BioGecko

Vol 12 Issue 03 2023

ISSN NO: 2230-5807

MICROBIOLOGICAL FEATURES OF THE NASOPHARYNX IN CHILDREN WITH BRONCHIAL ASTHMA

Zamirahon Olimjonovna Urumboeva
Andijan State Medical Institute,
Andijan, Uzbekistan

Furkat Mukhitdinovich Shamsiev
Republican Specialized Scientific and Practical Medical Center of Pediatrics, Ministry of Health of the Republic of Uzbekistan,
Tashkent, Uzbekistan.

Nilufar Irgashevna Karimova
Republican Specialized Scientific and Practical Medical Center of Pediatrics, Ministry of Health of the Republic of Uzbekistan,
Tashkent, Uzbekistan.

ABSTRACT
The objective of this research was to investigate the microbiological characteristics of the nasopharyngeal mucosa in children with bronchial asthma and provide a comparative analysis of the microbial flora. The study aimed to determine the prevalence of specific microorganisms and assess their significance in the context of bronchial asthma and recurrent bronchitis with bronchial obstruction syndrome. The results revealed a statistically significant elevation in the levels of Staphylococcus, Streptococcus, and Enterobacteriaceae, surpassing a threshold of 10^3 colony-forming units (CFU), in children with bronchial asthma. Similarly, in cases of recurrent bronchitis with bronchial obstruction syndrome, these microorganisms were found to be significantly elevated. However, there were distinct differences observed between the two conditions. Specifically, bronchial asthma exhibited a higher prevalence of Staphylococcus, Streptococcus, Enterococcus, and Enterobacteriaceae, along with a lower presence of Staphylococcus hominis compared to recurrent bronchitis with bronchial obstruction syndrome. Furthermore, the increased abundance of bacteria from the Enterobacteriaceae and Enterococcus families on the nasal mucosa indicates dysbiosis and underscores their crucial role in initiating allergic inflammation of the respiratory tract of atopic origin.

KEYWORDS: bronchial asthma, diagnosis, nasopharyngeal microflora, children

INTRODUCTION

Bronchial asthma (BA) is a prevalent respiratory allergic disease that demands ongoing investigation into its etiology and pathophysiology [1,2]. Extensive research has uncovered the heterogeneity of respiratory allergy symptoms, with the clinical and pathological changes in respiratory allergies being the outcome of the damaging effects of inflammatory mediators released through various atopic and pseudoatopic mechanisms. The specific factors that trigger the release of reagins determine the type of allergic reaction [3,5].

The mucous membrane of the nasopharyngeal cavity serves as a vital physiological barrier, effectively safeguarding against the entry of diverse environmental factors through the respiratory tract. This barrier is characterized by a consistently stable composition of microflora, which typically maintains a state of equilibrium.

Inflammatory processes within the upper respiratory tract emerge as a consequence of pathogenic microorganisms and disruptions in the local and systemic defense mechanisms of the body [6].

Scientific investigations have demonstrated the existence of distinct microorganisms that contribute to the formation of bacterial communities during the early stages of respiratory tract development [4,7,12]. Inflammatory processes within the nasopharynx compromise the nonspecific barriers that hinder the penetration of allergens and other pollutants. Alterations in the microbiome can be regarded as an indicator of respiratory dysbiosis in individuals with bronchial asthma.

The primary objective of this study is to determine the microbiological characteristics of the nasopharyngeal mucosa in children diagnosed with bronchial asthma and provide a comprehensive comparative analysis of the microflora, based on the underlying pathogenesis of the disease.

MATERIALS AND METHODS

A total of 28 children, aged 5 to 15 years, diagnosed with bronchial asthma (BA) were included in the study (Group I). This group was compared to a control group consisting of 30 children with recurrent bronchitis with bronchial obstruction syndrome (RB BOS) (Group II). The study was conducted at the Department of Pulmonology and Allergology of the Republican Specialized Scientific and Practical Medical Center of Pediatrics, Ministry of Health of the Republic of Uzbekistan.

The diagnosis of the disease in all examined children was established and verified based on diagnostic criteria and the current classification of the disease according to the "GINA 2022" guidelines and the National Program "Bronchial Asthma in Children: Treatment and Prevention Strategy." The diagnosis of BA was determined through a combination of medical history, clinical manifestations, functional tests, and specific allergic diagnostics.

The microbiomes of the nasopharyngeal mucosa were investigated, focusing on the bacterial species composition of the Staphylococcus genus. A comparative analysis of the microflora of the nasopharyngeal mucosa was conducted based on the pathogenesis of the disease. Microorganism isolation was performed using yellow salt agar, and the sectoral method was used for the inoculation. The seeded media were incubated in a thermostat at a temperature of 37°C for 24 hours. Microorganism counts were determined using a calculation table. Isolates were subcultured on slanted meat-peptone agar and nutrient semi-liquid agar (0.4%) to obtain pure cultures and investigate identification characteristics.

Statistical analysis of the research results was carried out using the Statistica 10.0 software package. The study sample was described by calculating the median (Me) and the interquartile range represented by the 25th and 75th percentiles (C_{25}–C_{75}).

RESULTS AND DISCUSSION

The study aimed to investigate the species composition of the microflora in the mucous membrane of the nasopharynx in patients with bronchial asthma (BA) and recurrent bronchitis with bronchial obstruction syndrome (RB BOS). The analysis revealed the presence of several microorganisms, including Streptococcus pneumoniae, Streptococcus haemolyticus, Enterococcus faecium, Enterococcus faecalis, Moraxella catarrhalis, and Haemophilus influenzae (Table 1).

In the case of BA, the content of conditionally pathogenic microorganisms was within the reference intervals, except for Haemophilus influenzae, which showed a statistically significant increase (28 × 104 CFU/mL) compared to the control group. Additionally, the nasal mucosa of BA patients exhibited the presence of Streptococcus haemolyticus (1.5 × 103 CFU/mL), Enterococcus faecium (103 CFU/mL), and Enterococcus faecalis (103 CFU/mL), which were not detected in the control group ($P < 0.001$).

Regarding RB BOS, the content of Streptococcus pneumoniae (25 × 104 CFU/mL) was significantly higher compared to the control group. Notably, the pathogens Haemophilus influenzae, Enterococcus faecium, and Enterococcus faecalis, which were found in BA patients, were absent in both the RB BOS group and the control group (Table 1).

Table 1.

Indicators of the species composition of the microflora of the nasal mucosa in BA and RB BOS, Me (C_{25}–C_{75})

Indicators,	Control	BA (n=25)	RB BOS	P	P1

CFU/mL	(n=30)		(n=30)		
S.pneumoniae	10^3 ($0–10^3$)	$8*10^3$ ($100–25*10^3$)	$25*10^4$ ($10^4–3*10^6$)	<0,01	<0,001
S.haemolyticus	0	$1,5*10^3$ ($10^3–2*10^3$)	10^4 ($10^3–15*10^3$)	<0,001	<0,001
E.faecium	0	10^3 ($10–5*10^3$)	0	<0,001	<0,001
E.faecalis	0	10^3 ($10–10^4$)	0	<0,001	<0,001
M.catarrhalis	10^3 ($0–10^3$)	10^4 ($100–15*10^3$)	$15*10^3$($10^3–42*10^3$)	<0,01	<0,01
H.influenzae	0	$28*10^4$ ($1,2*10^3–30*10^4$)	0	< 0,001	<0,001

Note: statistically significant differences in BA: P1 — with the control group, P2 — with the RB BOS group

In the subsequent analysis, we examined the species composition of Staphylococcus bacteria isolated from the nasal mucosa in patients with bronchial asthma (BA) and recurrent bronchitis with bronchial obstruction syndrome (RB BOS) (Table 2). A statistically significant increase in the number of Staphylococcus aureus strains was observed in the BA group compared to the control group. Furthermore, a wide variety of Staphylococcus species were identified, including S. epidermidis, S. haemolyticus, S. hominis, S. cohnii, S. capitis, and S. hyicus. Of particular interest is the presence of S. aureus (15.5 × 103 CFU/mL), S. epidermidis (104 CFU/mL), S. haemolyticus (104 CFU/mL), S. hominis (300 CFU/mL), S. cohnii (104 CFU/mL), S. capitis (104 CFU/mL), and S. hyicus (5.5 × 103 CFU/mL) on the nasal mucosa in BA patients. In the RB BOS group, S. aureus (52.5 × 103 CFU/mL), S. epidermidis (5 × 103 CFU/mL), S. haemolyticus (5.5 × 104 CFU/mL), S. hominis (75 × 104 CFU/mL), and S. capitis (103 CFU/mL) were detected ($P < 0.001$). Strains of S. capitis and S. hyicus were not found in the control group.

Table 2.

Indicators of the species composition of bacteria of the genus Staphylococcus isolated from the nasopharyngeal mucosa in BA and RB BOS, Me ($C_{25}–C_{75}$)

Indicators, CFU/mL	Control (n=30)	BA (n=25)	RB BOS (n=30)	P	P1
S.aureus	200 (0–200)	$15,5*10^3$ ($40–17*10^3$)	$52,5*10^3$ ($100–50*10^4$)	<0,001	<0,001
S.epidermidis	10^3 ($10^2–0^5$)	10^4 ($10^3–10^4$)	$5*10^3$ ($10^3–10^5$)	<0,001	<0,05
S.haemolyticus	100 ($10–10^2$)	10^4 ($10^2–10^5$)	$5,5*10^4$ ($7,5*10^3–25,5*10^5$)	<0,001	<0,05
S.hominis	$5,5*10^3$ ($10^2–1,1*10^3$)	300 ($90–10^4$)	$75*10^4$ ($5*10^5–10^6$)	<0,001	<0,05
S.cohnii	10^4 ($10^4–10^4$)	10^4 ($10^3–10^5$)	0	<0,001	<0,001
S.capitis	0	10^4 ($5,5*10^3–20,5*10^3$)	10^3 ($10^3–10^3$)	<0,001	<0,001
S.hyicus	0	$5,5*10^3$ ($10^3–10^4$)	0	<0,001	<0,001

Note: statistically significant differences in BA: P1 — with the control group, P2 — with the RB BOS group

The next stage involved determining the composition of microorganisms belonging to the genera Staphylococcus, Streptococcus, Enterococcus, and Enterobacteriaceae in the nasal mucosa of patients with bronchial asthma (BA) and recurrent bronchitis with bronchial obstruction syndrome (RB BOS) (Table 3). It

was found that the alteration of the composition of the conditionally pathogenic microflora was characterized by a statistically significant increase in the total microbial flora in the BA and RB BOS groups compared to the control group, exceeding 106 CFU/mL. Specifically, a statistically significant increase in the presence of microorganisms belonging to the genera Staphylococcus, Streptococcus, and Enterobacteriaceae (exceeding 103 CFU/mL) was observed in patients with BA and RB BOS compared to the control group.

Table 3.

Composition of the microflora of the nasopharyngeal mucosa in BA and RB SBO, Me (C_{25}–C_{75})

Indicators, CFU/mL	Control (n=30)	RB BOS (n=30)	BA (n=25)	P	P1
*Staphylococcus*spp.	10^4 (10^3–10^4)	$5{,}2*10^5$ ($2*10^4$–$8{,}6*10^5$)	$5*10^6$ (10^6–10^8)	<0,001	<0,001
*Streptococcus*spp.	10^3 (550–10^3)	10^6 ($5*10^5$–$8*10^6$)	10^8 (10^7–$5*10^8$)	<0,001	<0,001
*Enterococcus*spp.	0 (0–0)	0(0–0)	$5*10^4$ (10^4–$5*10^5$)	<0,001	<0,001
*Enterobacteriaceae*s pp.	10^3 (10^2–10^4)	10^5(10^5–10^5)	$2{,}7*10^6$ (10^6–$5*10^7$)	<0,001	<0,001
Thetotalcount	$1{,}6*10^5$ (10^3–$2{,}1*10^5$)	$2{,}2*10^6$ (10^6–$6*10^6$)	$2{,}8*10^8$ (10^8–$5*10^8$)	<0,001	<0,001

Note: statistically significant differences in BA: P1 — with the control group, P2 — with the RB BOS group

The study revealed a statistically significant increase in the total microbial flora exceeding 10^6 CFU/mL in patients with BA. Furthermore, a statistically significant increase in the levels of Staphylococcus ($5*10^6$ CFU/mL), Streptococcus (10^8 CFU/mL), and Enterococcus ($5*10^4$ CFU/mL) was observed in the BA group compared to the control group ($p<0.001$). Additionally, the concentration of Enterococcus was significantly higher in the BA group compared to the RB BOS group ($p<0.001$). It was observed that the nasal mucosa of BA patients exhibited a statistically significant increase in all investigated microorganisms (Staphylococcus, Streptococcus, Enterococcus, and Enterobacteriaceae) and, consequently, in the overall microbial count compared to the control group. In the RB BOS group, there was a statistically significant increase in Staphylococcus spp. ($5.2*10^5$ CFU/mL), Streptococcus spp. (10^6 CFU/mL), and Enterobacteriaceae spp. (10^5 CFU/mL) compared to the control group ($p<0.001$). No Enterococcus species were detected in the RB BOS group, similar to the control group.

Therefore, our study identified qualitative and quantitative changes in the composition of nasal microbiota in patients with bronchial asthma (BA) and non-BA small airway obstruction (RB BOS). One notable feature of the dysbiosis in BA and RB BOS was the presence of Staphylococcus species such as Staphylococcus epidermidis, Staphylococcus haemolyticus, and Staphylococcus hominis. Even minor deviations in the microbial composition of the nasal mucosa can serve as markers of dysbiotic changes. Analysis of other microbial genera revealed a statistically significant increase in Staphylococcus, Streptococcus, and Enterobacteriaceae (exceeding 10^3 CFU/mL) in both BA and RB BOS groups compared to the control group. BA patients exhibited a statistically significant increase in Staphylococcus, Streptococcus, and Enterococcus compared to the control group ($p<0.001$). Respiratory dysbiosis can be a consequence of both immune system stress and bacterial sensitization. The investigated microorganisms belong to the conditionally pathogenic microflora, and their increased abundance represents dysbiotic changes in the nasal mucosa, possibly resulting from impairment of both systemic and local immunity. Enterococcus species were found significantly more frequently in BA patients compared to RB BOS. These bacteria have been shown to possess sensitizing activity and may initiate allergic inflammation [5, 14]. In the RB BOS group, a disruption of the nasal mucosa microbial landscape was observed, characterized by an increase in the CFU of Staphylococcus, Streptococcus, and Enterobacteriaceae, as well as the total microbial count. The aforementioned microorganisms are representatives of the nasal mucosa's normal flora, but their combined count should not exceed 10^3 CFU/mL.

CONCLUSIONS

1. Our comparative microbiological analysis revealed the most pronounced changes in the microbiota of patients with bronchial asthma (BA). The total microbial count in the BA group exceeded 10^8 CFU/mL, while in the non-BA small airway obstruction (RB BOS) groups, it exceeded 10^6 CFU/mL. However, the total microbial count was statistically significantly higher in BA compared to RB BOS. The increase in the number of conditionally pathogenic microorganisms may indicate more pronounced immunological changes in atopy (BA) compared to pseudoatopy (RB BOS).
2. BA showed a predominance of conditionally pathogenic microorganisms compared to RB BOS. The results of our study can be interpreted as dysbiosis resulting from a decrease in local and systemic immunity due to trophic disturbances of the nasal mucosa.
3. BA was associated with a higher abundance of Enterobacteriaceae and Enterococcus families and a lower quantity of Staphylococcus hominis compared to RB BOS. The increased numbers of bacteria from the Enterobacteriaceae and Enterococcus families on the nasal mucosa characterize dysbiosis and emphasize the important role of these families in triggering allergic inflammation of the respiratory tract of atopic origin.

REFERENCES

1. Baturo A.P., Romanenko E.E., LeonovaA.Yu., Yartseva A.S., Savlevich E.L., Mokronosova M.A. Dominance of staphylococcus aureus in the microbiocenosis of the nasal cavity in children and adults with infectious and allergic rhinitis // Journal of Microbiology, Epidemiology and Immunobiology. 2015. No. 1. pp. 72-74.
2. Dobretsov K.G., Makarevich S.V. The role of staphylococci in the development of chronic polypous rhinosinusitis // Russian rhinology. 2017. vol. 25, No. 1. pp. 36-40.
3. Zakharova I.N., Kasyanova A.N., Klimov L.P., Kuryaninova V.A., Simakova M.A., Dedikova O.V., Koltsov K.A. Microbiome of the respiratory tract: what is known today? // Pediatrics. AppendixtothejournalConsiliumMedicum.
4. 2018. Vol. 4. pp. 10-17.
5. Melnik A.M., Voronov A.V., Dvoryanchikov V.V., Isachenko V.S., Achba R.R. The state of the microflora of the nasal cavity with polypous rhinosinusitis // Russian otorhinolaryngology. 2017. Vol. 1, No. 86. pp. 73-82.
6. Puhaeva M.O., Galueva Z.R., Mikhailidi E.F. Microbial biocenosis in allergic rhinitis in children // Almanac of World Science. 2017. Vol. 5, No. 20. pp. 21-22.
7. Fedoseev G.B., Trofimov V.I., Golubeva V.I., Timchik V.G., Negrutsa K.V., Razumovskaya T.S., Alexandrin V.A., Kryakunov K.N. On the role of bacteria in patients with bronchial asthma, chronic obstructive pulmonary disease // Russian Allergological Journal. 2018. Vol. 15, No. 6. pp. 65-78.
8. Anderson M., Stokken J., Sanford T., Aurora R., Sindwani R. A systematic review of the sinonasal microbiome in chronic rhinosinusitis. Am. J. Rhinol. Allergy, 2016, vol. 30, no. 3, pp. 161–166. doi: 10.2500/ajra.2016.30.4320
9. Carr T.F., Alkatib R., Kraft M. Microbiome in mechanisms of asthma. Clin. ChestMed., 2019, vol. 40, no. 1, pp. 87–96. doi: 10.1016/j.ccm.2018.10.006
10. Chalermwatanachai T., Velásquez L.C., Bachert C. The microbiome of the upper airways: focus on chronic rhinosinusitis. WorldAllergyOrgan J., 2015, vol. 8, no. 1: 3. doi: 10.1186/s40413-014-0048-6
11. Durack J., Lynch S.V., Nariya S., Bhakta N.R., Beigelman A., Castro M., Dyer A.M., Israel E., Kraft M., Martin R.J., Mauger D.T., Rosenberg S.R., Sharp-King T., White S.R., Woodruff P.G., Avila P.C., Denlinger L.C., Holguin F., Lazarus S.C., Lugogo N., Moore W.C., Peters S.P., Que L., Smith L.J., Sorkness C.A., Wechsler M.E., Wenzel S.E., Boushey H.A., Huang Y.J.; NationalHeart, LungandBloodInstitute's "AsthmaNet". Features of the bronchial bacterial microbiome associated with atopy, asthma, and responsiveness to inhaled corticosteroid treatment. J. Allergy Clin. Immunol., 2017, vol. 140, no. 1, pp. 63–75. doi: 10.1016/j. jaci.2016.08.055

12. Fazlollahi M., Lee T.D., Andrade J., Oguntuyo K., Chun Y., Grishina G., Grishin A., Bunyavanich S. The nasal microbiome in asthma. J. Allergy Clin. Immunol., 2018, vol. 142, no. 3, pp. 834–843. doi: 10.1016/j.jaci.2018.02.020
13. Frati F., Salvatori C., Incorvaia C., Bellucci A., Di Cara G., Marcucci F., Esposito S. The Role of the microbiome in asthma: the gut–lung axis. Int. J. Mol. Sci., 2018, vol. 20, no. 1: 123. doi: 10.3390/ijms20010123
14. Teo S.M., Mok D., Pham K., Kusel M., Serralha M., Troy N., Holt B.J., Hales B.J., Walker M.L., Hollams E., Bochkov Y.A., Grindle K., Johnston S.L., Gern J.E., Sly P.D., Holt P.G., Holt K.E., Inouye M. The infant nasopharyngeal microbiome impacts severity of lower respiratory infection and risk of asthma development. CellHostMicrobe, 2015, vol. 17, no. 5, pp. 704–715. doi: 10.1016/j.chom.2015.03.008

CURRENT POSITIONS OF AUTHORS:

1. Urumboeva Zamira Olimzhonovna
Assistant of the Department of "Hospital Pediatrics" of Andijan State Medical Institute, Tashkent, Uzbekistan (ORCID ID: 0000-0003-2997-7926)
2. Shamsiev Furkat Mukhitdinovich MD, Professor, Head of the Department of Pulmonology of the Republican Specialized Scientific and Practical Medical Center of Pediatrics, Uzbekistan, Tashkent
3. Karimova Nilufar Irgashevna
PhD, doctoral student of the Department of Pulmonology of the Republican Specialized Scientific and Practical Medical Center of Pediatrics, Uzbekistan, Tashkent (ORCID ID: 0000-0002-3687-4995)

Corresponding author, E-mail:
Urumboeva Zamira Olimzhonovna