Introduction
Saliva plays an important role in the oral environment and contributes to protection and homeostasis in the oral cavity [1]. Secretory immunoglobulin A (S-IgA) is the most frequently found immunoglobulin in mixed saliva and is considered to be a secretory factor for acquired immunity in the oral cavity. Antibodies of this type participate in the preservation of the integrity of the oral surfaces (enamel and mucous membrane) and, through restriction of microbial adhesion, become part of the first line of defence. S-IgA antibodies independently, or in complexes, participate in antigen-antibody reactions on the mucous membrane (and partly on the enamel too), thus limiting the penetration of bacteria and toxins [2, 3, 4, 5, 6, 7].

Secretory IgA antibodies are produced by the salivary glands. Lymphocytes and plasma cells

Secretory immunoglobulin A (S-IgA) in the Saliva of children with Type 1 Diabetes, Asthma, Systemic Health and Systemic Health but Wearing Removable Orthodontic Appliances

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Abstract
Aim: The aim of this study was to quantify secretory immunoglobulin A (S-IgA) in the saliva of children with type 1 diabetes, asthma, no systemic disease, and no systemic disease but wearing a removable orthodontic appliance.

Methods: The study recruited 116 children who had either type 1 diabetes or asthma, were systemically healthy, or were systemically healthy but wore a removable orthodontic appliance. All children were assessed for risk of dental caries and provided saliva samples for assay for S-IgA.

Results: The results indicated that the average values of S-IgA in the saliva of the healthy children and of the children with diabetes were 121.3 ±15.0 µg/ml and 133.9 ±160.5 µg/ml, respectively. Children with asthma and removable orthodontic appliances had statistically higher values for S-IgA: 196.4 ±145.3 µg/ml and 208.8 ±125.9 µg/ml, respectively. The average values of S-IgA in all four groups varied widely. The differences were greatest in children with removable orthodontic appliances and in children with asthma.

Conclusions: In the relatively small samples studied: (1) The average values of S-IgA in the saliva of healthy children and in the saliva of children suffering from diabetes were lower than those of children in the groups who had asthma and who were systemically healthy but wore removable orthodontic appliances. (2) The children with asthma and who wore removable orthodontic appliances showed statistically significant higher values of S-IgA: 196.4±145.3 µg/ml and 208.8±125.9 µg/ml, respectively. (3) The individual values of S-IgA in the different children in all four groups studied varied within broad limits. The differences were greatest in children with orthodontic appliances and in children with asthma. (4) Removable orthodontic appliances appeared to be a local immunogenic factor, which provided a stronger stimulus for oral secretory immunity than systemic factors such as diabetes and asthma. (5) Secretory immunity as a marker for local acquired immunity in the oral cavity may be affected by systemic and local factors.

Key Words: Secretory Immunoglobulin A, Saliva, Diabetes, Asthma, Removable Orthodontic Appliances, Oral Health, Secretory Immunity, Acquired Immunity

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accumulate around the epithelial acinar systems of the major salivary glands (parotid, submandibular, sublingual), as well as in the small submucous salivary glands. Secretions are mainly S-IgA and some smaller amounts of immunoglobulin M and immunoglobulin G [8, 9].

The largest amount (90%) of S-IgA is produced by the parotid and submandibular salivary glands. The plasma cells of these glands secrete dimeric immunoglobulin A (IgA-dimer), which associates with a secretory particle (SP), is resistant to proteolysis, and is secreted by the epithelial cells of the acinar channels [10, 11, 12].

The amounts of immunoglobulins in saliva and in serum are different. In some pathological processes, this relationship changes, and could have diagnostic value. In other situations, the reduced production of S-IgA, as a consequence of altered oral immunity [13, 14], may be the cause of some oral pathological processes.

The role of S-IgA in the development of dental caries is determined by the capacity of these antibodies to prevent the colonisation of the enamel surface by binding with the plaque microorganisms. Thus microbial adhesion is inhibited and the formation of new plaque biofilm is prevented. The secretory IgA binds mostly with Streptococcus mutans and its antigenic enzymes and metabolic products [15, 11].

Secretory IgA does not enter the gingival sulcus and so cannot control subgingival plaque. However, it is possible for S-IgA to modulate the accumulation of subgingival plaque, and thus control the latter’s formation and composition. During gingival inflammation, there is increased permeability of the gingival blood vessels and of the gingivae. As a result, larger quantities of serum IgA antibodies are found in the gingival sulcus [16, 17, 18].

It is clear that S-IgA plays an important role in oral homeostasis and is an important indicator of the defensive status of the oral cavity, where the rich oral microbiota has antigenic potential and can stimulate secretory antibodies related to the different factors involved in oral homeostasis. Because different methods of evaluation and calculation of S-IgA in saliva have been used, it was not possible to find standardised reference values serving as a norm for secretory antibodies in children’s saliva. This led to our interest in studying S-IgA in the oral cavity.

Aim

The aim of this study was the quantitative measurement of the S-IgA in the saliva of children with different diseases and conditions affecting the oral environment.

In order that this aim was achieved the following objectives were set:
1. Determination of the average values of S-IgA in the saliva of children with diabetes, asthma, removable orthodontic appliances as compared with a control group of healthy children,
2. Study of the distribution of children examined in the groups with lower (up to 100 µg/ml), medium (100-300 µg/ml) and high (>300 µg/ml) values of S-IgA.

Methods

1. Study population

The study population consisted of 116 children (6-17 years of age). The children were divided into four groups, two groups had either type 1 diabetes or bronchial asthma. The other two groups were either systemically healthy or systemically healthy but had worn a removable orthodontic appliance for the previous two years. Ethical approval for the study was obtained from the Ethics Committee of Medical University of Sofia. Consent for the inclusion of all children under the age of 16 years was sought from a parent or guardian, and for those aged 16 years or older, from the patients themselves.

1.1 Group 1 (T1): Systemic disease (type 1 diabetes)

Group 1 comprised 30 children, aged 7-17 years (average age 12.20 ±3.53 years), with non-controlled diabetes (type 1) from the Clinic for Children’s Endocrinology, Diabetes and Genetic Diseases, Department of Paediatric Medicine, Medical University of Sofia. The children in this group were selected against the following parameters:
1. Minimum two years duration of disease.
2. Laboratory parameters characterising diabetes at the time of the study:
- HbA1c (claycaded) >8.0%
- Blood glucose:
  - on empty stomach >8.0 µmol/l
  - two hours after food >10.0 µmol/l
- Acidic-alkali condition (AAC):
  - pH 7.35
  - BE (base excess) >2.5 mEq/l
  - SB (standard bicarbonates) >22 mEq/l

All children treated at the clinic over a six-month period who met the inclusion and whose parents gave consent were included in the study.

1.2 Group 2 (T2): Systemic disease (bronchial asthma)
Group 2 comprised children, aged 7-17 years (average age 8.84 ±3.02 years), with bronchial asthma who had a history of prolonged use of inhaler corticosteroids. They were recruited from the Clinic for Children’s Endocrinology, Diabetes and Genetic Diseases, Department of Paediatric Medicine, Medical University of Sofia. The children were selected against the following inclusion criteria:
- Diagnosed bronchial asthma.
- Moderately heavy clinical form of the disease.
- Asthma duration of at least two years.
- Treatment with inhaled corticosteroids (at the time of the study and for not less than six months previously).
- Inhaled corticosteroid (Flixotide®; GSK, Brenford, UK).

All children treated at the clinic over a six-month period who met the inclusion and whose parents gave consent were included in the study.

1.3 Group 3 (T3): Healthy children (without systemic disease)
A random sample of 34 healthy children without systemic disease and whose parents consented to their inclusion in the study were recruited from the Department of Paediatric Dentistry, Faculty of Dental Medicine, Medical University of Sofia. Their age range was 7-17 years (average age 10.47 ±2.75 years).

1.4 Group 4 (T4): Healthy children (wearing removable orthodontic appliances)
A random sample of 27 healthy children who had worn removable orthodontic appliances for a minimum of two years and whose parents consented to their inclusion in the study were recruited from the Department of Paediatric Dentistry, Faculty of Dental Medicine, Medical University-Sofia. Their age range was 7-17 years (average age 11.07 ±1.27 years).

2. Assessment
All the children underwent a three-part assessment. The first part consisted of a full oral examination, caries risk assessment, and salivary analysis, as previously described by Rashkova et al. (2008) [19]. In summary, this involved charting the teeth for caries, followed by the use of a caries risk-assessment questionnaire, which included questions on caries risk and protective factors such as oral hygiene, use of fluorides, parental caries experience, and so on.

The second part involved the use of Saliva Check Buffer Tests (in vitro test for checking the quality, pH and buffering capacity of saliva) (GC, Tokyo, Japan) to assess resting and stimulated saliva. Saliva was taken in the morning (before food) after stimulating salivation by means of chewing standard chewing gum for two minutes. The resulting saliva was collected in a 5 ml plastic container, from which a 2-3 ml was taken and frozen in a refrigerator at -10°C).

The third part involved the study of S. Mutans and Lactobacillus spp. using a CRT test (Ivoclar-Vivadent, Liechtenstein). Detailed analysis of the results obtained of this evaluation will be subject of a subsequent publication.

The ELISA (Enzyme-Linked ImmunoSorbant Assay) method [20] was used to quantify S-IgA in the saliva. A salivary secretory IgA kit (Salimetrics, State College, PA, USA) was used and a standard curve was prepared (Figure 1). Based on this, the concentrations of S-IgA in µg/ml saliva were calculated.

3. Statistical analysis
Data were analysed using statistical software (SPSS Version 16; SPSS Inc, Chicago, USA). The following tests were applied: chi-square, t-test, and Pearson’s coefficient. Dispersal of values about the mean are presented as a standard deviation; differences between the groups were examined using a value of P<0.05 being considered significant.

Results
The standard curve referred to in the methods section is at Figure 1.

1. Comparison of S-IgA in the saliva of the groups of children studied
The average values of S-IgA in the saliva of the children examined are presented in Table 1.

2. Visual distribution of S-IgA values in all children

The average values of S-IgA in the saliva of healthy children (group T3) was 121.3 ± 15.0 µg/ml. The highest values were registered in children with orthodontic treatment (group T4) at 208.9 ± 125.9 µg/ml and in children with asthma (group T2) at 196.4 ± 145.3 µg/ml. These values were significantly higher (*P < 0.05) than those for the control group (T3). In children with diabetes (group T1), the average values of S-IgA were 133.9 ± 160.5 µg/ml. They were not statistically different to those in the control group.

The distribution of S-IgA for each child in the different groups is shown in Figures 2-5. From these figures, it can be seen that the values of S-IgA within each group are very different and vary within wide limits. The variations are greatest in those children with orthodontic appliances (group T4) and those with asthma (group 2). These were the groups with the highest average values for S-IgA. In the control group (group T3), as indicated by the standard deviation and Figure 4, the distribution was more even.

3. Distribution of conditions by S-IgA level

Because of the wide range of S-IgA, it was decided to group the children into three groups according to the quantities of S-IgA:
- Low values of S-IgA (up to 100 µg/ml).
- Medium values of S-IgA (between 100-300 µg/ml).
- High values of S-IgA (>300 µg/ml).

The distribution of the children in these three groups is shown in Figure 6. These results indicated that in the group of healthy children (T3) there were no high values of S-IgA (>300 µg/ml). Two thirds (63.3%) of these children manifested low and one third (35.3%) medium values of S-IgA (up to 300 µg/ml). In the two groups (T2 and T4) with statistically higher mean values of S-IgA (children with asthma and with removable orthodontic appliances), a very interesting distribution of data is observed. Half of the children with asthma (56%) were in the category with medium values and half of the children with removable orthodontic (48.1%) were in the category with high values. The distribu-

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**Table 1. Average values of S-IgA in the saliva of the children examined**

<table>
<thead>
<tr>
<th>Groups of children</th>
<th>n</th>
<th>Average S-IgA ±SD</th>
<th>∆</th>
</tr>
</thead>
<tbody>
<tr>
<td>(T1) diabetes</td>
<td>30</td>
<td>133.9 ± 160.5</td>
<td>29.3</td>
</tr>
<tr>
<td>(T2) asthma</td>
<td>25</td>
<td>196.4* ± 145.3</td>
<td>29.1</td>
</tr>
<tr>
<td>(T3) healthy children</td>
<td>34</td>
<td>121.3 ± 15.0</td>
<td>87.6</td>
</tr>
<tr>
<td>(T4) with orthodontic. appliances</td>
<td>27</td>
<td>208.9* ± 125.9</td>
<td>24.2</td>
</tr>
</tbody>
</table>

T and P

<table>
<thead>
<tr>
<th>T 1,3</th>
<th>P</th>
<th>T 2,3</th>
<th>P</th>
<th>T 3,4</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.396</td>
<td>0.693</td>
<td>2.469</td>
<td>0.017</td>
<td>-3.196</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*P < 0.05
tion of the low values in the children with diabetes (group T1) was similar to that of healthy children (T3), but unlike the group of healthy children a fifth (20%) of children with diabetes had high values of S-IgA (>300 µg/ml).

**Discussion**

The rationale for the selection of a group of healthy children with removable orthodontic appliances was that the antigenic action of acrylic has been shown to have a strong antigenic stimulus [15,21]. The relatively small numbers recruited to the study reflect the numbers of children with either type 1 diabetes or asthma who were treated at the clinic concerned over the six-month period and who met the inclusion criteria. However, it must be accepted
that far larger samples are necessary before firm conclusions can be drawn.

The average values determined for the healthy children in the current study are at the lower end of the range that have been described by other authors; for example, the values for S-IgA in the saliva of children older than 16 years have been given as 102-471 µg/ml [10]. Bearing in mind that the average age of the children in the current study was less than 16 years, it is possible that the values (121.3 ±15.0 µg/ml) are within the norm for healthy children of this age. The children with asthma showed statistically higher (P<0.05) levels of S-IgA (196.4 ±145.3 µg/ml) compared with the healthy children. The increase in the secretory antibodies is indicative of a strong antigenic irritation in the rhinopharynx and the pulmonary mucous membrane, as is expected in asthmatics. Ongoing
usage of inhaled corticosteroids appears to have no immunosuppressive influence on oral secretory immunity.

In the literature, there are no unified referent values for the S-IgA in the saliva of children. This is due to the difference between the methodologies that have been used, including the absence of standards for the collection of saliva and the use of different units of measurement [1,2,10,20]. The average values of S-IgA in the saliva given by the firm Salimetrics are 367 µg/ml for adults; the corresponding values for children are (according to this company) much lower [20].

Even higher were the average values of S-IgA in children with orthodontic appliances (208.8 ±125.9 µg/ml). Although these children were healthy, the average values of S-IgA were significantly higher than the values recorded in the control group of children. This finding can be explained by the stimulatory effect exerted directly by the acrylic or by the conditions created in the mouth by the presence of the removable appliances, which make good oral hygiene more difficult to achieve and thus change the microflora and oral homeostasis.

Different authors have studied the influence of orthodontic appliances on the oral environment of children [5,22,23]. Most have investigated the toxic effects of orthodontic materials and have been conducted in vitro on a chosen cell population. Furthermore, in orthodontic therapy, different materials are used and subjected to a damp oral environment [23]. The use of biomaterial components in orthodontic practice was shown to release potential allergens such as metal ions from base metal alloys in fixed appliances, methylmethacrylate monomers and other organic substances from chemically-curing removable appliances and resin-based bonding materials. The results of preliminary investigations in another study [24] indicated that allergic patients with orthodontic appliances exhibit changes in the morphology and composition of salivary cells as compared to control patients. Differences were most pronounced in the first months of orthodontic treatment [24]. Kasacka et al. [21] also investigated saliva, which—apart from various biologically active substances—is affected by mucous epithelial cells and numerous haematologic factors. The aim of their study was to assess the changes in salivary cells of orthodontically treated allergic patients.

Intra-oral orthodontic appliances, frequently used in the treatment of malocclusions, may cause pathomorphological changes in the mouth and can be a potential source of antigen stimulation [24].

In the current study, the average of S-IgA values for children with diabetes was 133.9 ±160.5 µg/ml, close to the average for healthy children. The children were selected as non-controlled diabetics but nevertheless were in the initial phase of the disease. It may be that systemic endocrine illness at an early stage does not affect the local secretory oral immunity [7].
The values of S-IgA within in each separate group are very different and vary within wide limits. This may be because the investigated population was small. The variations were greatest in the groups with the highest average values. However, the finding in all groups of children with very low and very high values suggests the presence of other factors that affect the secretory oral immunity [6].

Analysis of the results suggests that removable orthodontic appliances may provide a significant stimulus for oral secretory immunity. In a previous, at present unpublished study by the authors of the current investigation [25], in which the same groups of children were studied, it was found that, excluding a reduced saliva buffering capacity, all the other parameters characterising the saliva of children with orthodontic appliances were similar to the corresponding parameters in the control group. It may therefore be that S-IgA is not affected by the quality of the saliva and is stimulated by acrylic removable orthodontic appliances which exert a stronger influence than so either type 1 diabetes or asthma on oral secretory immunity. This possible effect and the results of the microbiological investigations described in the methods section of this paper will be reported in a subsequent paper.

Conclusions

In the relatively small samples studied:
1. The average values of S-IgA in the saliva of healthy children and in the saliva of children suffering from diabetes were lower than those of children in the groups who have asthma and who were systematically healthy but wore removable orthodontic appliances.
2. The children with asthma and who wore removable orthodontic appliances showed statistically significant higher values of S-IgA, 196.4 ±145.3 µg/ml and 208.8±125.9 µg/ml, respectively.
3. The individual values of S-IgA in the different children in all four groups studied varied within broad limits. The differences were greatest in those children with orthodontic appliances and in those with asthma.
4. Removable orthodontic appliances appeared to be a local immunogenic factor, which provided a stronger stimulus for oral secretory immunity than systemic factors such as diabetes and asthma.
5. Secretory immunity as a marker for local acquired immunity in the oral cavity may be affected by systemic and local factors.

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