



COMPARATIVE ANALYSIS OF SALIVARY BIO-CHEMICAL, MICROBIOLOGICAL, AND IMMUNOLOGICAL PARAMETERS IN CHILDREN WITH BRONCHIAL ASTHMA AND HEALTHY CONTROLS

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ABSTRACT:

Bronchial asthma in children is a chronic inflammatory condition that may influence the composition and function of saliva, potentially impacting oral health. Altered salivary parameters, such as flow rate, pH, buffer capacity, microbiological profile, and immune components, are increasingly recognized as relevant in asthmatic populations.

Objective: To perform a comparative analysis of the biochemical, microbiological, and immunological characteristics of saliva in children with bronchial asthma versus healthy controls.

Material/Methods: The study included 116 children aged 5–17 years—58 with clinically diagnosed bronchial asthma and 58 healthy controls. Salivary parameters assessed included stimulated salivary flow rate, pH, buffer capacity, consistency, colony-forming unit (CFU) per ml saliva of *Streptococcus mutans* and *Lactobacillus* spp., and secretory immunoglobulin A (SIgA) concentration. Standardized in vitro tests and ELISA were used for measurements. Statistical analyses were performed using variation and alternative analysis methods.

Results: Children with asthma exhibited significantly lower stimulated salivary flow (mean: 4.78 ml/5min) compared to controls (mean: 8.05 ml/5min, $p < 0.05$). Saliva in asthmatics was more frequently acidic or slightly alkaline ($p = 0.019$). No significant differences were observed in buffer capacity or consistency. The prevalence of increased *S. mutans* and *Lactobacillus* spp. was higher in the asthma group, although not statistically significant. SIgA levels were significantly higher in children with asthma (mean: 208.82 µg/ml) compared to controls (mean: 138.83 µg/ml, $p < 0.05$).

Conclusion: Bronchial asthma in children is associated with reduced salivary flow and increased salivary acidity, along with elevated SIgA levels, indicating possible compensatory immune activation. These changes may predispose to oral health complications and warrant targeted preventive strategies.

Keywords: Pediatric asthma, Salivary biomarkers, Oral health,

INTRODUCTION:

Changes in the qualitative and quantitative composition of saliva disrupt its balance and protective properties. Some of the most sensitive parameters of saliva in asthmatics are changes in the quantity, composition, and pH of saliva. The antibacterial activity of saliva is associated with limiting the growth of microorganisms, their aggregation, and elimination. Among the immune antibacterial factors in saliva, secretory IgA plays the most important role, the amount of which is strongly influenced by immunopathological processes.

The aim of this study is to compare the level of biochemical, microbiological, and immunological indicators in the saliva of children with asthma and healthy individuals.

MATERIALS AND METHODS:

The study included 58 children aged 5–17 years with established bronchial asthma, treated at the Department of Pediatrics and Medical Genetics at the Medical University of Plovdiv, and 58 healthy children of the same age who visited the Department of Pediatric Dental Medicine for a preventive examination. Bronchial asthma was diagnosed according to GINA (Global Initiative for Asthma) criteria, including mild, moderate, and severe degrees. Exclusion criteria were the presence of neoplastic diseases, autoimmune diseases, and collagenoses. Data collection began after discussion with parents and obtaining informed consent for inclusion in the study. Data on the duration and severity of the disease, the type, form, and frequency of medication use, the patient's oral hygiene and dietary habits, and a detailed intraoral examination were collected.

The units of observation were the amount of stimulated saliva, pH, buffer capacity and consistency of saliva, colony-forming unit (CFU) per ml of saliva of *Streptococcus mutans* and *Lactobacillus* spp., and secretory

IgA. The study was conducted in the Department of Pediatric Dental Medicine, Department of Medical Microbiology and Immunology, and Department of Pediatrics and Medical Genetics at the Medical University of Plovdiv after approval by the ethics committee.

The Saliva Check Buffer Test (GC, Japan), which is an in vitro test for determining the quantity, acid-alkaline reaction, and buffer capacity of saliva, was used to examine the qualities of the saliva. Patients were instructed in advance not to consume food and beverages, as well as not to apply oral hygiene procedures for at least one hour before the procedure.

The amount of stimulated saliva was determined after stimulating salivation by chewing a paraffin gum with a neutral taste for 5 minutes. The secreted saliva was collected in a graduated container. Depending on the amount of saliva secreted, the children were divided into three groups - below 3ml/5min, between 3 and 5ml/5min, and above 5ml/5min. The total amount of stimulated saliva was calculated for children with asthma and the control group.

To determine the acidity of mixed saliva, a litmus test strip was used, which was placed under the tongue for a few seconds. The pH value was determined by comparing the color of the indicator with the color scale provided in the kit. Depending on the pH value of saliva, the children were divided into two groups - with alkaline (at values above 6.8), with slightly alkaline (at values between 5.8 and 6.8) and with acidic reaction of saliva (at a value below 5.8).

The consistency of saliva was measured visually, being evaluated in one of the categories - "liquid," "with bubbles," or "viscous."

The buffer capacity of stimulated saliva was measured immediately after taking the sample using the special buffer strip included in the test kit. Depending on the color of each of the three parts of the strip, a certain number of points from 0 to 4 were given. The result was recorded as the sum of the points of the three parts of the buffer strip from 0 to 12 points. Depending on the result, the children were divided into three groups - with high

buffer capacity (from 10 to 12 points), with medium-high buffer capacity (from 6 to 9 points), and with very low buffer capacity (from 0 to 5 points).

The selective semiquantitative dip-slide method (CRT Bacteria Test - Ivoclar Vivadent), created as a risk assessment tool for dental decay, was used to examine the CFU/ml of *S. mutans* and *Lactobacillus* spp. in saliva. After thoroughly wetting both agar surfaces with the collected stimulated saliva, the microorganisms were incubated (using Cultura/Ivoclar Vivadent) at 37 °C for 48 hours. After removal of the vial from the incubator, the density of *S. mutans* and *Lactobacillus* spp. colonies was compared with the corresponding evaluation pictures in the enclosed model chart. The children were divided into two groups depending on whether the number of respective microorganisms was above or below 10⁵ CFU/ml saliva.

An enzyme-linked immunosorbent assay (ELISA) was used for the quantitative determination of secretory IgA, for which 2-3ml of the salivary samples were frozen immediately after collection.

The results were processed through descriptive and alternative analysis. Statistical methods included descriptive statistics, independent samples t-tests for continuous variables, two-proportion Z-tests for comparisons of categorical frequencies between groups, and one-proportion Z-tests for within-group distribution analysis. The significance level was set at $p < 0.05$. Stimulated salivary flow rate and SIgA concentrations were presented as mean (M), standard deviation (SD), and standard error mean (SEM).

RESULTS:

The mean amount of stimulated saliva in children with asthma was lower than the established norms (Table 1). In the control group, the collected saliva was statistically significantly greater in quantity. Both in children with asthma and in the control group, the highest relative share was of children with an amount of stimulated saliva above 5ml for 5 min. The proportion of children with asthma with a low amount of stimulated saliva was higher, but without statistical significance of the difference (Table 2).

Table 1. Comparison of Stimulated Salivary Flow Rate Between Children with Asthma and Healthy Controls

	M	N	SD	SEM	t	
controls	8.0483	58	9.32725	1.22473		
asthma	4.7759	58	2.04376	0.26836	2.62	p<0.05

There were statistically significant differences in the acidity of saliva between children with and without asthma (Table 2). In the group of children with normal saliva acidity, children from the control group predominated, while in the group of children with slightly alkaline and acidic saliva, children with asthma predominated. In the control group, no child had a saliva pH below 5.8.

The distribution of children from both groups -

asthma and control - depending on the buffer activity of saliva, was without statistical significance. In both groups, the highest was the share of children with medium-high buffer capacity (Table 2).

The results of the visual examination of the consistency of saliva were similar, and there was no significant difference between the groups, with a higher relative share of children with liquid saliva (Table 2).

Table 2. Distribution of Children According to Amount of Stimulated Saliva, Salivary pH Levels, Buffer Capacity and Consistency in Asthma and Control Groups

Parameter	Groups/Values	Asthma - Relative Share %	Control - Relative Share %	p
Amount of stimulated saliva	>5 ml	55.2	51.7	p=0.691
	3.5-5 ml	29.3	39.7	p=0.238
	< 3.5 ml	15.5	8.6	p=0.271
pH	alkaline	77.6	93.1	p=0.019
	slightly alkaline	20.7	6.9	p=0.042
	acidic	1.7	0	p=0.317
Buffer capacity	low	37.9	37.9	p=1.00
	medium-high	50	48.3	p=0.845
	high	12.1	13.8	p=0.774
Consistency	liquid	67.2	60.3	p=0.44
	with bubbles	25.9	31	p=0.588
	viscous	6.9	8.6	p=0.739

When examining the CFU/ml of *S. mutans* in saliva, in both groups – asthma and control, children with CFU/ml > 10⁵ predominated. Although there was a slight predominance of CFU/ml > 10⁵ in children with asthma,

there was no statistically significant difference in the distribution of *S. mutans* CFU/ml < 10⁵ or CFU/ml > 10⁵ between the two groups of children (Table 3 & 4).

Table 3. Prevalence of *S. mutans* CFU/ml saliva in Asthma and Control Groups

<i>S. mutans</i> CFU/ml	Asthma		Control		p
	N	%	N	%	
<10 ⁵	16	27.6	21	36.2	p=0.589
>10 ⁵	42	72.4	37	63.8	p=0.32
		p=0.0006		p=0.036	

Table 4. Prevalence of *Lactobacillus* spp. CFU/ml saliva in Asthmatic and Control Groups

<i>Lactobacillus</i> spp. CFU/ml	Asthma		Control		P
	N	%	N	%	
<10 ⁵	18	31	26	44.8	p=0.121
>10 ⁵	40	69	32	55.2	p=0.121
		p=0.003		p=0.327	

When determining the CFU/ml of *Lactobacillus* spp. in saliva, children with CFU/ml > 10⁵ predominated in both groups, but without statistical significance of the difference (Table 4).

The measured mean values of secretory IgA in children with asthma were statistically significantly higher (p < 0.05) in asthmatic children (Table 5).

Table 5. Secretory Immunoglobulin A (SIgA) Concentrations in Saliva of Asthmatic and Healthy Children

	N	M	SD	SEM	t	
controls	52	138.83	80.30	11.14		
asthma	52	208.82	191.57	26.57	2.43	p<0.05

DISCUSSION:

The present study reinforces the growing body of evidence suggesting that bronchial asthma significantly affects the physicochemical and immunological properties of saliva in pediatric patients. The observed decrease in stimulated salivary flow and the shift toward a more acidic pH in asthmatic children are congruent with prior research linking asthma and its pharmacotherapy—particularly the use of β 2-agonists and inhaled corticosteroids (ICS)—with salivary gland dysfunction and reduced oral pH [1, 2, 3, 4, 5, 8]. The mechanism is hypothesized to involve a reduction in parasympathetic stimulation of the salivary glands as well as a systemic effect of ICS on mucosal tissues, leading to glandular atrophy and decreased secretion [4, 11].

A lower salivary flow rate not only impairs the self-cleansing of the oral cavity but also alters the concentration of critical components such as bicarbonate ions, which contribute to buffering capacity, and salivary proteins involved in host defense, including secretory IgA. Despite this, our findings show that not all asthmatic children exhibit significantly reduced buffering capacity, suggesting a degree of individual variability in glandular compensation or response to pharmacotherapy, as also indicated in other studies [3, 10, 12].

The higher prevalence of acidic and slightly alkaline saliva in asthmatic children, as compared to the predominantly alkaline pH in healthy controls, is clinically relevant given that low pH favors the proliferation of acidogenic bacteria. The non-significant, yet consistently elevated CFU/ml saliva of *S. mutans* and *Lactobacillus* spp. observed in the asthma group support this inference. These microorganisms are major etiological agents in dental caries and thrive in low pH environments [6, 7, 8, 13].

The immunological component of the study revealed a statistically significant increase in secretory IgA (SIgA) levels in asthmatic children. This may reflect an upregulation of mucosal immune responses due to chronic antigenic stimulation in the airways. Elevated SIgA in saliva has been previously associated with mucosal hyper-reactivity and is viewed as an indicator of the local immune system's activation against persistent antigen ex-

posure [9, 14]. It is also notable that the use of inhaled corticosteroids (ICS) did not appear to suppress salivary SIgA production, supporting earlier findings that suggest ICS have a limited immunosuppressive effect on mucosal immunity [9, 14].

Nevertheless, the relationship between SIgA levels and oral health outcomes in asthmatic children remains complex. Some authors suggest that excessive mucosal immune activation may predispose dysbiosis, altering the equilibrium between commensal and pathogenic species in the oral cavity [15]. Additionally, methodological inconsistencies such as differences in saliva collection protocols, diurnal variation, and assay standardization contribute to the variability in reported SIgA concentrations and should be considered in the interpretation of the data [14].

While the present study provides valuable insights, it also highlights the need for more nuanced longitudinal research integrating salivary biomarkers with clinical oral health indices and asthma control parameters. The absence of significant changes in some salivary indicators may be attributable to compensatory mechanisms, improved preventive oral hygiene practices, or insufficient sample size to detect subtle biochemical variations [10].

CONCLUSION:

This study demonstrates that children with bronchial asthma exhibit significant alterations in salivary characteristics compared to healthy peers, including reduced stimulated salivary flow, increased acidity, and elevated levels of secretory IgA. These findings support the hypothesis that asthma and its pharmacological management influence both the physicochemical and immunological properties of saliva. Although changes in buffer capacity and microbial load were not statistically significant, the trend toward higher cariogenic bacterial titers in asthmatic children may contribute to an increased risk of oral disease. These results underscore the importance of integrated preventive dental care in pediatric asthma management and highlight salivary parameters as potential biomarkers for monitoring oral health in this population.

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