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Article

Microbions and Their Interrelation in the Development of Respiratory Allergy in Children

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Abstract: Respiratory allergic diseases in children represent one of the most pressing issues in modern pediatrics and allergology, characterized by steady growth in prevalence, early manifestation, and significant impact on the quality of life of both patients and their families. According to WHO data, the prevalence of allergic diseases of the respiratory system in the child population ranges from 10-20% in developed countries to 5-10% in developing countries, with a steady increase in the prevalence of this pathology observed over the last decade. In the Republic of Uzbekistan, the prevalence of bronchial asthma in children is 4.2–5.8 per 1,000 children (National Clinical Protocol of the Ministry of Health of the Republic of Uzbekistan, 2024), and the incidence of allergic rhinitis in the child population reaches 25–35%.

Keywords: *microbiota, respiratory allergy, children, immune tolerance.*

1. Introduction

Modern achievements in microbiology, immunology, and molecular genetics open up fundamentally new ideas about the role of the microbial factor in the development of tolerance and, conversely, in predisposition to allergic diseases [1]. The implementation of high-tech research methods, such as 16S rRNA sequencing, metagenomic analysis, and culture studies of microbial communities, allows for a detailed study of the composition, functional activity, and dynamics of microbial changes in both the intestinal tract and the respiratory tract during the early stages of ontogenesis [2]. A growing body of evidence indicates that forming a healthy microbiome during critical periods of immune system development is crucial for developing immune tolerance and preventing the development of allergic diseases [3].

At the same time, despite significant progress in understanding the mechanisms of interaction between the microbiota and the immune system, many issues remain insufficiently studied [4]. There is no single algorithm for assessing the state of the microbiome in respiratory allergies in children, the critical time windows for microbial colonization are not sufficiently clear, the mechanisms through which certain microbial taxa prevent the development of allergies are not fully understood, and microbiome-oriented approaches to the prevention and treatment of respiratory allergic diseases have not been developed [5]. Environmental and socio-economic factors, including the nature of feeding, the use of antibiotics, living conditions, and exposure to air pollutants, significantly influence the formation of the microbiome during critical developmental periods; however, the relationship between these factors and the development of allergies requires further study.

The role of the microbiota in the development of respiratory allergy in children represents one of the most promising areas of modern allergology and immunology. According to major international studies, dysbiosis of the gut microbiome and respiratory

tract in early childhood correlates with an increased risk of developing allergic diseases in later years of life[6]. In the Republic of Uzbekistan, there is little data on the impact of microbial colonization on the development of allergies in children, which determines the need for comprehensive research in this area [7].

Mechanisms through which the microbiota influences the development of immune tolerance include the production of short-chain fatty acids, modulation of innate immunity through pattern-recognizing receptors, changes in intestinal barrier permeability, and the formation of specific cellular immunity. These and other mechanisms require further detailed study to develop preventive and therapeutic strategies. This has determined the relevance of this study.

2. The aim of the study is to study

The role of microbial factors and microbial diversity in the development and progression of respiratory allergies in children and to substantiate microbiome-oriented approaches to the prevention of respiratory allergic diseases.

3. Materials and methods.

The study was conducted at the Republican Children's Medical Center and the Department of Pediatric Allergology and Immunology of the Tashkent Pediatric Medical Institute between 2024 and 2026. The research type is prospective controlled with elements of comparative analysis.

The study included 240 children aged from birth to 14 years, divided into four groups: primary group 1 (60 children diagnosed with bronchial asthma), primary group 2 (65 children with allergic rhinitis), primary group 3 (60 children with combined lesions of the upper and lower respiratory tract), and control group (55 healthy children). The average age of patients in the main groups was 7.2±3.4 years, and in the control group, it was 7.6±3.3 years. Inclusion criteria: age from birth to 14 years; presence of a verified diagnosis of respiratory allergic disease or absence of allergic symptoms (for the control group); informed consent of parents or guardians. Exclusion criteria: severe comorbidities, primary immunodeficiencies, use of antibiotics for 4 weeks before inclusion in the study, and mental illnesses that hinder cooperation. Clinical methods included a thorough anamnesis collection, paying special attention to early childhood factors (feeding patterns, age of supplemental feeding, use of antibiotics, presence of intestinal infections, living conditions), conducting an allergological examination, assessing the severity of respiratory allergic disease according to the GINA (Global Asthma Initiative) scales, and assessing quality of life using the PAQLQ/PADQLQ questionnaire. Instrumental methods included spirometry with determination of FEV1 and FVC, and skin allergological testing with primary aeroallergens. Microbiological methods included obtaining samples for microbiome analysis in two ways. For the analysis of intestinal microbiota, samples of feces were obtained, for the analysis of respiratory microbiome, samples of nasopharyngeal rinses and sputum from the lower respiratory tract were obtained. DNA was isolated from the obtained samples using standard methods. The composition of the microbial community was analyzed using the 16S rRNA sequencing method, determining the following parameters: (1) α-diversity of the microbial community with the calculation of Shannon and Simpson indices; (2) β-diversity of the microbial community with the construction of PCoA graphs; (3) relative abundance of dominant taxa at the level of phylums (Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria) and families (Lachnospiraceae, Ruminococcaceae, Bacteroidaceae, Prevotellaceae, Enterobacteriaceae); (4) identification of functional groups of microorganisms with an emphasis on short-chain fatty acid (CSF) producers, specifically butyrate. Additionally, culture analysis of respiratory tract samples was conducted to identify conditionally pathogenic microorganisms and determine their antibiotic sensitivity.

Immunological methods included determining the level of total IgE and specific IgE to the main aeroallergens using enzyme-linked immunosorbent assay, determining the level of short-chain fatty acids (acetate, propionate, butyrate) using gas chromatography in fecal samples, analyzing the cytokine profile (IL-4, IL-10, IFN-γ, TNF-α) using the Luminex method, and determining T-lymphocyte function (CD4+, CD8+, Treg) using flow cytometry. Statistical processing of the obtained data was performed using the SPSS software package version 26.0 and R version 4.0. For quantitative variables, Shapiro-Wilk

tests for distribution normality were conducted, followed by parametric (Student's t-test) or non-parametric (Mann-Whitney test) comparison methods. Qualitative indicators were compared using the $\chi 2$ (chi-square) criterion. Correlation analysis was conducted using the Pearson or Spearman method. Differences were considered statistically significant at $p < 0.05$.

4. Research results.

During the study, a comprehensive analysis of the microbiota composition was conducted in 240 children. By age group, patients were distributed as follows: children aged from birth to 3 years accounted for 64 patients (26.7%), 3–6 years – 78 patients (32.5%), and 6–14 years – 98 patients (40.8%).

When analyzing early childhood factors, it was established that 156 patients (65.0%) with respiratory allergies had a complicated inheritance of allergic diseases. Breastfeeding for less than 6 months was noted in 124 children in the main groups (58.5%) compared to the control group (20.0%; $p < 0.001$). The use of antibiotics in the first year of life was recorded in 98 patients in the main groups (46.2%), compared to 8 patients in the control group (14.5%; $p < 0.001$). When analyzing the α-diversity of the microbial community, significant differences were identified between patients with allergies and healthy children[8,9,10]. The Shannon index in the primary groups with allergies was 2.4 ± 0.7, which is significantly lower than in the control group (4.1 ± 0.8; $p < 0.001$). The Simpson index also decreased in the allergy groups (0.58 ± 0.12 vs 0.78 ± 0.11 in the control group; $p < 0.001$). This indicates a significant decrease in microbial diversity and the dominance of individual microbial taxa in children with respiratory allergies.

Analysis of β-diversity (qualitative composition of the microbial community) established significant differences between groups according to PCoA analysis (ANOSIM $R = 0.62$; $p < 0.001$), indicating qualitatively different microbiota composition.

When evaluating the relative abundance of dominant phylums, the following patterns were identified. The proportion of Firmicutes was significantly reduced in children with allergies (18.4±6.3%) compared to the control group (32.8±7.5%; $p < 0.001$). The opposite trend was observed for Proteobacteria: in children with allergies, their share was 36.2±9.4%, which significantly exceeded the control values (15.8±5.9%; $p < 0.001$). The proportion of Bacteroidetes was slightly higher in the allergy groups (28.6±7.2% vs. 26.4±6.8% in the control group; $p > 0.05$), while Actinobacteria was also slightly higher in the main groups (16.8±5.2% vs. 14.2±4.3%; $p < 0.05$).

Detailed analysis at the level of microorganism families revealed a significant decrease in the proportion of Lachnospiraceae (known butyrate producers) in children with allergies: 6.2±2.8% versus 18.4±4.6% in the control group ($p < 0.001$). Similarly, the share of Ruminococcaceae decreased ($7.4 \pm 3.1\%$ vs $14.6 \pm 4.2\%$; $p < 0.001$). At the same time, a relative predominance of Enterobacteriaceae (18.6±6.2% vs 8.4±3.5%; $p < 0.001$) and Pseudomonadaceae (8.2±3.4% vs 2.8±1.6%; $p < 0.001$) was noted.

When determining the level of short-chain fatty acids, a significant decrease in butyrate concentration was established in children with allergies: 1.8 ± 0.6 mmol/kg compared to 4.2 ± 1.1 mmol/kg in the control group ($p < 0.001$). The concentration of propionate was also lower (0.8 ± 0.3 mmol/kg vs 1.6 ± 0.5 mmol/kg; $p < 0.001$). Acetate levels did not differ between groups ($p > 0.05$).

When analyzing immunological parameters, it was established that a low content of Firmicutes and a decrease in butyrate levels correlated with a decreased number of regulatory T-cells (Treg): $r = 0.58$ ($p < 0.001$) and $r = 0.64$ ($p < 0.001$) respectively. The average Treg content in the main groups was $4.1 \pm 1.2\%$, which is significantly lower than the control values ($8.6 \pm 2.1\%$; $p < 0.001$). An increase in the pro-inflammatory cytokine IL-4 level correlated with a decrease in the proportion of Lachnospiraceae ($r = -0.52$; $p < 0.001$) and an increase in the proportion of Proteobacteria ($r = 0.48$; $p < 0.001$).

When analyzing the influence of early childhood factors on the microbiota, it was established that children who received breastfeeding for less than 6 months had a significantly reduced microbial diversity (Shenon index 2.8±0.7 vs 3.6±0.8 for breastfeeding ≥ 6 months; $p < 0.01$). The use of antibiotics in the first year of life was associated with a decrease in the proportion of Firmicutes (14.2±4.6% vs 22.8±6.2%; $p < 0.01$) and a decrease in Lachnospiraceae (4.8±2.1% vs 7.6±3.2%; $p < 0.05$).

During the culture analysis of respiratory tract samples, conditionally pathogenic microorganisms were primarily identified in children of the main groups: Haemophilus influenzae (44.8% of cases), Streptococcus pneumoniae (36.4%), Staphylococcus aureus (20.5%), and Pseudomonas aeruginosa (7.8%). In the control group, such microorganisms were identified significantly less frequently (in 4.5%, 2.7%, 1.8%, and 0% of cases, respectively; $p < 0.001$)[11,12].

Correlations have been established between the composition of the respiratory microbiome and the intestinal microbiome. Children with low microbial diversity in the respiratory tract also had reduced diversity in the intestinal microbiome ($r = 0.46$; $p < 0.001$). This indicates the systemic nature of dysbiosis in respiratory allergies.

5. Discussion.

The results obtained indicate the leading role of the microbial factor in the development and progression of respiratory allergies in children. The identified decrease in microbial diversity in children with allergies aligns with the hygiene hypothesis and its modern development—the theory of microbial deprivation. According to this theory, decreased contact with diverse microorganisms in early childhood leads to incorrect programming of the immune system and its tendency toward an allergic response.

The detected predominance of Proteobacteria and a decrease in Firmicutes, specifically the Lachnospiraceae and Ruminococcaceae families, in children with allergies has clear pathogenetic foundations. These bacterial families are the primary producers of short-chain fatty acids, especially butyrate. Butyrate is a critical metabolite that maintains Treg cell function through the mechanism of histone deacetylation and the activation of GPR43 receptors. A decrease in these bacteria leads to butyrate deficiency, which in turn affects the number and function of Treg cells, reducing immune tolerance and increasing the risk of developing allergic inflammation[13,14].

The identified negative impact of early cessation of breastfeeding and the use of antibiotics on microbiome composition emphasizes the critical role of the first months and years of life in the formation of a healthy microbiome. Breast milk contains special carbohydrates (oligosaccharides) that serve as prebiotics, contributing to the growth of beneficial bacteria of the Bifidobacteriaceae family. The use of antibiotics, even for the purpose of treating infections, leads to the disruption of the microbial community, which can persist for a long time.

The established correlations between respiratory microbiome composition and intestinal microbiota indicate the systemic nature of dysbiosis in allergy and suggest the presence of a unified intestine-lung axis in the regulation of immune homeostasis[15].

6. Conclusions

Microbiota plays a key role in the development and progression of respiratory allergies in children. A decrease in microbial diversity, a change in the composition of the microbial community with a predominance of conditionally pathogenic microorganisms and a decrease in the number of short-chain fatty acid producers, as well as the impairment of immune system function associated with these changes, serve as important mechanisms for the pathogenesis of respiratory allergic diseases. Preventive strategies aimed at maintaining a healthy microbiome during critical developmental periods (specifically through supporting breastfeeding, rational use of antibiotics, and the use of probiotics and prebiotics) can serve as the foundation for primary and secondary prevention of respiratory allergies in children.

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