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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

THE RELATIONSHIP BETWEEN GUT MICROBIOTA AND THE IMMUNE SYSTEM AND ITS CORRECTION IN CHILDREN WITH ACUTE URTICARIA

Speciality: 3220.01 – "Pediatrics"

Field of science: Medical Sciences

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GENERAL CHARACTERISTIC OF THE WORK

Relevance of the research. One of the priorities of healthcare in the field of pediatrics in modern times is to eliminate the percentage of complications resulting from allergic diseases among children. Thus, according to statistics from the World Health Organization, the incidence of these chronic diseases ranges between 15-35%.

The progressive increase in the mentioned percentage among children every year is explained by the role of many additional markers, in addition to allergic inflammatory mediators, which are involved in the allergic inflammatory processes that occur during these diseases. Among the allergic diseases found in children, acute urticaria is of particular importance, after bronchial asthma and allergic rhinitis. According to recent statistics, the incidence of acute urticaria among children is 6.7%[1].

According to internationally agreed upon health system documents, acute urticaria is pathogenetically heterogeneous and is described as a typical skin reaction characterized by angioedema, observed in the form of wheals[2].

Recent literature has highlighted the role of cytokines synthesized by T lymphocytes in the formation of the immune response during acute urticaria and in the transition of the allergic inflammatory process to a chronic form[3]. Cytokines are produced by cells with different morphological and functional activity, sufficiently activating

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the cytokine focus, leading to the formation of severe pathologies in the immune system[4].

Thus, the transformation of IgE-dependent allergic reactions during urticaria into slow allergic reactions creates an allergic inflammatory process of a cellular effector nature, which promotes the chronicity of the disease[5]. The role of many cytokines, especially IL-9, IL-17, and TNF-β, in this process is theoretically explained in many literatures[6].

Hypersecretion of these mediators is clinically reported as an increase in vascular permeability and vasodilation, causing irritation of sensory nerve fibers. Despite the data obtained, the role of cytokines, in particular IL-9, IL-17, TNF-β in acute urticaria, has not been practically fully studied. Information about cytokines and their biological effects still remains limited.

According to the International Expert Group on Acute Urticaria (GA^{2}LEN Global Urticaria Forum 2016), the modern concept of pathogenesis is multifactorial. The basis of these factors is explained by the role of the gut microbiota in the detection of visceral manifestations in the clinic of acute urticaria.

The human gut microbiota is a complex and dynamic ecosystem composed of numerous and diverse microorganisms. Although up to 400 bacterial isolates have been detected in the gastrointestinal tract during routine investigations, it is estimated that there are over 35,000 bacterial species in total. In 60% of cases, the microbiota is located in different parts of the gastrointestinal system. The gastrointestinal microbiota is composed of anaerobic,

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facultative anaerobic, and aerobic bacteria[7,8]. The gut microbiota resides in the intestinal tract of the human body and plays an important role in regulating the organism's metabolism and immune system. Its main role is to regulate many metabolic systems of the organism by maintaining intestinal homeostasis. Thus, the interaction of the intestinal microbiota with the immune system creates the basis for further exacerbation of the allergic inflammatory process and its transition to chronic. Considering the influence of the gut microbiota on the immune system, it is currently being discussed whether it plays a key role in the pathogenesis of allergic diseases[9].

Thus, acute urticaria in children is described as one of the most complex and ambiguous problems of modern healthcare. Acute urticaria is of particular interest in pediatric practice, as it is associated with significant diagnostic and therapeutic difficulties. From this perspective, the main goal of our research work was to study the relationships between clinical symptoms, immune markers, and gut microbiota in children diagnosed with acute urticaria.

Object and subject of research.

The study included 80 patients diagnosed with acute urticaria, and the clinical and immunological characteristics of the gut microbiota in the treatment and pathogenesis of the disease in these patients were studied as the subject of the study.

Purpose of the study: It consists of studying the interaction of the intestinal microbiota with the immune system during acute urticaria in children, as well as evaluating the clinical and immunological characteristics of pharmacocorrection carried out in their treatment.

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The tasks of the research:

1. Determination of IL-17, IL-9 and TNF-β cytokines in the blood serum of children with acute urticaria.
2. Determination of secretory IgA in the oral cavity of children with acute urticaria.
3. Study of gut microbiota (Bifidobacterium spp, E.coli, C.difficile, B fragilis, Lactobacillus spp, total bacterial count) in children with acute urticaria at the DNA gene sequence level.
4. Evaluation of the effectiveness of a symbiotic drug on the gut microbiota and immune system, administered alongside basic treatment, to children with acute urticaria.
5. Evaluation of correlations and ROC analysis between gut microbiota and immune indicators in children with acute urticaria.

Research methods: The research methods included clinical, laboratory, instrumental, and statistical methods.

Provisions of the dissertation submitted for defense:

1. Molecular study of the factors leading to acute urticaria creates conditions for a selective approach to the treatment of the disease.

2. Assessment of immunodeficiency in acute urticaria in addition to the determination of cytokines IL-9, IL-17 and TNF-β in the blood serum, the study of secretory IgA, which are alternatively markers of the activated immune system, in oral secretion also reveals a large role of local immunity in violation of general homestasis in children with acute urticaria.

3. The interaction between the immune system and the gut microbiota plays a major role in the development of the organism's defense function during acute urticaria.

4. The use of symbiotic drug in the complex treatment of acute urticaria helps to restore intestinal microbiota and indirectly impaired parameters of the immune system, improving the quality of treatment.

Scientific novelty of the work:

– The role of cytokines IL-9 , IL-17 and TNF-β in the clinical and diagnostic aspects of acute urticaria was revealed.

– The role of the influence of gut microbiota at the DNA gene sequence level in the clinical and diagnostic aspects of acute urticaria has been elucidated.

– For the first time, a scientific research study has evaluated the effectiveness of a symbiotic drug used in the treatment of children with acute urticaria, its effect on the gut microbiota and the immune system.

– The correlation between the gut microbiota and the immune system during acute urticaria was assessed.

Theoretical and practical significance of the study:

Studying the gut microbiota and immune system indicators during acute urticaria and assessing the correlation between them will be considered an additional diagnostic criterion and will provide a basis for early diagnosis of the disease and timely and selective implementation of the principle of adequate immunotherapeutic treatment.

Approbation of the research and its practical application:

The main provisions of the dissertation were presented at the international conference "Current Problems of Medicine" dedicated to the 100th anniversary of the birth of Professor Tamerlan Aliyev on October 6-8, 2021, at the "18th National Uludağ Pediatrics Winter Congress" (poster, Uludağ, Turkey) on March 13-16, 2022, at the XII International Conference "Current Problems of Pediatrics" on October 11-13, 2022, and at the "Rational Antibiotic Use Congress" on October 26-30, 2022.

The results of the research are used in the practical work of Educational Therapeutic Clinic of AMU and Children's Clinical Hospital No. 6, and the theoretical and practical recommendations of the dissertation are used in the educational process of the "II Children Diseases" department of AMU.

The organization in which the dissertation work was carried out. The research was carried out at the Educational Therapeutic Clinic of Azerbaijan Medical University, Children's Clinical Hospital No. 6, Scientific Research Immunology Laboratory of Azerbaijan Medical University, and the National Hematology and Transfusiology Center.

Published scientific works:

According to the results of the research, 13 scientific works have been published, 8 of which are articles and 5 are theses.

The scope and structure of the dissertation.

The dissertation work is written in Azerbaijani on 158 pages (202 000 characters), Chapter I, consisting of an introduction a literature review (65000 characters), Chapter II and materials and methods (13000 characters), Chapter III (34000characters), Chapter IV (27000 characters), Chapter V (27500characters), conclusion (24000 characters), results (2200 characters), practical recommendations (600 characters), a list of literature sources and conventional abbreviations. The dissertation consists of a bibliography that includes 200 literature sources. The dissertation is illustrated with 24 tables and 27 figures.

MATERIAL AND METHODS OF THE RESEARCH

The research work was carried out at the "II Children Diseases" Department of Azerbaijan Medical University during 2018-2020. The study subjects were 80 children diagnosed with acute urticaria at Children's Clinical Hospital No. 6 in Baku and the Educational Therapeutic Clinic of Azerbaijan Medical University. 20 practically healthy children were included in the control group.

Laboratory studies were carried out at the Educational Therapeutic Clinic of Azerbaijan Medical University, Children's Clinical Hospital No. 6, the Immunology Laboratory of Azerbaijan Medical University, and the National Hematology and Transfusiology Center. The children's parents were informed about the aims of the research and written consent was obtained from them. The ethical principles set forth in the World Medical Association Declaration of Helsinki (1964, 2000) were adhered to during the research. The diagnostic methods used were in accordance with the principles of bioethics and medical deontology.

Patient inclusion criteria:

- children from 1 to 18 years old;
- confirmation of the diagnosis of “acute urticaria”;
- absence of concomitant acute infectious diseases;
- absence of concomitant dermatological diseases;
- consent form from the children's parents.

Exclusion criteria:

- absence of a confirmed diagnosis of “acute urticaria”;
- confirmation of chronic urticaria diagnosis;
- the presence of infectious diseases 1 month before the examination;
- the presence of antibacterial treatment 3 months before the examination;
- presence of intestinal infections during the last 3 months;
- absence of consent form from the children's parents;

Children included in the control group were selected based on the following criteria:

- Age from 1 to 18 years;
- Denial of the diagnosis of acute urticaria;
- Denial of allergic diseases in the anamnesis;

The diagnosis was made based on the International Classification of Diseases (ICD-10), presented on the basis of clinical symptoms and anamnestic data.

Determination of IL-9, IL-17 and TNF- β cytokine levels in all children included in the study was determined by immunoenzymatic analysis from a single blood serum sample using a Medispec-6000 (RT-6000, Microplate Reader) apparatus (kits from Invitrogen by Thermo Fisher Scientific, USA). In our study, the "sandwich" type of solid-phase immunoenzymatic assay was used to conduct the analyses. The results were evaluated on a semi-automatic spectrophotocolorimetric analyzer and expressed in pg/ml.

The degree of immunodeficiency for each cytokine was calculated using the formula proposed by A.M. Zemskova: (patient group indicator / control group indicator – 1) x 100. Parameter values up to 33% are considered grade 1, 34-66% are considered grade 2, and above 66% are considered grade 3. If the result we get is evaluated as –, it is hypofunction, and if it is evaluated as +, it is hyperfunction.

The secretory IgA used for the research was measured in 1 ml of oral fluid taken from children for immunological examination. The study of sIgA in oral fluid was determined by immunoenzyme analysis method using reagents manufactured by the company "Xema-Medica" on the Medispec-6000 (RT-6000, Microplate Reader) apparatus.

The study of gut microbiota was performed in the laboratory of the Thalassemia Center of the Ministry of Health of the Republic of Azerbaijan. For all children, stool samples were examined using the ZPR-PV method, manufactured in Germany and owned by QIAGEN, to determine the microbiota of the colon.

The research study was identified as analytical by design; clinical by method; sample by volume; scientific by type; prospective by material; cross-sectional and longitudinal by duration; and clinical by location.

Statistical studies were conducted using the methods of variation in cross-sectional studies (t-Student-Bonferroni, U-Mann-Whitney, z-Sign), longitudinal studies (W-Wilcoxon), discriminant (Chi-square Pearson, Q-Cochran), and dispersion (test ANOVA). The significance between indicators was assessed using the non-parametric rho-Spearman rank correlation method. In ROC analysis, ROC curves were constructed, which are an integral indicator of the sensitivity and specificity of each test. In all statistical methods, the null hypothesis was rejected at $p < 0.050$.

Calculations were performed in MS EXCEL - 2019 and IBM Statistics SPSS - 26 programs.

RESEARCH RESULTS AND DISCUSSION

The study included 80 children with acute urticaria who developed urticarial rashes from the 1st and 2nd days. Of these, 37 (46.3%) were girls and 43 (53.7%) were boys (ratio 1:1.2). The age of the children varied from 2 to 16 years, with a mean age of 6.5±0.4 [95% CI: 5.7 - 7.3] years. The age structure of children examined with a diagnosis of acute urticaria was dominated by those aged 3 to 6-42 patients (52.5%). The average age of children included in the control group was 6.6±1.0.

The etiology of the disease was determined in 98.7% of the children included in our study. The study of the anamnesis showed that in most cases food allergens (n=57) were identified, and in rare cases, drug allergies (n=15). In 2 children, the acute allergic reaction was caused by insect allergens. In 6 children, the etiological factor was not identified.

Analysis of clinical and anamnestic data of 80 children diagnosed with acute urticaria included in the study showed that 43 (53.8%) of the children had allergic diseases in their parents. No predisposition to allergic diseases was detected in the parents of 37 (46.2%) children. In the study group of patients, allergic heredity was observed in 8 (18.6%) children from both the maternal and paternal lines. It should be noted that in 19 (44.2%) children, paternal relatives and in 16 (37.2%) children, maternal relatives suffered from atopic disease.

According to the results of our study, among the clinical signs in children diagnosed with acute urticaria, rash was noted in 77 (96.3%) children, itching in 73 (91.3%) children, edema in 3 (3.8%) children, and Quincke's edema in 25 (31.3%) children. Other clinical signs included allergic rhinitis in 1 (1.3%) child's history, acute bronchitis in 5 (6.3%) children's history, and anemia in 2 (2.5%) children's history.

The distribution of children according to the severity of urticaria was as follows: 18 (22.5%) had a mild degree of the disease, 47 (58.8%) had a moderate degree of the disease, and 15 (18.7%) had a severe degree of the disease (Figure 1).

Figure 1. Percentage indicators of severity of acute urticaria in children

Analysis of clinical manifestations of acute allergic reactions showed that 25 (31.3%) children had acute urticaria and various localized angioedema. The mean age of children with angioedema was 6.00±0.64 [95% CI: 4.68-7.32] years.

Considering the pathogenetic role of cytokines in the development of urticaria, the levels of IL-9, IL-17, and TNF-β from the cytokine cascades in the serum of both children diagnosed with acute urticaria and healthy children were studied (Table 1).

Table 1.

Comparative characteristics of cytokine status of children with acute urticaria M±m (min – max)

Indicator		Number	Average indicator	Standard error	Mini-mum	Maxi-mum	P_f	P_u
TNF-β	control	20	64,6	4,1	32	94	0,750	0,339
	main	70	62,0	4,2	4	181		
IL-9	control	20	0,97	0,07	0	2	0,000	0,000
	main	70	3,84	0,24	1	9		
IL-17	control	20	4,55	0,51	3	14	0,037	0,000
	main	70	6,69	0,52	4	33		

Note: The statistical integrity of the difference between group indicators according to the Pf -t-Anova criterion and the Pu-U-Mann-Whitney criterion

During the comparative analysis of serum IL-9 levels, it was determined that in the group of children diagnosed with acute urticaria, its average value was 3.84±0.24 pg/ml. This indicator was 0.97±0.07 pg/ml in the control group, which is explained by its lower level compared to the main group (p=0.000). Comparative analysis of IL-17 level indicators showed that its average value in the main group was 6.69±0.52 pg/ml. This indicator was 4.55±0.51 pg/ml in the control group. This also indicates that it is higher compared to the control group ($p<0.05$). Comparison of TNF- β levels in serum of the main (62.0±4.2 pg/ml) and control groups (64.6±4.1 pg/ml) did not reveal any statistically significant difference ($p>0.05$). In addition, a wider range of TNF-β levels was noted in the blood of children with acute urticaria compared to the control group.

As can be seen from the results of our research, in the group of children diagnosed with acute urticaria, the levels of IL-9 and IL-17 cytokines were significantly increased compared to the control group,

while the level of TNF-β in the blood serum was slightly increased compared to the control group.

In the next stage of our research, we assessed the level of sIgA (an indicator of mucosal immunity) in the oral cavity of children with acute urticaria. In children diagnosed with acute urticaria, the level of secretory IgA was lower than in the control group (median 91.5pg/ml, quartiles Q1 and Q3 40.7-197.0), which in turn confirms the weakening of local immunity against the antigen entering the organism. Thus, the average structural level of sIgA in the main group was ±91.5 g/l, which is 1.3 times (31.1%) lower than in the control group (±109.0 g/l) (Table 2).

Table 2.

Evaluation of sIgA levels in children with acute urticaria

İndicator	Number	Control	main	P_t	P_u
SIgA		20	48	p>0,05	p>0,05
	average	113,9	127,1		
	average structural indicator	109,0	91,5		
	Q 1	77,5	40,7		
	Q 3	142,3	197,0		

Note: The statistical integrity of the difference between group indicators according to the Pt -t-Student-Bonferroni and Pu-U-Mann-Whitney criteria

In modern times, it has been established that the gut mic-robiota, in addition to participating in the digestive process, also performs an immunomodulatory function, as well as participating in the development and functioning of various organs and systems. Given the influence of the gut microbiota on the immune system, its role in the pathogenesis of allergic diseases is currently widely debated.

Fecal samples were examined for the purpose of determining the colonic microbiota for all children. The obtained materials were studied using the ZPR method for the four main types of microorganisms belonging to the bacterial domain (*Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria*).

The composition of the gut microbiota of the children included in the study group is presented in Table 3.

The most common types of bacteria in the main group are the Actinobacteria phylum, Actinobacteridae class, Bifidobacteriaceae family, and Bifidobacterium phylum. Bifidobacterium phylum is a non-spore-forming gram-positive polymorphic rod, most often strict anaerobes.

Table 3.

Composition of gut microbiota in examined children

Name		Group 1				P_{x^2}
		Control		main		
		Number	%	Number	%	
Bifidobacterium	Decrease	12	60,0%	2	2,5%	p<0,001
	Increase	8	40,0%	78	97,5%	
E.coli	Decrease	7	35,0%	9	11,3%	p<0,05
	Increase	13	65,0%	71	88,8%	
B.fragilis	Decrease	10	50,0%	3	3,8%	p<0,001
	Increase	10	50,0%	77	96,3%	
C.difficile	Decrease	20	100,0%	74	92,5%	p>0,05
	Increase	0	0,0%	6	7,5%	
Lactobacil	+	7	35,0%	31	38,8%	p>0,05
	++	13	65,0%	49	61,3%	
	+++	0	0,0%	0	0,0%	
Total bacteria	+	6	30,0%	37	46,3%	p>0,05
	++	14	70,0%	43	53,8%	
	+++	0	0,0%	0	0,0%	

Note: PX2 – Statistical significance of the difference between the test results according to the Chi-square Person criterion:
** -The "0" hypothesis is rejected*

The growth of Bifidobacterium species was recorded in the examined material of 78 (97.5%) children diagnosed with acute urticaria. During the analysis by age group, it was determined that in children aged 0-3 years, Bifidobacterium species bacteria were found in 3 children (3.75%). It occurs in 41 (51.25%) children aged 3-6, 26 (32.5%) children aged 7-11, and 8 (10.0%) children aged 12-18. Two (2.5%) children diagnosed with acute urticaria had a decrease in bifidobacteria in their gut microflora.

The next type of bacteria that is more prevalent in the intestinal microflora of children in the main group is Bacteroidetes, class Bacteroidia, family Bacteroidaceae, species B. fragilis - gram-negative, rod-shaped bacteria. They were observed in the stool material of 77 (96.3%) children diagnosed with acute urticaria. During the analysis by age group, it was determined that B. fragilis bacteria were found in 3 (3.75%) children under the age of 3. It was observed in 39 (48.75%) children aged 3-6, 26 (32.5%) children aged 7-11, and 9 (11.25%) children aged 12-18. A decrease in B. fragilis bacteria was detected in 3 (3.8%) children.

The third most common type of bacteria in the intestinal microflora is Proteobacteria, family Enterobacteriaceae, genus Escherichia, which is gram-negative, short-rod, facultative anaerobe. In 71 (88.8%) children, the growth of E. coli bacteria was recorded in the studied material. During the analysis by age groups, it was determined that *E.coli* bacteria were found in 3 (3.75%) children under 3 years of age. It was observed in 38 (47.5%) children aged 3 to 6 years, in 23 (28.75%) children aged 7 to 11 years, and in 7 (8.75%) children aged 12 to 18 years. It was not detected in 9 (11.3%) children.

Thus, the main bacteria of the microbial class in the feces of children with acute urticaria were *Bifidobacterium* - observed in 78 (97.5%) patients. Then, in terms of numerical composition, *B. fragilis* was found in 77 (96.3%) and E. coli in 71 (88,8%) children.

The Firmicutes bacteria type, class Clostridia, type Clostridium - gram-positive anaerobes, which were not detected within the norm, were identified in the intestinal microbiota of children with acute urticaria, and C. difficile was observed in 6 (7.5%) children. When analyzing by age group, it was determined that *C. difficile* bacteria were found in only 6 (7.5%) of children aged 3-6.

A decrease in the amount of *Lactobacillus*, assessed as 1+, was recorded in 38.8% of children, and a decrease in the amount of *Lactobacillus*, assessed as 2+, was recorded in 61.3% of children. The deficiency of lactobacteria suggests an important role of this type of obligate flora in the pathogenesis of intestinal dysfunction in children with acute urticaria. A decrease in the total bacterial count by 1+ was

recorded in 46.3% of children, and a decrease by 2+ was recorded in 53.8% of children with acute urticaria.

A change in the quantitative ratio of Firmicutes and Bacteroidetes in the intestinal microbiota can be one of the indicators of a disruption in the state of the microbiota, and in this case, a change in this mutual ratio is observed both in the direction of increase and decrease. When comparing the frequency of occurrence of various taxa of microorganisms in the colon of children in the main and control groups, clear differences were found for three microorganisms. The most common type of bacteria in the group of children with acute urticaria was represented by the Actinobacteria type, which includes gram-positive bacteria. Compared to the control group, an increase in Bifidobacterium genus bacteria was recorded in children diagnosed with acute urticaria ($m\pm56.25$; $p<0.001$). The next most common type of intestinal microflora bacteria is the Bacteroidetes, which consists of gram-negative, strict anaerobes with saccharolytic activity. Compared to the control group, an increase in the proportion of bacteria of the genus *Bacteroides* (B.fragilis) ($m\pm55.13$; $p<0.001$) was observed in children diagnosed with acute urticaria. In the group of children with acute urticaria, a statistically significant increase in the proportion of bacteria belonging to the *Proteobacteria phylum* was also recorded in the composition of the intestinal microbiota. The phylum *Proteobacteria* includes most gram-negative microorganisms that belong to facultative or obligate anaerobes. In children diagnosed with acute urticaria, the increase in *Proteobacteria* occurs due to bacteria of the genus *Escherichia (E.Coli)* ($m\pm52.88$; $p < 0.05$).

Thus, as a result of the analysis of the distribution of species, the dominance of bifidoflora was revealed in children diagnosed with acute urticaria, while in the group of children without allergic pathology, the dominance of Escherichia coli and the subdominance of B. fragilis were revealed.

The changes detected in the gut microbiota suggest the need for the prescription of symbiotic drugs to correct the gut microflora of such patients. Overall, positive changes in the state of the intestinal microbiocenosis were observed in children after the administration of the symbiotic drug. A controlled analysis after correction of intestinal

dysbiosis showed that 50.3% ($p<0.001$) of children had a clear increase in *Lactobacillus* counts, 95.2% ($p<0.001$) of children had a decrease in *E.coli*, and 66.7% ($p<0.001$) of children with acute urticaria had a decrease in *B.fragilis*. Comparative analysis of the microbial background of the colon of patients and healthy children after treatment revealed statistically significant differences for *Bifidobacterium* ($p<0.05$), *E.coli* ($p<0.05$), *B.fragilis* ($p<0.05$), *Lactobacillus* ($p<0.05$). The state of the intestinal microbiota, which plays a leading role in ensuring homeostasis of the child's organism, is of particular importance in the pathogenesis of allergic skin diseases. In modern times, the study of the influence of the intestinal microbiota on the immune system in the pathogenesis of allergic pathologies is of a debatable nature. Currently, various symbiotic drugs are used to treat intestinal dysbiosis.

The clinical effectiveness of symbiotic drugs is explained precisely by the fact that its strains of microorganisms participate in the suppression and inhibition of pro-inflammatory cytokines, as well as have an immunomodulatory effect. In the next phase of our study, intergroup and within-group comparative assessment of the amount of IL-9, IL-17, TNF-ß and secretory IgA in the blood serum of children diagnosed with acute urticaria during treatment with symbiotic drug (up to 1 year old - ½ packet 1-2 times, 1-3 years old - ½ packet 2 times, 3-6 years old - 1 packet 1-2 times, over 6 years old - 1 packet 1-2 times during meals) was carried out.

The intergroup comparison confirmed a statistically significant increase in interleukin IL-9 and secretory IgA levels after treatment compared to the control group ($p<0.001$). During the intergroup comparison after treatment compared to the control group, no statistically significant differences were found in the mean levels of TNF-β and IL-17 in the serum of children ($p > 0.05$).

During the treatment dynamics, a 1.3-fold decrease in serum IL-9 levels was observed during the within-group comparison ($p=0.024$). In a comparative analysis of the average level of IL-17 in the blood serum, a decrease of 37.3% was observed ($p=0.003$). No statistically significant difference was detected in the within-group comparative analysis of the mean serum TNF-β levels. Based on the results of the

applied statistical analysis, during the within-group comparison, a high statistical significance was confirmed for interleukin IL-9 and interleukin IL-17 indicators ($p<0.05$). As seen from the obtained data, a decrease in sIgA levels was observed in children diagnosed with acute urticaria before treatment. Thus, low sIgA levels confirm the weakening of the local immune functions in the existing disease. On the background of treatment with the application of the symbiotic drug, the sIgA level in the main group increased from 59.1±5.3 g/l (range 22.0-301.0 g/l) to 93.9±15.6 g/l (range 24.0-112.0 g/l), which can be explained as a 1.6-fold increase compared to the control group.

In the next stage of the study, a more sensitive ROC analysis of the gut microbiota and immune indicators was conducted by us. Based on the results of the statistical analysis, the area of the ROC curve of the SIgA indicator for Bifidobacterium was determined as 0.368±0.073; 95% CI: upper and lower limits 0.225-0.510: p=0.151, and the area of the ROC curve for the TNF-β indicator was determined as 0.495±0.084; 95% CI: upper and lower limits 0.330 - 0.659: p=0.954. The area of the ROC curve for the IL-9 indicator was 0.884±0.049; 95% CI: upper and lower limits 0.788 - 0.980: p=0.000 and the area of the ROC curve for the IL-17 indicator was 0.774±0.079; 95% CI: upper and lower limits 0.618-0.929: p=0.003, which was considered statistically significant.

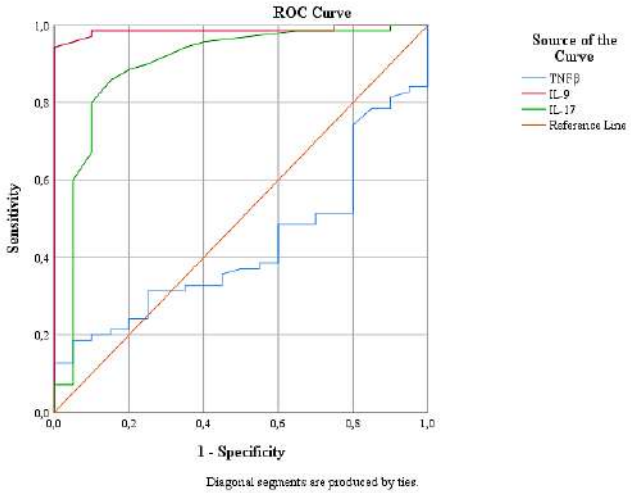
The area of the ROC curve of sIgA for E. coli was 0.338±0.074; 95% CI: upper and lower limits, 0.193–0.483, respectively: p=126, and was not statistically significant. The area of the ROC curve for TNF-β was 0.560±0.083; 95% CI: upper and lower limits 0.397–0.724: p=0.569, no statistically significant result was obtained. The area of the ROC curve for the IL-9 indicator was 0.768±0.072; 95% CI: upper and lower limits 0.627–0.909: p=0.011, which can be considered diagnostically significant. The area of the ROC curve for the IL-17 indicator was 0.727±0.086; 95% CI: upper and lower limits 0.558–0.896: p=0.032, which was considered statistically significant. During the ROC analysis of B. fragilis and immune indicators, the area of the ROC curve for the sIgA indicator was 0.279±0.067; 95% CI: upper and lower limits, 0.147–0.411: p=0.024, which was considered statistically significant. The area of the ROC curve for TNF-β was

0.486±0.084; 95% CI: upper and lower limits 0.321–0.651: p=0.885, and a statistically significant result was not obtained.

The area of the ROC curve for the IL-9 indicator was 0.840±0.058; 95% CI: upper and lower limits 0.727 – 0.954: p=0.001, a statistically significant result was achieved. The area of the ROC curve for the IL-17 indicator was 0.761±0.090; 95% CI: upper and lower limits 0.585–0.936: p=0.008, which was considered statistically significant. When analyzing the ROC analysis between C. difficile and immune indicators, the ROC curve area for the sIgA indicator was 0.365±0.146; 95% CI: upper and lower limits, 0.078–0.651: p=0.434, and for TNFβ, the ROC curve area was 0.327±0.162; 95% CI: upper and lower limits 0.009–0.645: p=0.317, and the ROC curve area for the IL-17 indicator was 0.774±0.090; 95% CI: upper and lower limits 0.597–0.950: p=0.113, indicating that no reliable results were obtained for these indicators. The area of the ROC curve for the IL-9 indicator was 0.858±0.072; 95% CI: upper and lower limits 0.718–0.999: p=0.038, resulting in a statistically significant diagnostic indicator. The area of the ROC curve of the sIgA index for *Lactobacillus* was 0.652±0.075; 95% CI: upper and lower limits 0.505-0.799: p=0.056-"The null hypothesis can be conditionally rejected. The area of the ROC curve for the TNF-β indicator was 0.429±0.081; 95% CI: upper and lower limits 0.270 - 0.588: p=0.374, the area of the ROC curve for the IL-9 indicator was 0.507±0.084; 95% CI: upper and lower limits 0.343 - 0.672: p=0.926, the area of the ROC curve for the IL-17 indicator was 0.492±0.081; 95% CI: upper and lower limits 0.332 - 0.652: p=0.920, so we could not evaluate it as a statistically significant diagnostic indicator. Based on the analysis of the ROC analysis of the total number of bacteria, the area of the ROC curve for the sIgA indicator was 0.596±0.079; 95% CI: upper and lower limits 0.411-0.750: p=0.230, the area of the ROC curve for the TNF-β indicator was 0.643±0.074; 95% CI: upper and lower limits 0.498-0.788: p=0.073, the area of the ROC curve for the IL-9 indicator was 0.396±0.080; 95% CI: upper and lower limits 0.238-0.553: p=0.191, the area of the ROC curve for the IL-17 indicator was 0.392±0.079; 95% CI: upper and lower limits 0.237-

0.546: p=0.174 and we could not evaluate it as a statistically significant diagnostic indicator.

Figure 2 shows the ROC curve for the IL-9, IL-17, and TNF-β parameters in the group of patients with acute urticaria. The area under the ROC curve for the TNF-β indicator was 0.430±0.065; 95% CI: upper and lower limits 0.303 - 0.556; p=0.339, which was not statistically significant. The area under the ROC curve for the IL-9 indicator was 0.986±0.011; 95% CI: upper and lower limits 0.964 - 1.000; p=0.000, which was considered statistically significant. The area under the ROC curve for the IL-17 indicator was 0.894±0.049; 95% CI: upper and lower limits 0.797 - 0.990; p=0.000, which can be considered a statistically significant diagnostic indicator.



Indicators	Area	Standart error	P – statistical integrity	95% CI	
				Lower limit	Upper limit
TNF-β	0,430	0,065	0,339	0,303	0,556
IL-9	0,986	0,011	0,000	0,964	1,000
IL-17	0,894	0,049	0,000	0,797	0,990

Figure 2. ROC analysis of cytokines in the diagnosis of acute urticaria in children

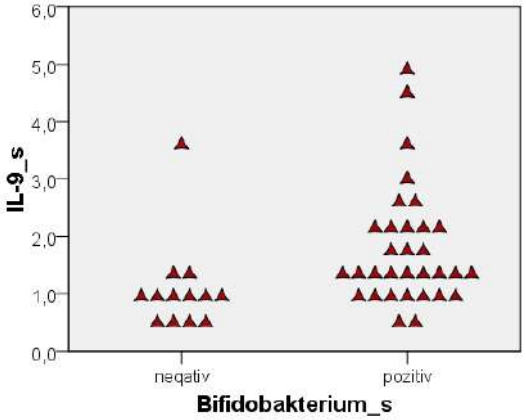
The results of the ROC examination confirm that gut microbiota has high informativeness and accuracy in diagnosing immune indicators.

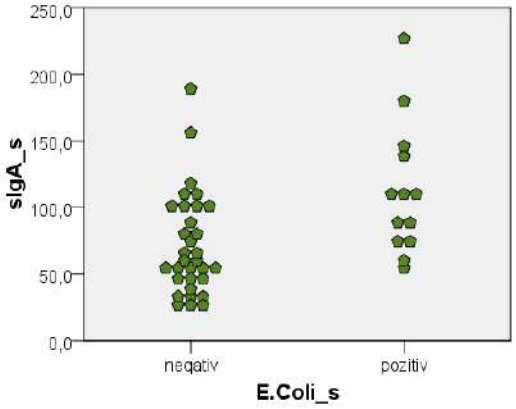
Multiple correlation analysis was performed using the Sperman correlation coefficient to assess the interaction of microorganisms with clinical and laboratory indicators and to understand their interaction with each other.

Thus, the analysis of the results obtained showed that acute urticaria in children is accompanied by hypersecretion of pro-inflammatory cytokines in the blood. A clear increase in the levels of IL-9 ($p < 0.001$) and IL-17 ($p < 0.001$) in the blood of children diagnosed with acute urticaria confirms the important role of these cytokines in the pathogenesis of the disease. The established intersystem relationships revealed a clear correlation between high levels of pro-inflammatory cytokines such as IL-9 ($p < 0.001$) and IL-17 ($p < 0.001$) and clinical symptoms of acute urticaria.

Intrasystem correlations have also been observed among gut microbiota. The correlation index between conditionally pathogenic microorganisms and representatives of obligate microflora decreased with a decrease in the degree of dysbiotic manifestations. We recorded equal inverse correlations between the amounts of *Lactobacillus* and *E. coli* ($\rho = - 0.417$; $p = 0.004$).

Figure 3 provides a graphical representation of the resulting direct and reverse correlation between microbiotes and immune indicators in treatment dynamics.

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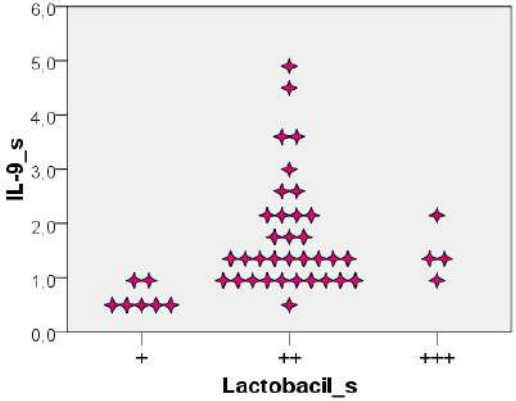
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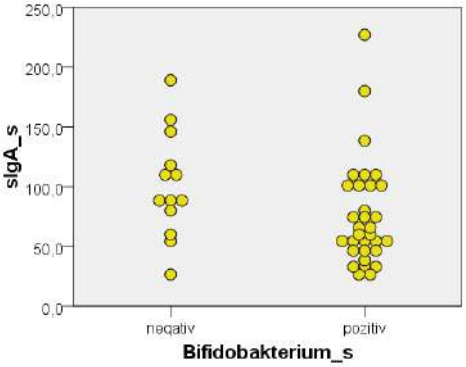
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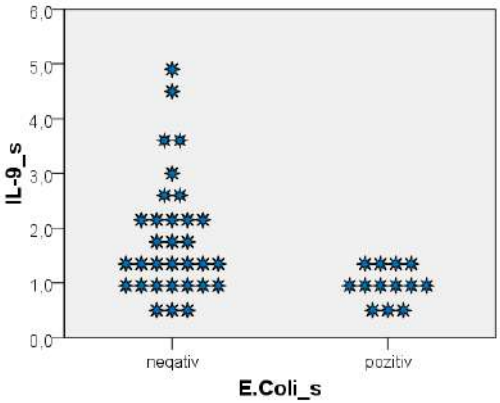

Figure 3. Direct and inverse correlation between microbiota and cytokines

According to the results of the study, a positive correlation was recorded between *Bifidobacteria* and IL-9 cytokine (ρ=0.396; $p<0.05$), and a negative correlation was recorded with sIgA (ρ=-0.315; $p<0.05$). There was a positive correlation between *E. coli* and sIgA (ρ=0.410; $p<0.05$), a negative correlation with the cytokine IL-9 (ρ=-0.417; $p<0.05$), and a positive correlation between *Lactobacillus* and IL-9 (ρ=0.423; $p<0.05$).

By evaluating the dynamics of changes in intermicrobiota correlations, it is possible to confirm that during treatment with symbiotic drugs, an increase in obligate flora occurs in the colon of children diagnosed with acute urticaria, and a large part of the connections between the conditionally pathogenic flora of the intestine are lost. Therefore, during the treatment period, the interaction between microorganisms is replaced, and new correlations between opportunistic and obligate representatives appear.

Overall, positive changes in the state of the intestinal microbiota were observed in children after the administration of the symbiotic drugs. A controlled analysis after correction of intestinal dysbiosis showed that in the microbial background of children, a clear increase in the number of *Lactobacillus* was recorded in 50.3% ($p<0.001$), a decrease in *E. coli* in 95.2% ($p<0.001$), and a decrease in *B. fragilis* in

66.7% ($p<0.001$) in children with acute urticaria. Comparative analysis of the microbial background of the colon of patients and healthy children after treatment revealed statistically significant differences for *Bifidobacterium* ($p<0.05$), *E.coli* ($p<0.05$), *B.fragilis* ($p<0.05$), *Lactobacillus* ($p<0.05$).

Thus, our study revealed that the relationship between cytokine cascades and intestinal microbiota in the children with acute urticaria is of a correlational nature. By prescribing a symbiotic drug to such patients, we also influence the level of cytokines by normalizing the intestinal microbiota, and this creates a basis for preventing the chronicization of the allergic inflammatory process.

RESULTS

1. A study of cytokines such as IL-9, IL-17, and TNF-β in children diagnosed with AU revealed that the deficiency among them is of a compensatory nature. As a result of the study, it was determined that the serum IL-9 concentration in children with acute urticaria was 4 times higher than in the control group (3.84±0.24 pg/ml, $p<0.001$), and the IL-17 concentration was 1.5 times higher than in the control group (6.69±0.52 pg/ml, $p<0.01$), and their levels were significantly higher. No statistically significant difference was found in the concentration of TNF-β (62.0±4.2pg/ml, $p>0.05$) compared to the control group. The observed fluctuations in the level of interleukins characterize the exacerbation of the allergic inflammatory process and indicate the progression of the disease [2,4,10,13].

2. Determination of sIgA ($p>0.05$) in the oral cavity is an alternative substrate for assessing the mucosal immune response in children with AU. The level of the studied indicator was 1.3 times lower than in the control group. This indicator is considered an informative indicator for monitoring the severe course of the disease, as a result of our study [12].

3. The intestinal microbiota of children with AU is characterized by a deficiency of *Lactobacillus* (+ 38.8%, ++ 61.3%) against the background of an increase in the number of *Bifidobacterium* (97.5%), *B. fragilis* (96.3%), and *E. coli* (88.8%) species, which confirms that

the intestinal microbiota is relatively unstable and requires correction of the detected deficiencies [7, 9].

4. Considering the role of gut microbiota in the immune mechanism of AU, the inclusion of the symbiotic drug in the basic treatment resulted in positive dynamics between the conditionally pathogenic representatives of the gut microflora (*B.fragilis, E.coli, Lactobacillius, Bifidobacterium*) and immune indicators (IL-9, IL-17, sIgA) and clinically manifested improvement ($p<0.005$) [3, 5, 6].

5. According to the results of the study, during the correlation between gut microbiota and immune indicators, a positive correlation was recorded between *Bifidobacterium* and the level of cytokine IL-9 ($r=0.396$; $p<0.05$), and a negative correlation was recorded with sIgA ($r= - 0.315$; $p<0.05$). There was a positive correlation between *E. coli* and sIgA ($r=0.410$; $p<0.05$), a negative correlation with the cytokine IL-9 ($r= - 0.417$; $p<0.05$), and a positive correlation between *Lactobacillus* and IL-9 ($r=0.423$; $p<0.05$) [8, 11].

6. According to the conducted ROC analysis, we can conclude about a highly informative relationship between IL-9 and *Bifidobacterium*; IL-17 and *Bifidobacterium* indicators. The average informativeness of the analysis was determined for the indicators IL-9 and *B.fragilis*; IL-17 and *B.fragilis*; IL-9 and *C.difficile*; IL-17 and *E.Coli*, IL-9 and *E.Coli*. For the remaining indicators, the informativeness of the analysis was insignificant or insufficient [3].

PRACTICAL RECOMMENDATIONS

1. It is recommended to study the gut microbiota in children diagnosed with acute urticaria to optimize treatment and assess the clinical course of the disease.

2. During the complex clinical-laboratory evaluation of children with acute urticaria, the study of pro-inflammatory cytokines IL-9, IL-17 and sIgA is recommended as an additional diagnostic criterion to assess the intensity of the allergic inflammatory process.

3. In the treatment of children with acute urticaria, it is more appropriate to prescribe a symbiotic drug to restore the immune system and colon microbiota.

PUBLISHED SCIENTIFIC WORKS ON THE TOPIC OF THE DISSERTATION

1. N.H.Sultanova, A.C.Şixəmmədova. Uşaqlarda kəskin övrə zamanı bağırsaq mikroflorası ilə sitokin kaskadları arasında qarşılıqlı əlaqə. // «Azərbaycan Təbabətinin Müasir Nailiyyətləri" jurnalı.», 2021, №1, səh. 142-145. Bakı.

2. N.H.Sultanova, A.C.Şixəmmədova. Uşaqlarda kəskin övrə zamanı sitokin statusunun qiymətləndirilməsi. / Tamerlan Əliyevin 100 illiyinə həs olunmuş "Təbabətin aktual problemləri" Beynəlxalq kongres, 6 oktyabr 2021 materialları, səh. 31-34. Bakı.

3. A.C.Şixəmmədova. Kəskin övrə zamanı immun sisteminin göstəriciləri ilə bağırsaq mikrobiotasının səviyyələrinin qiymətləndirilməsi. // "Sağlamlıq" jurnalı. 2022, Cild 28, № 1, səh 115 - 118. Bakı.

4. N.H.Sultanova, A.C.Şixəmmədova. Akut ürtikerli çoçuklarda IL-17, IL-9, TGF-B değerlendirilmesi. / "18 Ulusal Uludağ Pediatri" kış kongresi" 2022, 13 - 16 mart, səh. 278 Türkiyə,Uludağ.

5. A.C.Şixəmmədova. Клинико-анамнестическая характеристика детей с острой крапивницей. // "Azərbaycan Allerqologiya və Klinik İmmunologiya" jurnalı. Cild 10; №1, 2022, səh. 39 – 43. Bakı.

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7. N.H.Sultanova, A.C.Şixəmmədova. Особенности микробиоты кишечника у детей с острой крапивницей. // "Медицинские новости". 2022, 9 sentyabr, səh 49 – 51. Belarus, Minsk.

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9. N.H.Sultanova, A.C.Şixəmmədova. Uşaqlarda kəskin övrənin diaqnostikasında bağırsaq mikrobiotlarının rolu. / "Azərbaycan

allerqoloq,immunoloq və immunoreabilitoloqların VI milli beynəlxalq" konfransı, 2022, 4 noyabr, səh 105. Bakı.

10. N.H.Sultanova, A.C.Şixəmmədova. Особенности цитокинового статуса у детей с острой крапивницей. // Журнал "Педиатрия". 2022, Том10, №4. Səh 538 – 545. Belarus.

11. A.C.Şixəmmədova. Uşaqlarda kəskin övrə zamanı IL-17 ilə bifidobacterium səviyyəsinin qarşılıqlı əlaqəsi. // "Azərbaycan Pediatriya" Jurnalı, 2023, Cild1, №3, səh 32 - 36. Bakı.

12. A.C.Şixəmmədova. Uşaqlarda kəskin övrənin klinik-anamnestik xüsusiyyətləri və xəstələrin ağız boşluğunda sekretor IgA səviyyəsi. // "Azərbaycan Tibb" jurnalı. 2024, №4, səh 111 - 114. Bakı

13. A.C.Şixəmmədova. Kəskin övrəli uşaqlarda IL-17 səviyyəsinin qiymətləndirilməsi. / Azərbaycan-Türkiyə Allerqoloq və İmmunoloqlarının 1-ci Beynəlxalq Konfransı, 2024, 07 dekabr, səh.61. Bakı.

LIST OF CONVENTIONAL ABBREVIATIONS

ARI	–	acute respiratory infection
AU	–	acute urticaria
CI	–	confidence interval
Ig	–	immunoglobulin
IL	–	interleukin
NSAID	–	non-steroidal anti-inflammatory drug
sIgA	–	secretory immunoglobulin A
Th	–	T helper cells
TNFβ	–	tumor necrosis factor beta
Treg	–	T-regulatory cells

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