Secretory Immunoglobulin A (S-IgA) and the Oral Risk Markers: Quality of Saliva, Dental Biofilm, Oral *Candida* and *Lactobacillus spp.*

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Abstract

Aim: The aim of this study was to determine the dependency of secretory immunoglobulin A (S-IgA) on the risk markers of the oral environment, such as the quality of the saliva, the dental biofilm, the oral *Candida* and *Lactobacillus spp.* in children. ***Methods:*** The study was conducted on a total of 116 children who were divided into four groups, which were: diabetes, asthma, orthodontic appliances, and no systemic disease or local oral factors. All children were subjected to a clinical assessment for dental caries and oral cleanliness. Samples of stimulated saliva and smears were taken for microbiolgical examination (for oral *Candida* and *Lactobacillus spp.)* and immunological examination (for S-IgA). For each parameter examined (salivary flow, pH, buffering capacity, Simplified Oral Hygiene Index, oral *Candida* and *Lactobacillus spp.)* the children were divided into two subgroups: one with low and the other with high values of the corresponding parameter. ***Results:*** The results demonstrated that (1) the speed of salivation, the pH, and the buffering capacity of the saliva did not influence the secretion of S-IgA; (2) secretory oral immunity did not correlate with the markers of the resident oral microflora and the dental biofilm in the children. ***Conclusions:*** Even though, in terms of classical immune reactions, the defence potential of S-IgA is restricted, it may be that these reactions could be used as diagnostic indicators for an adaptive oral immunity of the saliva in children with different oral pathologies.

Key Words: Secretory Immunoglobulin A, Oral Risk Markers, Quality of Saliva, Dental Biofilm, Oral Candida, Lactobacillus spp.

Introduction

The oral environment is formed as a result of complex interrelations between saliva, microorganisms, defence mechanisms, systemic factors, and the external environment. The mouth is the gateway for many external pathogenic factors, which can cause both local and general pathology [1,2,3]. Recently there has been growing interest in saliva as a medium for monitoring a broad spectrum of physiological and pathological conditions, not only in the mouth but also in the whole body [4,5,6,7].

The basic physical and chemical markers that characterise the liquid oral environment are salivary secretion, pH, consistency and buffering capacity. The quality of saliva has an effect on the dental biofilm and thus—directly or indirectly—on oral pathology [5,8].

Saliva is affected by the general condition of the host and in the case of certain general diseases this fact must be taken into consideration when the oral environment is evaluated in terms of the risk of oral pathology [9,10,11,12,13].

It has been shown that oral candida is one of the microbial species that are the earliest colonisers of the oral cavity. The process begins as early as childbirth, especially in children born during a risk

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pregnancy, with systemic damage and chronic illnesses [14]. The influence and interaction of oral candida on general health status continues in later childhood. Oral candida proliferates in illnesses and conditions that create a favourable ground for the growth of opportunistic pathogens. The interactions, observed by the authors, lead them to regard oral candida as a microbial marker to show the influence on the general health status of the organism on the oral environment and on oral eubiosis (balance between the host and microorganisms) [15,16,17,18,19,20].

Studies on oral *Lactobacillus spp.* have shown that they are influenced by local factors such as acidity, elimination of carbohydrates, dental caries. Because of this, these microorganisms can also be used as microbial markers but as ones reflecting the local oral environment [21].

Signs of the numerous components in the composition of saliva provide information on local and systemic diseases or pathological processes [22]. The secretory immunoglobulin antibody (S-IgA) is the prevalent immunoglobulin in mixed saliva and is considered to be a basic factor for adaptive immunity in the oral cavity. Antibodies of this type participate in the preservation of the integrity of the oral surfaces (enamel and mucous membrane). Through a restriction of microbial adhesion, they become a part of the first defence line of the host. S-IgA plays an important role in the oral homeostasis [23,24,25,26]. It is an indicator of the adaptive local immunity of the mouth. It depends on the condition of the immune system of the host as well as on the rich oral antigenic potential. S-IgA acts synergetically with other antibacterial factors such as lysosym, lactopherin, salivary peroxidase, mucine, etc., thus preventing the penetration of antigens through the oral mucous membrane [27,28,22].

A previous evaluation of risk factors in the oral environment of children with different diseases assessed oral secretory adaptive immunity [23,24]. The differences in the average values of S-IgA in the different groups of children suggested that secretory immunity is stimulated mostly by local immunogenic factors and immunopathological processes (such as asthma) and is not influenced by systemic diseases such as diabetes [28].

However, this area of research is complex. Against this background, the current study investigated relationships and dependencies of S-IgA with the other markers of the oral risk environment in childhood.

Aim

The aim of this study was to determine the dependency of S-IgA on the following risk markers of the oral environment: quality of the saliva, dental biofilm, oral candida and *Lactobacillus spp.* in children.

Within this aim, the study had the following objectives:

1. To examine the concentration of the S-IgA in the saliva of children.

2. To examine the interdependence between the concentration of S-IgA and the qualities of the saliva (salivary flow, pH, buffering capacity).

3. To examine the interdependence between the S-IgA and the dental biofilm.

4. To examine the interdependence between the S-IgA and the microbial markers for oral eubiosis *(Candida* and *Lactobacillus spp.*).

Methods

1. Study population

The study population consisted of 116 children (7-17 years of age), who were divided into two groups. Children in the first group had either Type 1 diabetes (30 children, average age 12.20±3.53 years) or bronchial asthma (25 children, average age 8.84±3.02 years). Those in the second group were either systemically healthy (34 children, average age 10.47±2.75 years) or were systemically healthy but had worn a removable orthodontic appliance for the previous two years (27 children, average age 11.07±1.27 years). The children were recruited from the different children's clinics within the Department of Paediatric Medicine, Medical University of Sofia, and from the Department of Paediatric Dentistry, Faculty of Dental Medicine, Medical University of Sofia.

Ethical approval for the study was obtained from the ethics committee of the 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.

For each parameter examined (salivary flow, pH, buffering capacity, Simplified Oral Hygiene Index (OHI-S), oral *Candida* and *Lactobacillus spp.)* the children were divided into two subgroups: one with low and the other with high values of the corresponding parameter.

2. Evaluation and recording of the oral risk environment of the children

An evaluation of the oral medium of the children was made. Data concerning the risk oral profile and status were recorded in the following order:

- Caries risk assessment as described in a previous paper [18].

- Evaluation of the dental biofilm using the Simplified Oral Hygiene Index (OHI-S) [29].

- Evaluation of the qualities of the saliva by means 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.

- Microbiological examination of the oral microorganisms serving as markers *(Candida* and *Lactobacillus spp.*).

- Quantitative determination of S-IgA in the saliva by means of the ELISA (Enzyme-Linked ImmunoSorbant Assay) method with a salivary S-IgA kit (Salimetrics, State College, PA, USA).

3. Examination of the saliva

3.1. Examination of stimulated salivation.

The stimulated salivary flow rate was determined after stimulation with chewing gum. The saliva secreted for five minutes was collected in a graduated (from 0 to 5 ml) container. The children were divided into two groups:

(1) With normal salivary flow rate: >5 ml-3.5 ml/5 min (0.7-1.0 ml/min).

(2) With weak salivary flow rate: <3.5 ml/5 min (below 0.7 ml/min).

3.2. Examination of the pH of the saliva.

A litmus paper strip was put under the tongue for a couple of seconds. The pH of the saliva was assessed as:

(1) When the litmus paper strip turned green: normal saliva.

(2) When the litmus paper strip turned yellow or red: acidic saliva.

3.3. Examination of the buffering capacity.

As detailed in the manufacturer's instructions, colour change was evaluated from the three tampons of the test-strips sprinkled with saliva. For each change of colour, according to the instructions for the test, a certain number of points were scored. The sum total of these points showed the buffering capacity of the saliva sample. The evaluation was made in accordance with the following scale: (1) 0-9 points: low buffering capacity; (2) 10-12 points: normal buffering capacity.

4. Microbiological methods

For the microbiological examination, saliva was taken from the mouths of the children as well as a smear by means of a sterile tampon. In order to ensure a high microbial count, the procedure was performed in the morning when the children had an empty stomach and before the teeth were brushed. The quantitative determination of candida was carried out through the stroke method of Gould [36]. After a 48-hour cultivation at 35°C, the resulting colonies were counted by means of standard tables and a microbial score was registered. For yeast identification, BBL™ CHROMagar™ candida and API candida were used.

On removal from the mouth, the specimens for *Lactobacillus spp.* identification were placed immediately in a special solution: RTF (reduced transport fluid). Cultures were grown in an LBS (Lactobacillus Selection) Agar environment and were cultivated in aerobic and anaerobic conditions for two days. From the colonies that emerged, a preparation was produced for Gram staining. The microbial score was determined by means of the stroke method as employed with candida. For a positive result, we accepted the highest microbial score arising from the results from the two cultures (aerobic and anaerobic).

From the results of the microbial cultures, the children were classified as either:

(1) With *Candidia* or *Lactobacillus spp.:* with a microbial score over 10^4 cells/ml.

(2) Without *Candidia* or *Lactobacillus spp.:* with a microbial score below 10^4 cells/ml.

5. The ELISA method of examining the S-IgA in the saliva

The ELISA method for detecting S-IgA in saliva [27] was used to quantify S-IgA in the saliva. A salivary S-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.

6. 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 of correlation. 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.

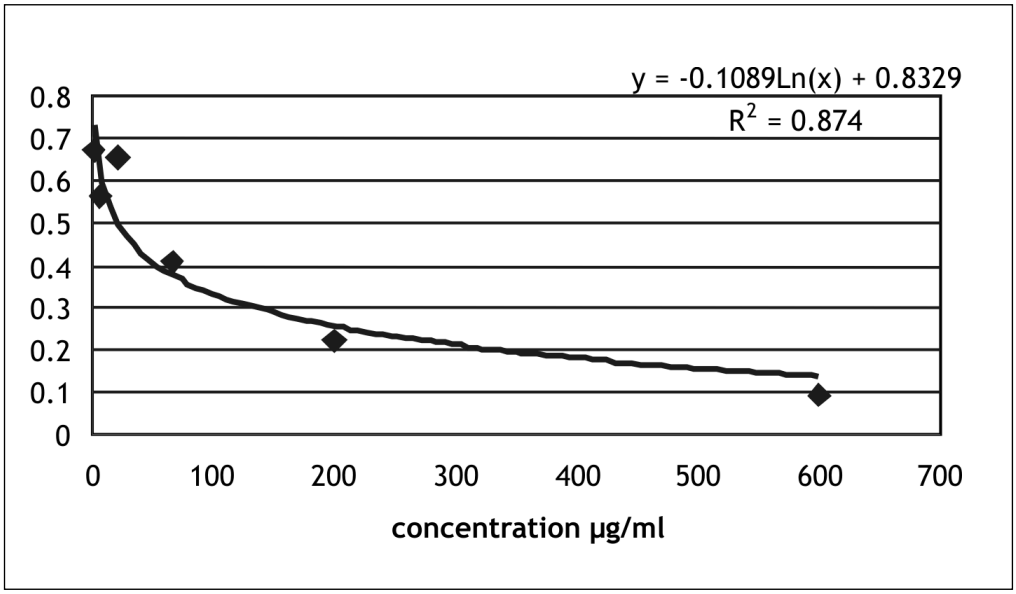

Figure 1. *Standard curve for concentrations of S-IgA in µg/ml.*

Results

1. Concentration of S-IgA in the saliva of children

The average values of the S-IgA antibodies in mixed saliva in the children studied were 154.60±135.34 µg/ml. The differences in S-IgA in the groups of children with different diseases have been presented in a previous publication [28].

2. Concentration of S-IgA and the qualities of the saliva (salivation, pH, buffering capacity) in children

2.1. Dependence of S-IgA on the stimulated salivary flow rate. *Table 1* gives the average values of S-IgA in the stimulated saliva. In children with normal salivation, the amount of S-IgA in the saliva was 161.85±135.44 µg/ml, whereas in children with reduced salivation, it was 150.16±136.04 µg/ml. The difference was not statistically significant (P>0.05), supporting the conclusion that the speed of salivation is not of importance for the secretion of S-IgA in the children's mouths.

2.2. Dependence of S-IgA on pH and the buffering capacity of the saliva. Normal pH of mixed saliva is considered to be a pH of around neutral (6.8-7.2), a level which is supported by different buffering systems. The acidification of saliva during eating or for other reasons relating to a deficit in the buffering capacity of saliva (such as a higher quantity of glucose in the saliva in diabetes, reduced salivary clearance when using local corticosteroids) is a precondition for the stimulation of acidogenic–cariogenic oral microorganisms.

Comparing the average values of S-IgA in the groups of children with normal and acidic pH, lack of a statistically significant difference was observed (P>0.05). The results are shown in *Table 2.* These results suggest the lack of any impact on the part of the physical and chemical qualities of the saliva on secretory oral immunity.

The buffering capacity of saliva also had no effect on secretory oral immunity. The difference between the average values of the S-IgA in the group with normal buffering capacity and the group with reduced buffering capacity was not statistically significant (P>0.05). The data are shown in *Table 3.*

On the other hand, the buffering capacity correlated with the pH of the saliva—Pearson's Correlation Index = 0.045 (P<0.05)—suggesting that both parameters do not affect the quantity of S-IgA in the mouth.

3. Interdependence between S-IgA and the dental biofilm

The concentration of S-IgA in the saliva and the quantity of dental biofilm, assessed using the OHI-S [29], is shown in *Table 4.* In children with a high OHI-S score, which is an indication of bad oral hygiene, the S-IgA antibodies were within the limits of the norm (138.67±138.68 µg/ml). The quantity of such antibodies was lower when the accumulation of biofilm was smaller (160.60± 141.71 µg/ml). Nevertheless, the difference was not statistically significant (P>0.05).

Table 1. *Stimulated Saliva and S-IgA in the Children Examined*

Stimulated salivation	**Children**	**S-IgA µg/ml**		
	(n)	**X**	**±SD**	**Δ**
Normal salivation	44	161.85	135.44	20.41
Reduced salivation	72	150.16	136.04	16.03
T P	T=0.450	P=0.654	(*P*>0.0)	

X=mean; SD=standard deviation; Δ=standard error; T=result of *t*-test; P=Pearson's coefficient of correlation

Table 2. *pH of Saliva and S-IgA in the Children Examined*

pH of saliva	**Children**	**S-IgA µg/ml**		
	(n)	**X**	**±SD**	**Δ**
Norm (pH6.8-7.2)	44	147.43	132.04	19.90
Acidic (pH5.6-6.8)	72	135.12	138.03	16.26
T P	T=-0.468	P=0.641	(P>0.05)	

X=mean; SD=standard deviation; Δ=standard error; T=result of *t*-test; P=Pearson's coefficient of correlation

Table 3. *Buffer Capacity of Saliva and S-IgA in the Children Examined*

Buffer capacity	**Children**	**S-IgA µg/ml**		
	(n)	**X**	**±SD**	**Δ**
Norm	21	143.64	112.07	24.45
Reduced	95	157.70	140.37	14.40
T P	T=0.409	P=0.684	(P>0.05)	

X=mean; SD=standard deviation; Δ=standard error; T=result of *t*-test; P=Pearson's coefficient of correlation

Table 4. *Dental Biofilm and S-IgA in the Children Examined*

Oral hygiene	**Children**	**S-IgA µg/ml**		
	(n)	**X**	**±SD**	**Δ**
Good (OHI-S=0-2)	47	160.60	141.71	20.67
Bad (OHI-S=2-3)	45	138.67	138.68	19.92
T P	T=0.763	P=0.448	(P>0.05)	

X=mean; SD=standard deviation; Δ=standard error; T=result of *t*-test; P=Pearson's coefficient of correlation

4. Dependence between S-IgA and the oral microorganisms (MO), indicative of oral microbial homeostasis

The results are shown in *Table 5.* The correlation between S-IgA and *Lactobacillus spp.* in the mouth cavity is shown in *Table 6.*

There was no statistically significant difference between the average values of S-IgA in children with and without isolated *Lactobacillus spp.,* suggesting the lack of a link between the secretionary oral immunity and the *Lactobacillus spp.* in the mouth cavity.

Table 5. *Oral Candida and S-IgA in the Children Examined*

Candida	**Children**	**S-IgA µg/ml**		
	(n)	**X**	**±SD**	**Δ**
Without candida	72	145.99	124.43	14.66
With candida	44	168.67	168.67	22.91
T P	T=-0.875	P=0.384	(P>0.05)	

X=mean; SD=standard deviation; Δ=standard error; T=result of *t*-test; P=Pearson's coefficient of correlation

Table 6. *Lactobacillus spp. and S-IgA in the Children Examined*

***Lactobacillus* spp.**	**Children**	**S-IgA µg/ml**		
	(n)	**X**	**±SD**	**Δ**
Without Lactobacillus spp.	83	145.99	140.97	15.47
With Lactobacillus spp.	33	168.67	116.38	20.25
T P	T=1.551	P=0.125	(P>0.05)	

X=mean; SD=standard deviation; Δ=standard error; T=result of *t*-test; P=Pearson's coefficient of correlation

Discussion

In the relevant literature, there are no unified reference values for S-IgA in the saliva of children. This is due to the differences in the methodologies used, the absence of standards concerning the collection of material (saliva), and the use of different units of measurement [29,30,31,32]. In the current study, the average value of the S-IgA antibodies in mixed saliva in the children was 154.60±135.34 µg/ml. The average values of the S-IgA in the saliva given by Salimetrics (the company that provided the ELISA-test) for adults is 367 µg/ml; the corresponding values in children (according to the firm itself) are much lower [33]. According to other authors, in children over 16 years the relevant value of S-IgA is 102-471 µg/ml [30]. The value we arrived at (154.60 µg/ml) was within the limits cited in previous studies; the deviations from the average values were relatively large (±135.34 µg/ml). This was possibly due to the fact that although some of the children studied were healthy, others had different diseases and conditions, which probably affected their oral immune response.

Oral health is sustained by means of complex processes of interaction between different elements in the oral environment, which ensure the necessary homeostasis. One of the most important factors is saliva. The role of saliva in the development of oral pathology is mainly a defensive one—it contains buffers which maintain the oral environment at around neutral pH, directly counter caries attack via the dental biofilm, and indirectly affect the formation of the subgingival biofilm, connected with periodontal pathology. The physical and chemical qualities of the saliva (salivary flow rate, pH, buffering capacity) have certain individual specifics determined by the genotype of the person concerned. They also change in accordance with the type of the food, any medicines taken and any systemic diseases from which the individual suffers, as well as with local factors. As a result of this the defence qualities of the saliva change [18].

In the current study, there was no correlation between the S-IgA in the mouth and the physical and chemical qualities of the saliva. Likewise, there was no correlation between the S-IgA antibodies in the mouth and the pH of the saliva and its buffering capacity. Because the children examined had different illnesses and conditions that affected the qualities of the saliva [28], the lack of dependence on the S-IgA antibodies indicated the relatively autonomous character of these antibodies associated with immunogenic stimuli of a mainly local character. This was borne out by the finding that the S-IgA increased considerably in children with removable orthodontic appliances, confirming the role of the local factors with regard to the secretory oral immunity [28].

The dental biofilm is the basic aetiological factor for dental caries and periodontal diseases both in children and in adults. It has a three-dimensional structure with specific ecological, physiological, and biochemical characteristics and represents an accumulation of microorganisms situated in a polysaccharide matrix and attached (through adhesion) to a tooth surface. Selective connection of the plaque microorganisms with S-IgA prevents the colonisation of the enamel surface by the microorganisms. The antibodies counteract all the other mechanisms of microbial adhesion and prevent the formation of dental biofilm. S-IgA connects mostly with *Streptococcus mutans* and antigens of its enzymes and metabolic products. However, the seemingly large prophylactic effect of S-IgA thus described is not actually so large. The real oral biological environment is not that efficient because of the constant detergent effect of the saliva and the impossibility of maintaining sufficient concentration of S-IgA on the enamel surface [34,35].

Our results, which suggest that the qualities of S-IgA-antibodies do not affect the qualities of the dental biofilm, support such a conclusion. This view is also supported by a number of contradictory studies, which sought to establish the potential of secretory immunity so that it could be used for

inhibiting the formation of plaque and thus secure a method for immunoprophylaxis against dental caries and periodontal diseases.

In the oral environment, there are microorganisms from the resident microflora such as oral candida that are potentially pathogenic. They can stimulate an immune reaction in the mouth. In immunocompromised individuals they can cause opportunistic infections (oral candidosis). The correlations demonstrated in previous studies suggested that oral candida could be used as a marker to show the influence of the general immunologic status of the patient on the sustenance or disturbance of the oral eubiosis [17,19].

As an opportunistic pathogen, candida is strongly affected by the immune response. That is why we compared the average values of S-IgA in the saliva of children with and without oral candida. There was no statistically significant correlation between S-IgA in in the two groups of children. This implies that no correlation was found between the oral candida and secretory immunity in the mouth. Such a connection of candida with the secretory antibodies is often commented on in a contradictory way in the relevant literature [26,22].

Our previous research on the oral lactobacilli showed that these microorganisms can also be used as a marker of risk dental caries [28]. Unlike the case of candida, though, they correlate with local factors such as acidity, carbohydratic clearance and dental caries.

In summary this study aimed at finding a correlation between S-IgA and different microbial factors (biofilm, *Candida* and *Lactobacillus spp.)*. The conclusion can be reached that the oral microflora was not influenced by the secretory antibodies in the oral cavity. This conclusion supports the finding of increased resistance of the resident oral microflora towards the local defence factors in the mouth. The secretory antibodies are one of the most important indicators of an acquired local immunity. It is assumed that the resistance of the oral microflora as an antigenic stimulus for S-IgA is due to its reduced activity in causing immune responses. It is supposed that the larger part of the oral microorganisms are not immunogenic for the macroorganism concerned because during the long period of evolutionary adaptation they live in a symbiosis with its oral medium. The mechanisms of oral tolerance in the mouth are also of importance for the suppression of the antigenic stimulus for secretion of the S-IgA [24,25].

Conclusions

The results of the present study suggest the following conclusions:

1. The salivary flow rate, the pH, and the buffering capacity of the saliva were of no importance for the secretion the S-IgA antibodies in the oral medium in children studied.

2. The secretory oral immunity was not dependent on the oral microflora (biofilm and microbial markers) in children studied.

Even though, in terms of classical immune reactions the defence potential of S-IgA is restricted, it may be that these reactions could be used as diagnostic indicators for an adaptive oral immunity of the saliva in children with different oral pathologies.

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