ALTERATION IN SALIVARY COMPONENTS OF CHILDREN WITH ALLERGIC ASTHMA

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ABSTRACT
Asthma is a chronic inflammatory disorder of the airway causing recurrent episodes of wheezing, breathlessness, chest tightness, and coughing. Salivary analysis of oral inflammatory and local humoral immune response should give additional information on the role of salivary components in asthmatic children.

This study aimed to evaluate the oral inflammatory and humoral immune status in children with allergic asthma. Samples of unstimulated whole saliva from 32 children with allergic asthma (14 patients treated with corticosteroids and 18 patients treated only with antihistamines) and 20 control children were analyzed for salivary levels of total protein, secretory immunoglobulin A (sIgA), immunoglobulin G (IgG), C-reactive protein (CRP) and haptoglobin, determined by colorimetric, radial immunodiffusion and immunoturbidimetry methods.

Salivary CRP and haptoglobin were significantly higher among children with allergic asthma compared to controls. Lower sIgA and elevated levels of CRP were found in allergic children treated with corticosteroid. Salivary level of IgG, haptoglobin and CRP were significantly higher in allergic patients treated with antihistamine compared to healthy children. Children under corticosteroid therapy showed lower level of sIgA compared to asthmatic children under antihistamine therapy. A significant correlation between total protein/haptoglobin and IgG/sIgA for children with allergic asthma was found.

The results suggest that the higher salivary levels of CRP and haptoglobin may be an answer to allergic inflammation and severity of asthma.

Keywords: allergic asthma, saliva, CRP, haptoglobin, sIgA
Abbreviations: sIgA - secretory immunoglobulin A; IgG - immunoglobulin G; IgM - immunoglobulin M; CRP - C-reactive protein

Introduction
Asthma is a chronic airway inflammatory disease caused by immune cells such as T-lymphocytes and Eosinophils. Asthmatic inflammation is responsible for vital features of the disease, including bronchial hyperresponsiveness.

A study across 56 countries demonstrated large variations in the prevalence of asthma. The highest prevalence was documented in Australia, Peru, New Zealand and the lowest recorded prevalences were found in Albania and Russia (18). In Bulgaria childhood asthma represents 4-6% of all chronic disease in children (16).

Human saliva contains several factors with protective or antibacterial properties in the oral cavity. Usually these factors are divided into immune and nonimmune agents. While the IgA and IgG are examples of local immune response, CRP and haptoglobin take part in inflammatory defence.

Salivary IgA, which predominantly is secretory (sIgA), constitutes the main specific immune defense mechanism in saliva (15). Low sIgA have been associated with atopic eczema (13). In children with chronic airways diseases there was only a slight increase of secretory IgA during the first 4 years of live (14). The mean concentrations of sIgA in patients with pneumonia were lower, compared to the healthy subjects (17). The authors suggest that low sIgA has an important role in the course of the disease in patients with pneumonia.

Some authors surmise that serum CRP levels may be related to the state of asthma exacerbation and allergic inflammation (9).

The present investigation was designed with the aim to evaluate the salivary levels of a larger constellation of proteins that take part in inflammatory mechanisms in oral cavity of children with allergic asthma. It included the following parameters: total protein, sIgA, IgG, CRP and haptoglobin.

Materials and Methods
Patients were recruited from the Pediatric Clinic, Faculty of Medical University, Sofia, Bulgaria for a period of 5 months.

The procedure for collecting saliva was explained to the parents of the involved subjects and an informed written consent was obtained prior to the investigation.

Thirty two children with allergic asthma (14 patients under oral corticosteroid therapy and 18 patients under antihistamine...
therapy (average age 9.7 and range 2-15) and twenty control children were included in the study (average age 8.9 and range 3-16).

Diagnoses (allergic asthma) were made with the help of a clinical questionnaire, physical examination and skin prick tests to 10 most common allergens.

All selected children were required to show: normal patterns of growth and development; absence of congenital or systemic disease; absence of dental abscess; any mucosal lesions; lack of any medication therapy (only for controls); no prior dental treatment by the time of the exam, according to the instruction of De Farias and co-workers (8).

The dental examination was performed in a dental chair, using a dental mirror and an explorer and no stimulation was utilized before the collection of the saliva.

Whole unstimulated salivary probe was collected as described by Dawes and Weatherell (7). Children were asked to refrain from eating, drinking for at least 1 hour before the collection. The last oral hygiene procedure had been accomplished in the previous night. Salivary samples were collected into 15 ml test tubes for 10 min between 7.30 a.m. and 8.30 a.m. In 4 children (aged 2-4 years) saliva was collected by cotton tampons placed in the oral cavity and by wringing them (3). The specimens (approximately 5 ml) were stored and kept at -70°C until further analysis.

The levels of IgG were assessed by radial immunodiffusion method with high sensitivity by Manchini (Immunotest kits, Sofia, Bulgaria). Before the analysis, samples were thawed at room temperature. Then they were centrifuged at 4000 g for 15 min. Five µl of the supernatant were used for radial immunodiffusion. The kits were incubated for 7 days at room temperature (22-25°C) and were stained with 4% water solution of tannin for 30 min. The diameters of the precipitation rings (mm) were assessed. To calculate the concentrations of IgG (mg/l), the diameters were multiplied by a factor, deduced from the constructed standard linear curve. It was drawn from 6 standard solutions with concentrations from 20 mg/l to 790 mg/l.

sIgA concentration in saliva was measured by enzyme-linked immunosorbent assay (ELISA) (Salimetrics kits, USA) according to the instructions to producer.

The levels of haptoglobin and CRP in saliva were determined by imunoturbidimetric method. Salivary samples were centrifuged at 10 000 x g for 10 min to avoid visible precipitates. Cobas Integra 400 - Roche Diagnostic analyser was used (lower detection limit for haptoglobin was 0.102 g/l and for CRP 0.85 mg/l). Total protein was determined by colorimetric method using Cobas Integra 400 - Roche Diagnostic analyser (lower detection limit: 0.8 g/l).

Statistical analysis was performed using SPSS package for Windows 11.5. T-test was used for comparison of the data between the tested groups and values lower than 0.05 were considered statistically significant. Spearman correlation coefficients (r) were calculated to assess possible association between tested parameters.

Results and Discussion
Clinical studies of oral or systemic disease in relation to salivary proteins are usually complicated by the difficulties in standardizing sampling methods and laboratory tests, which contribute to a diversity of findings.

In the present study, a great variability of values from the immunological assays in children was detected, which is in correlation with the results of Ben-Aryeh et al. (4). These authors speculate that the alterations can be related to the biological maturation of the immune system, which varies in different individuals. In our study, only 2 children younger than 4 years old were included after consideration that their individual values of the salivary components did not significantly influence the mean values when compared to intra and inter groups.

Mean values and standard deviation of the studied variables are presented in Table 1, Table 2, Table 3 and Table 4.

Previous studies have measured the salivary level of IgA in patients with asthma. Low IgA concentrations in saliva have been reported (6).

Transient absence of salivary IgA in the first year of life was associated with an increased risk of developing atopy, asthma, or bronchial hyperreactivity later in life (10).

A statistically significant association between decreased or absent levels of sIgA in patients with bronchial asthma and recurrent pneumonias was observed (6), but other authors did not found differences between the salivary IgA levels of normal and atopic children (21).

In the present study the salivary level of sIgA were low in allergic children treated with corticosteroid compared to children under antihistamine therapy (Table 4).

Patients with pollinosis, bronchial asthma, urticaria and Quincke’s oedema, presence of polyclonal hypergammaglobulinemia was detected, which was manifested by a rise in the levels of IgG, IgA IgM; only the level of IgD was lowered. The authors surmised that an essential increase in the level of IgG in saliva was due to the local synthesis of this immunoglobulin (2). In the present study we observed a significant increase of salivary IgG only in allergic children under antihistamine therapy (Table 3).

CRP and haptoglobin are acute phase reactants and their blood level is elevated in several inflammatory diseases. However, little is known about its association with asthma. We were able to demonstrate the presence of CRP and haptoglobin in saliva using imunoturbidimetry method.

In the literature we found that serum CRP concentration is not a good marker of bronchial hyperresponsiveness, which is mainly dependent on asthmatic inflammation (16). Buyukozturk et al. measured CRP in serum and they did not find significantly different in patients with allergic airway disease compared to the control group (5).
### TABLE 1

Salivary components in patients with allergic asthma compared with controls

<table>
<thead>
<tr>
<th>Parameters/Patients</th>
<th>Total protein (g/l)</th>
<th>IgG (mg/l)</th>
<th>sIgA (mg/l)</th>
<th>Haptoglobin (mg/l)</th>
<th>CRP (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic asthma (n=32)</td>
<td>0.525 ± 0.952</td>
<td>28.4 ± 20.36</td>
<td>79.3 ± 32.6</td>
<td>35.2 ± 56.1</td>
<td>0.29 ± 0.091</td>
</tr>
<tr>
<td>Controls (n=20)</td>
<td>0.395 ± 0.262</td>
<td>18.3 ± 18.2</td>
<td>86.7 ± 42.6</td>
<td>9.7 ± 8.34</td>
<td>0.151 ± 0.060</td>
</tr>
<tr>
<td>P</td>
<td>p=0.553</td>
<td>p=0.077</td>
<td>p=0.604</td>
<td>p=0.050</td>
<td>p=0.000</td>
</tr>
</tbody>
</table>

Results are expressed by mean ± SD; number of a studied individuals is given in parenthesis.

### TABLE 2

Salivary components in allergic children under corticosteroid therapy and controls

<table>
<thead>
<tr>
<th>Parameters/Patients</th>
<th>Total protein (g/l)</th>
<th>IgG (mg/l)</th>
<th>IgA (mg/l)</th>
<th>sIgA (mg/l)</th>
<th>Haptoglobin (mg/l)</th>
<th>CRP (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated with corticosteroid (n=14)</td>
<td>0.429 ± 0.42</td>
<td>17.9 ± 5.66</td>
<td>62 ± 23.6</td>
<td>65.6 ± 28.8</td>
<td>34.1 ± 55.6</td>
<td>0.299 ± 0.098</td>
</tr>
<tr>
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<td>18.3 ± 18.2</td>
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<td>9.7 ± 8.34</td>
<td>0.151 ± 0.060</td>
</tr>
<tr>
<td>P</td>
<td>p=0.780</td>
<td>p=0.931</td>
<td>p=0.129</td>
<td>p=0.041</td>
<td>p=0.06</td>
<td>p=0.000</td>
</tr>
</tbody>
</table>

Results are expressed by mean ± SD; number of a studied individuals is given in parenthesis.

### TABLE 3

Salivary components in allergic children under antihistamine therapy and controls

<table>
<thead>
<tr>
<th>Parameters/Patients</th>
<th>Total protein (g/l)</th>
<th>IgG (mg/l)</th>
<th>IgA (mg/l)</th>
<th>sIgA (mg/l)</th>
<th>Haptoglobin (mg/l)</th>
<th>CRP (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated with antihistamines (n=18)</td>
<td>0.594 ± 1.2</td>
<td>35.7 ± 23.7</td>
<td>78.2 ± 33.6</td>
<td>91.9 ± 31.7</td>
<td>36 ± 58.2</td>
<td>0.286 ± 0.089</td>
</tr>
<tr>
<td>Controls (n=20)</td>
<td>0.395 ± 0.262</td>
<td>18.3 ± 18.2</td>
<td>77.3 ± 26.6</td>
<td>86.7 ± 42.6</td>
<td>9.7 ± 8.34</td>
<td>0.151 ± 0.060</td>
</tr>
<tr>
<td>P</td>
<td>p=0.476</td>
<td>p=0.019</td>
<td>p=0.734</td>
<td>p=0.732</td>
<td>p=0.05</td>
<td>p=0.000</td>
</tr>
</tbody>
</table>

Results are expressed by mean ± SD; number of a studied individuals is given in parenthesis.

### TABLE 4

Salivary components in allergic children under corticosteroid therapy compared to allergic children under antihistamine therapy

<table>
<thead>
<tr>
<th>Parameters/Patients</th>
<th>Total protein (g/l)</th>
<th>IgG (mg/l)</th>
<th>IgA (mg/l)</th>
<th>sIgA (mg/l)</th>
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<td>91.9 ± 31.7</td>
<td>36 ± 58.2</td>
<td>0.286 ± 0.089</td>
</tr>
<tr>
<td>P</td>
<td>p=0.608</td>
<td>p=0.017</td>
<td>p=0.129</td>
<td>p=0.041</td>
<td>p=0.932</td>
<td>p=0.717</td>
</tr>
</tbody>
</table>

Results are expressed by mean ± SD; number of a studied individuals is given in parenthesis.
Contrariwise, adults with asthma and asthma symptoms have higher levels of CRP in serum. Some authors suppose that the potential use of CRP as a clinically useful marker for asthma severity should be further explored (1).

In the present study salivary levels of CRP were higher in asthmatic children compared to the controls (Table 1) and lower slgA and elevated levels of CRP were found in treated with corticosteroid allergic children (Table 2).

Previous studies have measured haptoglobin levels in serum of asthmatic patients. Serum concentration of haptoglobin decreases at the time of late asthmatic response and is subsequently replenished during the ensuing time (11). Decreased haptoglobin levels are more frequently associated with young age, atopic antecedents, positive skin tests for pollens, higher IgE and higher RAST activity for pollens and housedust mite (20). But other authors suggest that elevation of haptoglobin level at acute exacerbation is more marked in the cases with poor response to initial bronchodilator therapy at acute exacerbation, which suggests that the increased haptoglobin level at acute exacerbation in asthma might reflect the degree of airway inflammation (12).

The results of this study showed that salivary haptoglobin levels in children with allergic asthma are elevated when compared to the healthy subjects. Statistical significance was found (Table 1).

Statistical analysis performing Spearman rank correlation showed a significant correlation between total protein/haptoglobin (r = 0.626, p = 0.000) and IgG/slglA (r = 0.672, p = 0.000) for children with allergic asthma.

Conclusions
In children with allergic asthma the salivary concentrations of CRP and haptoglobin were significantly higher. We can speculate that CRP and haptoglobin have been directly transferred from the blood into saliva. The higher levels of these parameters may be an answer to allergic inflammation and severity of asthma. We observed positive correlation between total protein/haptoglobin and slgA/IgG for children with asthma.

Low slgA level in children under corticosteroid therapy could be reflecting the local immune suppression.

Salivary analysis revealed an overall altered salivary composition in children with asthma indicating a compromised oral environment in these patients and suggesting salivary analysis as an additional diagnostic tool for allergic disease.

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