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IMMUNE RESPONSE OF THE BODY IN COLON DYSBIOSIS

Masharipov V.U.

Tashkent Medical Academy

Department of Microbiology, Virology, and Immunology

Objective To study the immune response of the body and evaluate the significance of serum antibody levels against antigens of conditionally pathogenic enterobacteria, as well as the level of sensitization to these antigens in individuals with intestinal dysbiosis.

Materials and Methods The study involved 138 children with grade III-IV colon dysbiosis, 102 children with diarrheal diseases, and 36 practically healthy children. The research focused on analyzing the normal microflora of the colon, determining the antibody titers in serum and coprofiltrates, and identifying anti-endotoxin antibodies in serum.

The study employed bacteriological, bacterioscopic, serological, immunological, ELISA, and statistical methods.

Results To achieve the objectives, we examined children aged 4 months to 14 years. Among them, 102 children were diagnosed with grade III-IV colon dysbiosis and included in the main group. The first control group consisted of 36 practically healthy children. To compare the main group's indicators, the second control group included children with various diarrheal diseases: 22 with bacterial dysentery, 28 with salmonellosis, and 52 with gastroenteritis caused by conditionally pathogenic enterobacteria (E. coli, Proteus sp., Klebsiella sp.). The age and gender distribution of children in the main and both control groups were identical.

The clinical diagnosis of diarrheal diseases was based on medical history, clinical symptoms, and laboratory tests. The etiological diagnosis was confirmed bacteriologically. Blood serum samples from both sick and healthy children were used for the analysis. For coprofiltrate studies, stool samples were collected according to standard procedures, diluted with saline, filtered, and analyzed for antibodies against conditionally pathogenic enterobacteria (CPE) using ELISA.

Indirect ELISA was used to detect antibodies against CPE antigens. The method is based on an indirect solid-phase enzyme immunoassay using polystyrene microplates. Results were recorded spectrophotometrically at a wavelength of 492 nm.

Prepared bacterial antigen complexes were adjusted to a concentration of 40 µg/ml, which was used for sensitizing the solid phase (polystyrene plates). After washing the antigen-sensitized plates with a washing solution and drying, test serum samples diluted in buffered saline (1:25 to 1:6400) were added. Plates were incubated for 60 minutes, washed, and then treated with horseradish peroxidase-labeled anti-human IgG antibodies. A substrate solution was added to visualize the reaction, and results were read visually based on color intensity.

Antibody titers were categorized into five groups:

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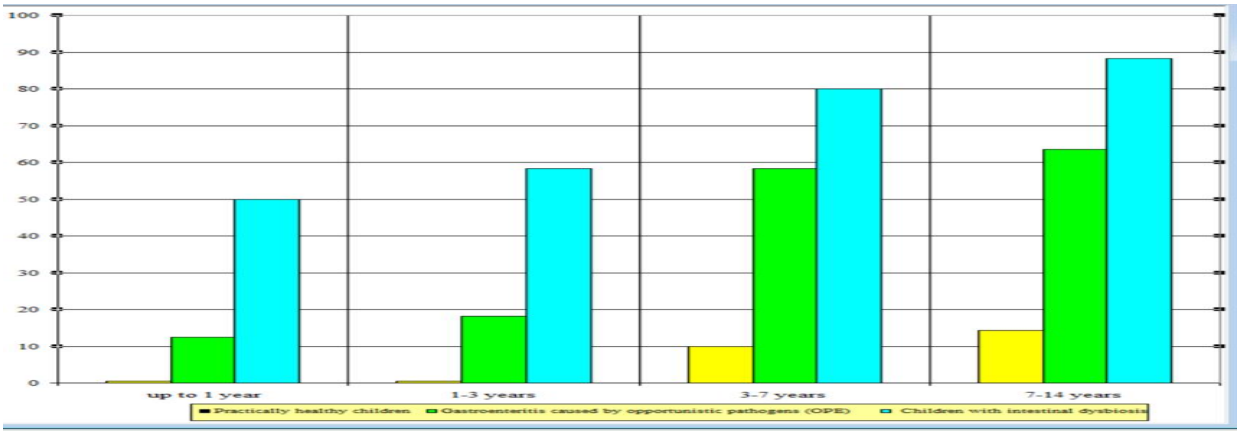

1. Strongly positive (≥1:1600)
2. Positive (1:400–1:800)
3. Weakly positive (1:100–1:200)
4. Doubtful (1:25–1:50)
5. Negative (0)

Conclusions

1. Antibody Frequency in Healthy Children: Significant variability in serum antibody titers was observed in practically healthy children, with a range of 13–29% for antigens of conditionally pathogenic microorganisms (E. coli, P. vulgaris, C. freundii, K. pneumoniae, E. aerogenes, E. cloacae, P. aeruginosa). Grouping based on antibody titers enabled relative normalization.
2. Immune Response in Dysbiosis: Specific antibodies to CPE antigens were detected in 80.3% of children with grade III-IV intestinal dysbiosis. A strong immune response was frequently observed in children with CPE associations. Seronegative cases were 2.5–3 times fewer than seropositive ones across all studied CPE antigens. Notably, P. aeruginosa antigens showed higher seropositivity ($p<0.05$). Antimicrobial antibody levels significantly increased with age ($p<0.05$).
3. Experimental ELISA Test System: The proposed ELISA test system using E. coli antigen strains demonstrated sensitivity and specificity for different conditionally pathogenic enterobacteria.
4. Enterotoxin-Specific Antibodies: Antibodies against CPE enterotoxins were found in all studied groups (excluding healthy children under 3 years). Antibody levels increased with age, while doubtful and negative results decreased. Children with colon dysbiosis had significantly higher levels of anti-enterotoxin antibodies ($p<0.05$) compared to healthy children and those with gastroenteritis caused by CPE.

(Figure 1 illustrates these findings.)

Figure 1. Levels of Anti-Endotoxic Antibodies Depending on Age

5 Protease-Producing Microflora in Dysbiosis: In children with colon dysbiosis, strains of intestinal microflora producing proteases with immunoglobulin-degrading activity emerge. This is particularly notable for general and thiol protease activities. The method for

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determining immunoglobulin protease activity in coprofiltrates can be used as an additional diagnostic test for detecting colon dysbiosis in children.

6 Effect of Biocorrection in Treatment: Introducing a biological preparation as part of the treatment normalizes the composition of the normal intestinal microflora. Positive effects of biocorrection were observed in the levels of secretory IgA (sIgA) in blood serum and coprofiltrates ($p<0.05$), as well as in the concentrations of serum immunoglobulins IgM, IgG, and IgA ($p<0.005$).

Table 1.1 presents the immune status indicators (M ± m) of children before and after treatment for dysbiosis

Examination Time	sIgA, g/L in Coprofiltrates	sIgA, g/L in Saliva	Serum Immunoglobulins, g/L	Complement Components, g/L
			IgG	IgM
Before Treatment	0.09 ± 0.04	0.16 ± 0.03	10.5 ± 0.7	0.88 ± 0.07
After Treatment (50 days)	0.14 ± 0.06*	0.21 ± 0.08*	12.4 ± 1.2	1.6 ± 0.2*

7 ELISA Analysis Results: Analysis of ELISA results revealed a reduction in the percentage of seropositive sera with antigens from conditionally pathogenic microorganisms (CPM). The reduction ranged from 1.9 to 2.7 times. Along with the decrease in seropositive sera, the intensity of antibody formation against CPM antigens also diminished.

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