M. Immune system of mucous membranes // immunology. - 2017. - №4. - p.7-13.
4. Bondarenko av, bondarenko vl.m., bondarenko vm ways to improve the etiopathogenetic therapy of dysbiosis // zhur. Microbiol. - 2016. - №5. - p.96-101.
5. Valenkovich ln, yakhontova on on the functional state of the small intestine in patients with diabetes mellitus. Kazan med zhurn 2011; 5: 87-88.

6. Vorobyov aa, abramov na, bondarenko vm, shenderov ba dysbacteriosis - an urgent problem of medicine // bulletin of the russian academy of medical sciences. - 2007. - №3. - p.4-7.
7. Highly sensitive immunochemical method for determining autoantibodies to endometrial tissue antigen and its use in diagnostics of gynecological diseases /khokhlov p.p., mikhnina e.a., kalinina n.m. Et al. // medical immunology. - 2007. - vol. 9. - no. 2-3. - p. 268-269.
8. Garib f.yu., shamsiev a.m., eliseeva m.r. Immune-dependent diseases // tashkent, 2010. - 72 p.
9. Gvasalia m.m. Determination of immunoglobulins for the purpose of predicting the course of diabetes mellitus // method. Rec. - tbilisi. - 2011.
10. Darsalia i.a., tsyvkina g.i. Immunodeficiency states in children and dysbacteriosis. Possibilities of immunocorrection // proceedings of the far eastern regional scientific and practical conference, vladivostok. - 2010. - p.11-14.
11. Enzyme immunoassay of antibodies to insulin in human blood serum / sorokina n.v., gavrilova e.m., egorov a.m. Et al. // laboratory work. - 2000. - no. 2. - p.25-27.
12. Enzyme immunoassay test system for determining staphylococcal enterotoxin type c / fluer f.s., prokhorov v.ya., vesnina a.f., akatov a.k. // journal of microbiology. - 2002. - no. 6. - p.65-68.
13. Enzyme immunoassay for determination of specific antibodies in saliva of patients with pollinosis / manzhos m.v., shkadov s.a., nikishin a.v. Et al. // clinical laboratory diagnostics. - 2006. - no. 5. - p. 44-45.
14. Yeager l. Clinical immunology and allergology // moscow: Medicine; 1990.
15. Kasatkina e.p., voronin a.a., taranenko l.a. Bacteriological diagnostics of intestinal dysbacteriosis in children with diabetes mellitus // method. Rec. - moscow - 1996.