GINGIVAL DISEASE AND SECRETORY IMMUNOGLOBULIN A IN NON-STIMULATED SALIVA IN CHILDREN

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ABSTRACT

AIM: To find the relationship of secretory immunoglobulin A (SIgA) to gingival diseases in childhood and adolescence by quantitative study of these antibodies in non-stimulated saliva.

PATIENTS AND METHODS: The survey included 30 somatically healthy children (mean age 15.37 ± 1.06 yrs) with clinically healthy gingiva and another 30 children (somatically healthy) (mean age 15.07 ± 0.69 yrs) with manifested plaque-induced gingivitis. The diagnosis of periodontal status was made on the basis of clinical criteria, the oral-hygiene index of Silness & Loe, the papilla bleeding index (PBI) of Saxer & Mulheman and the periodontal screening index for evaluation - Periodontal Screening and Registration (PSR, after ADA - American Dental Association).

SIgA in saliva was quantified by ELISA with salivary secretory IgA kit of Salimetrics LLC – USA.

RESULTS: In children with gingivitis the mean SIgA was 41.07 ± 32.14 μg/ml; it was higher in healthy children – 48.3 ± 32.41 μg/ml. A correlation was found between SIgA and the oral-hygiene index of Silness & Loe, (P < 0.05) and lack of dependence on the degree of gingival bleeding.

CONCLUSIONS: SIgA is a factor characterizing the local specific immunity which depends on local antigenic stimuli (plaque biofilm), but it does not affects the gingival pathology directly. SIgA can be considered an important part of an integrated assessment of oral risk environments.

Key words: secretory immunoglobulin A (SIgA), plaque-induced gingivitis, ELISA, biofilm

INTRODUCTION

Secretory immunoglobulin A (SIgA) is the main immunoglobulin isotype found in saliva and other body secretions.1-4

Biologically, SIgA provides the first line of immune defense in the oral environment. It is responsible for inhibiting the bacterial adhesion on the enamel and epithelial cells, acting in synergy with other defense mechanisms, making inactive bacterial enzymes and toxins and activating the complement. It is partially involved in cell-mediated immune responses. Thus, SIgA limits the invasion of various antigens in the mucosal epithelium and is involved in the maintenance of bacterial environment in the mouth and in the formation of biofilms on the enamel surface.5,6

In multi-factor periodontal pathology it is rather difficult to make a precise assessment of the importance of each risk factor in the oral environment, including the SIgA. However it has been found that these antibodies are in inverse relation with the salivary current, and the salivary current is essential for the accumulation of plaque on tooth enamel.7,8

Having in mind that SIgA do not enter the gingival sulcus, it is suggested that these antibodies, control the formation and composition of subgingival biofilm through modeling the accumulation of supragingival biofilm. They can affect the bacterial
depot of microorganisms, including the subgingival area and to prevent bacterial transmission from one gingival niche to another. Studies on dependencies of periodontal pathology and the concentration of SIgA in saliva are contradictory.9-12

Evaluation of oral risk environments is an important part of modern approach to diagnosis and preventive treatment of oral diseases. Determining the risk of periodontal disease includes detection of the factors that cause disturbance in the balance between local subgingival biofilm and local protective processes in the periodontium.

The aim of the study was to find if SIgA can be correlated with the gingival diseases in childhood and adolescence by studying these antibodies quantitatively.

PATIENTS AND METHODS

PATIENTS

The study was conducted among 60 schoolchildren from Ruse. The children were allocated to two clinical groups: group I included 30 children aged 15.37 ± 1.06 (13 boys and 17 girls), in somatic health and with clinically healthy gingiva; group II consisted of another 30 children (mean age 15.07 ± 0.69 yrs) (11 boys and 19 girls), somatically healthy but with clinically manifested plaque-induced gingivitis.

To form these two groups we examined 180 randomly selected children aged 15-16 with stabilized periodontium; the children were given a thorough examination of their mouths focusing on their periodontal health; they were clinically healthy and without systemic diseases. Sixty children were selected - 30 with absolutely healthy periodontium and 30 with plaque gingival diseases.

The study was conducted with the permission of the Ethics Committee of Scientific Research at Medical University-Sofia obtaining informed consent from each probant over 16 years and from their parents if the children were younger than 16 years of age.

EVALUATION OF BIOFILM IN THE STUDIED CHILDREN

Oral hygiene status of the children was determined by recording the oral hygiene index (OHI of Silness & Loe) using the classical methodology of the index determination.13

DIAGNOSING THE PERIODONTAL STATUS

Diagnosis of children with healthy periodontium and children with chronic plaque-induced gingivitis was conducted using the following criteria to distinguish between the two groups:

1. Diagnosed due to clinical examination using the clinical criteria for healthy and inflamed gingiva (Table 1).

2. Provoked bleeding on probing. We used the Papilla Bleeding Index (PBI) of Saxer & Mulheman.14

The obtained values were recorded on a pre-made card for evaluation and registration of oral status with periodontal orientation. To register the location of the gingival inflammation we grouped the children with gingivitis in 4 subgroups according to the proportion of bleeding papillae to the total number tested papillae for each child. This is an important indicator in children given that localized gingivitis in children is common and it must be registered as such. The idea was suggested by GBI of Aynamo & Bay.15

3. Determination of the depth of the gingival sulcus through probing (probing pocket depth). We used the index for screening periodontal assessment and registration - PSR (after ADA-American Dental Association).16 In this index, as in the CPITN, dentition is divided into six sextants, and probing is done within 6 points of every tooth representative for each sextant. For each sextant the highest measured value was considered, and for the tested child - the value of the worst affected sextant. We used the following coding: 0 - no specifics, 1 - up to 3 mm and bleeding; 2 - up to 3 mm, bleeding + tartar; 3 - 3 - 5 mm; code 4 - > 6 mm; code (*) - detection of furcations, mobility, recession and other periodontal lesions.

Table 1. Clinical criteria for identifying healthy gingiva and gingival inflammation

<table>
<thead>
<tr>
<th>Clinical criteria</th>
<th>For gingival inflammation</th>
<th>For healthy gingiva and periodontium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gingival papilla</td>
<td>edematous, shiny, red, detached, and with possible ulceration</td>
<td>pale pink, tight-fitting</td>
</tr>
<tr>
<td>Gingival edge (free gingiva)</td>
<td>swollen, thickened, hyperemic</td>
<td>pale pink, tight, adhering to the tooth</td>
</tr>
<tr>
<td>Attached gingiva</td>
<td>shiny, red, smooth contour</td>
<td>pale pink, orange peel</td>
</tr>
</tbody>
</table>
In our study we found no children with scores 3 and 4 suggesting initial periodontal destruction and confirming the clinical diagnosis of gingivitis.

EXAMINATION OF SECRETORY IMMUNITY (SIgA) IN SALIVA

Methods for collecting saliva. A sample of non-stimulated saliva was taken in the morning before breakfast, after washing the teeth at least half an hour before taking the sample. The saliva was collected in a 5 ml plastic container, from which a certain amount (1 ml) was taken by a pipette and placed in another container for freezing. The samples were frozen in refrigerator (-20°C).

ELISA method for examination of IgA-S in saliva. For quantification of SIgA in saliva we used the ELISA assay with salivary secretory IgA kit of SalimetricsLLC – USA. This is an indirect method.

RESULTS

CHARACTERIZATION OF THE BIOFILM IN THE STUDIED CHILDREN

Comparative analysis of accumulation of plaque between the two groups of children was performed using the OHI of Silness & Loe (Table 2).

Table 2. Oral-hygiene index of the examined children after Silness & Loe

<table>
<thead>
<tr>
<th>Children</th>
<th>Mean ± SD</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I - with gingivitis</td>
<td>2.61 ± 0.54</td>
<td>0.09</td>
</tr>
<tr>
<td>Group II - without gingivitis</td>
<td>1.32 ± 0.87</td>
<td>0.16</td>
</tr>
<tr>
<td>Significance</td>
<td>t = 6.877, P = 0.0001</td>
<td></td>
</tr>
</tbody>
</table>

There was a significant difference in OHI between the two groups of children (P < 0.05). The children with gingival disease had more biofilm and poorer oral hygiene which increases the risk of periodontal pathology and in the absence of other risk factors for such pathology proves the clinical diagnosis in children from the second group - plaque-induced gingivitis.

CLINICAL CHARACTERISTICS OF PERIODONTAL STATUS OF CHILDREN WITH GINGIVAL DISEASES

Clinical evaluation of periodontal health according to existing clinical criteria. In the group of children with periodontal pathology we observed only plaque-induced gingival diseases, and only one child was diagnosed with ulcerative necrotic gingivitis.
Clinical assessment of gingival bleeding using PBI. Gingival bleeding is the most characteristic symptom of gingival inflammation; its assessment using indices is the right way to measure it. The results showing the proportion of bleeding papillae compared with the total number of tested papillae are indicative of the degree of involvement of individual teeth in gingival inflammation (Table 3).

Almost half of children (43.33%) with gingival diseases presented with generalized gingivitis, affecting on an average more than 75% of the gingiva of each dentition. They are followed by the children with localized gingival diseases affecting from one fourth to half of the gingival papillae. They represent slightly more than one third of the whole group of children. The remaining children are equally distributed in children with bleeding in one fourth of the papillae and children with bleeding in more than half of the papillae. These are local gingival inflammations around individual teeth and inflammations involving more than half but no less than two thirds of the dentition. It is noteworthy that in all cases of localized plaque-induced gingivitis the localization of inflammation is related to available orthodontic anomalies and teeth, ectopically located to varying degrees (teeth with deviation from the dental arch).

Table 3. Distribution of children with gingivitis, according to the relative portion of bleeding papillae (after PBI)

<table>
<thead>
<tr>
<th>Grouping according to the percentage of bleeding papillae (after PBI)</th>
<th>n</th>
<th>%</th>
<th>± Sp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: 1 - 25%</td>
<td>3</td>
<td>10</td>
<td>5.47</td>
</tr>
<tr>
<td>Group II: 25 - 50%</td>
<td>11</td>
<td>36.66</td>
<td>8.95</td>
</tr>
<tr>
<td>Group III: 50 - 75%</td>
<td>3</td>
<td>10</td>
<td>5.47</td>
</tr>
<tr>
<td>Group IV: 75 - 100%</td>
<td>13</td>
<td>43.33</td>
<td>9.20</td>
</tr>
<tr>
<td>Statistical significance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t_{1,2} = 2.79</td>
<td></td>
<td>P &gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>t_{2,3} = 2.79</td>
<td></td>
<td>P &gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>t_{3,4} = 3.16</td>
<td></td>
<td>P &lt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Comparison of children, grouped according to PBI

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean ± SD</th>
<th>SEM</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children with code up to 1</td>
<td>10</td>
<td>1.007 ± 0.022</td>
<td>0.007</td>
<td>t_{1,4} = -27.398</td>
</tr>
<tr>
<td>Children with code up to 2</td>
<td>6</td>
<td>1.95 ± 0.260</td>
<td>0.106</td>
<td>P = 0.0001</td>
</tr>
<tr>
<td>Children with code up to 3</td>
<td>13</td>
<td>2.867 ± 0.212</td>
<td>0.590</td>
<td>t_{1,2} = -11.688</td>
</tr>
<tr>
<td>Children with code up to 4</td>
<td>1</td>
<td>3.56</td>
<td></td>
<td>P = 0.0001</td>
</tr>
</tbody>
</table>
children according to the PSR-index have at least one sextant with score 1 or 2 confirming the clinically detected gingival inflammation. If there are two children with score 0 it shows a discrepancy between the established clinical diagnosis (gingivitis) and healthy sextants and those with mild and more severe gingival inflammation. Thus the clinical diagnosis of plaque induced gingivitis is confirmed. Some of the children have generalized gingivitis, others have localized gingivitis.

The distribution of the sextants by severity of the pathology observed in the group of children with gingivitis is shown in Fig. 4.

Of all examined 30 children the biggest percentage of healthy children are in the fourth sextant - 15 children (50% of all cases). The least healthy are the fifth sextants - in 4 children (13.3%) and these refer to the lower front teeth. The difference between them is significant (P < 0.05). Similar difference, although smaller, is found between healthy second and fourth sextants. In this comparison as well the second sextants are more affected than the fourth ones. i.e. the upper front teeth are more severely affected than the lower front teeth. There is no significant difference in the number of healthy first, third, fourth and sixth sextants.

Gingival inflammation in the study children is mainly in the front - significantly more affected are second and fifth sextants, as inflammation is predominant in the upper frontal sections.

Quantifying SIgA in saliva of healthy children and children with plaque-induced gingivitis

Oral secretory immunity is characterized by antibodies of the SIgA type which are secreted in saliva and are the result of local antigenic stimuli. The most important among these are the bacterial plaque biofilm antigens. As a protective factor of the macroorganism, SIgA provides the most important specific immune protective factor in the mouth and plays an important role in the homeostasis of the oral microbial environment.

SIgA was studied by using samples of non-stimulated saliva. Mean values of SIgA in mixed saliva of the examined children were $44.93 \pm 32.24 \mu g/ml$. The obtained values vary between $1.2 \mu g/ml$ and $175.5 \mu g/ml$. The distribution of mean values of SIgA in both groups of children is shown in Table 5.

In children with gingivitis the mean SIgA is $41.07 \pm 32.14 \mu g/ml$, and in healthy children it is higher - $48.3 \pm 32.41 \mu g/ml$. Although the difference between them does not reach statistical significance (P > 0.05), there is a slight tendency to reduction of SIgA in children with plaque-induced gingivitis.

Given that gingivitis is plaque-dependent, using the correlation coefficient of Pearson we looked...
Gingival Disease and Secretory Immunoglobulin A in Non-Stimulated Saliva in Children

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Table 5. Mean SIgA values in saliva of children with and without plaque-induced gingivitis (μg/ml)

<table>
<thead>
<tr>
<th>Children</th>
<th>n</th>
<th>( \bar{x} \pm \text{SEM} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>With gingivitis</td>
<td>30</td>
<td>41.07 ± 32.14</td>
</tr>
<tr>
<td>Without gingivitis</td>
<td>30</td>
<td>48.3 ± 32.41</td>
</tr>
<tr>
<td>t / P</td>
<td></td>
<td>( t = -0.927, P = 0.358 )</td>
</tr>
</tbody>
</table>

Table 6. Correlation between SIgA, OHI and PBI

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Pearson Correlation coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>OHI - SIgA</td>
<td>30</td>
<td>-0.291</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>PBI - SIgA</td>
<td>30</td>
<td>-0.212</td>
<td>( P &gt; 0.05 )</td>
</tr>
</tbody>
</table>

Figure 4. Distribution of the sextants by severity of the pathology observed in the group of children with gingivitis.

A correlation was found between SIgA and the plaque biofilm with a level of significance \( P < 0.05 \) and lack of dependence on the degree of gingival bleeding.

DISCUSSION

Dynamically changing saliva as material for analysis...
the measurement of their quantity.\textsuperscript{21}

Our research shows significant differences in the concentration of SIgA using the same test, but applied to samples of stimulated and non-stimulated saliva. In the present study SIgA in somatically healthy children (15-16 yrs) was $44.93 \pm 32.24$ μg/ml and also in previous studies in healthy children (7-15 yrs), whose saliva is stimulated, it is 121.22. Although non-stimulated saliva shows lower levels of SIgA, their distribution is more even compared to SIgA from stimulated saliva (from a previous study\textsuperscript{23,24}, which is why we recommend non-stimulated saliva for the study of SIgA.

In the literature there is scanty information about the role of SIgA from saliva in the development of periodontal diseases. According to some authors, low levels of SIgA in saliva can be considered a potential risk factor for the development of periodontal disease and caries.\textsuperscript{12,22} It is very difficult to ascertain how SIgA can control the subgingival biofilm, because secretory antibodies do not enter the gingival sulcus or pocket. It is possible SIgA to control the formation and composition of subgingival plaque and its potential to cause disease by modulating the accumulation of supragingival plaque.\textsuperscript{11} The results of our study confirm this hypothesis because they prove the correlation between SIgA and the amount of biofilm. SIgA-antibodies could protect the proliferation of periodontopathogens in the tongue which is a reservoir for these organisms and a source for their colonization in the gingival sulcus.\textsuperscript{11}

The presence of correlation between SIgA and OHI shows that the biofilm is an important immunogenic factor for secretory antibodies in saliva, although their quantity can not be regarded as a risk marker of gingival inflammation.

Local antigens in the mouth are the most important stimulus for oral secretory immunity. This conclusion is supported by another study of ours, which demonstrated that the plastic orthodontic appliances are also local stimuli for secretory antibodies in the mouth in children.\textsuperscript{24}

**CONCLUSIONS**

SIgA is a factor characterizing the local specific immunity which depends on local antigenic stimuli, but does not affect directly the gingival oral pathology. This conclusion is supported by the described mechanisms of action of SIgA in pathological processes in the mouth. Its quantitative evaluation is highly dependent on salivary current and the method of analysis. SIgA is a factor that can be used in comparative studies on the effects of different antigenic stimuli on oral risk environments.

As an indicator of specific local immunity in the mouth, SIgA can be considered an important part of an integrated assessment of oral risk environments.

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**ACKNOWLEDGEMENTS**

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**REFERENCES**

Гингивальные заболевания и секре́тный иммуно́глобу́лин A (SIgA) в нестимулированной слюне у детей
М. Рашкова, А. Тошева

ЦЕЛЬ: Работа ставит себе целью провести коли́чественное исследование SIgA в нестимулиро́ванной слюне, установить связь этих антител с гингивальными заболеваниями в детско-юношеском возрасте.

ПАЦИЕНТЫ И МЕТОДЫ: В исследование включено 30 детей (средний возраст 15.37 ± 1.06), соматически здоровых с клинически здоровой гингивой и 30 детей (средний возраст 15.07 ± 0.69) соматически здоровых с клинически проявленным бляшко-инду́цированным гингивитом. Диагностирование пародонтального статуса проведено с применением клинических критериев, орально-гингивальным индексом Silness & Löe, Papilla Bleeding Index (PBI) в рамках стандартизованной методики Periodontal Screening and Registration (PSR, по ADA - Американская дентальная ассоциация). Содержание SIgA в слюне определено с помощью ELISA-метода.

РЕЗУЛЬТАТЫ: У детей с гингивитами средняя стоимость SIgA в границах 41.07 ± 32.14 μg/ml, а у здоровых детей она выше - 48.3 ± 32.41 μg/ml. Установлена корреляция между SIgA и орально-гингивальным индексом Silness и отсутствием зависимости со степенью гингивальной кровоточивости.

ЗАКЛЮЧЕНИЕ: SIgA представляет фактор, ха́рактеризующий локальный специфический имму́нитет, зависящий от локальных антигенных сти́мулов (бляшковый биофильм), но не оказывающий непосредственное влияние на гингивальную пато́логию. SIgA можно считать важной частью комплексной оценки оральной рисковой среды.