

SECRETORY IMMUNOGLOBULIN A (SIGA) AND PERIODONTAL STATUS IN CHILDREN WITH DISEASES AND CONDITIONS INFLUENCING THE ORAL ENVIRONMENT

Rashkova M.*, Baleva M.**, Toneva N.*, Jegova G.*

* Department of Paediatric Dentistry, Faculty of Dental Medicine, MU - Sofia

** Department of allergology, Laboratory of clinical immunology, MU - Sofia

SUMMARY

The secretory IgA-antibodies play an important role in the oral homeostasis. They are an indicator of the adaptive immunity in the mouth and influence the oral pathology by interaction with the oral microorganisms. It is the purpose of the present study to evaluate SIgA and the connection of those antibodies with the periodontal status of children with different diseases and conditions influencing their oral medium. The study was done with 116 children with diabetes, asthma, orthodontic treatment and healthy controls. The following methods were used: (1) ELISA with "Salivary secretory IgA kit" of Salimetrics LLC-USA was used to determine quantitatively the SIgA in the saliva; (2) evaluation of the oral risk medium and periodontal status (PSR, PBI, OHI) of the children studied. The results show that plaque-induced gingivitis is observed in 50% of the children with diabetes and 30% of the children with orthodontic treatment. The gingivitis of healthy children and children with asthma is considerably lower (10%). The gingival diseases observed vary. The indexes used, (Papilla Bleeding Index(PBI) and Periodontal Screening and Recording Index (PSR), confirm the extent of inflammation of children studied and also confirm the clinical diagnosis made. SIgA of children with gingivitis does not differ statistically from the SIgA of children without gingivitis. This is a confirmation of the opinion of the weak influence of the secretory immunity on the periodontal health.

Key words: secretory IgA-antibodies, oral homeostasis, diabetes, asthma, orthodontic treatment, periodontal status, gingival diseases, Papilla Bleeding Index(PBI), Periodontal Screening and Recording Index (PSR).

INTRODUCTION:

The oral environment is formed as a result of the complex interrelations between the saliva, the microorganisms, the defence mechanisms, the system factors and the external environment. The mouth is the gate for many external pathogenic factors, causing both a local and a general pathology(2, 12, 9).

The Secretory IgA- antibodies (SIgA) play an

important role in preserving the oral homeostasis. They certify the adaptive immunity of the mouth and affect the oral pathology by interacting with microorganisms from the dental biofilm and microorganisms colonizing the oral mucous membrane(3, 4, 5).

SIgA do not enter the gingival sulcus. Thus, they cannot control the subgingival biofilm. Nevertheless, it is possible that SIgA antibodies modulate the accumulation of supragingival biofilm and by so controlling the formation and structure of plaque-biofilm. Inflamed gingiva is more permeable due to higher permeability of blood vessels. IgA as well as other antibodies (IgG, IgM, IgD) are found in larger amounts in gingival sulcus(6, 10).

The aim of this research is understanding the meaning of SIgA-antibodies in the complex periodontal pathology in children with different diseases and conditions influencing the oral medium.

Tasks:

1. Quantitative assessment of SIgA in children with different diseases and conditions influencing the oral medium.
2. Examining periodontal status in children with different diseases and conditions influencing the oral medium.
3. Examining the dependency of SIgA of and periodontal status in children with different diseases and conditions influencing the oral medium.

MATERIALS AND METHODS

The study was performed on **116 children**:

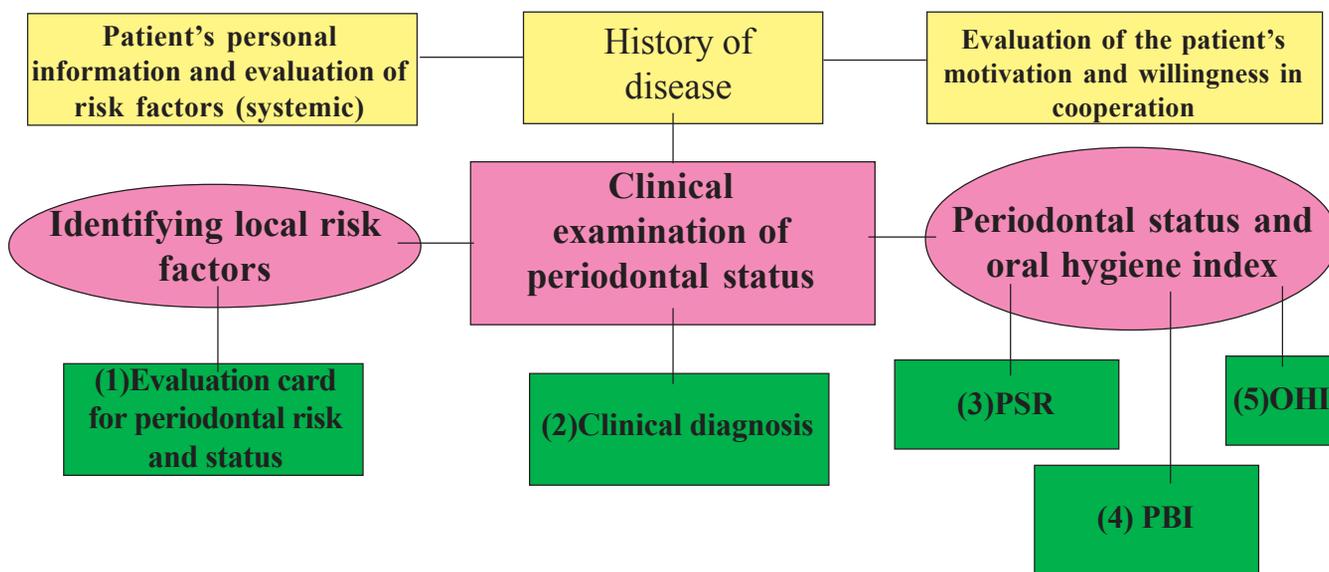
- 30 diabetics children (av. age $12,2 \pm 3,53$ years old);
- 25 children suffering asthma (av. age $8,84 \pm 3,02$ years old);
- 34 healthy children (av. age $10,47 \pm 2,75$ years old);
- 27 undergoing orthodontic treatment (av. age $11,04 \pm 1,26$ years old);

The selected groups of children are suitable for this study because in the three of them we can expect a change in the oral immunity. In **diabetic children** this change is in relation to the main disease; in **asthmatic children**- allergic

terrain and local influence of the inhalatory corticosteroids; the local irritating effect of the plastic in the **orthodontic appliances** may modify the oral medium.

1. Clinical examination and evaluation of periodontal status of examined children was made according to the following algorithm:

Fig. 1. Periodontal status examination algorithm and evaluation of the risky oral medium in children



The following indexes were used:

(3) PSR – Periodontal Screening and Recording Index(ADA) - Probing is made in 6 locations of every representative tooth(16,11,26,36,31,46). In 7- 12 year old children we use values in the range 0- 2(7,11).

(4) PBI - Papilla Bleeding Index (modified) - Probing is made in the gingival sulcus. We watch for provoked bleeding. The index is calculated in % .Code (-)no bleeding; (+)-there is bleeding(1,7).

(5) OHI Silness & Loe for calculating the quantity of the oral plaque biofilm(8).

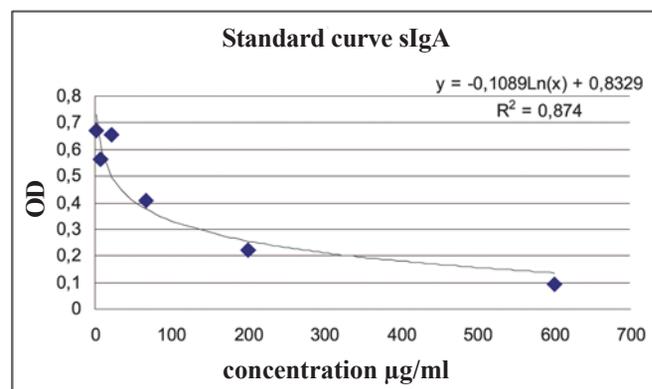
2. The ELISA method of examining the SIgA in the saliva(13):

The sample was taken in the morning on an empty stomach after the salivation has been stimulated for five minutes by chewing standard indifferent chewing gum (from the test for saliva “Saliva check” - GC). After that the saliva was collected in a plastic container. From it by means of a dropper a certain amount was taken that was then frozen in a refrigerator (- 10°C).

For the quantitative determination of the SIgA in the saliva we employed the ELISA method with „Salivary secretory IgA KIT” of Salimetrics_{LLC} - USA. The method is indirect, a constant quantity of goat anti-human SIgA conjugated with horseradish peroxidase(HRP) being used. The antibody-conjugate is added in test-tubes with specific dilutions of standards or samples from the saliva studied. From each sample sprinkling is made in separate small holes with human SIgA grouped on the same plaque. The

quantities of free antibodies are in reverse proportion to the quantity of SIgA. An enzyme reaction is evoked, which causes blue colouring that changes into yellow after the end of the reaction. By means of standard registration as per ELISA the optical thickness is measured with a length of the wave 450nm and 650 nm, the average value being taken into account. After certain calculations (described by the firm producer of the test) a standard curve is built from the values of the six controls and the standards. By means of the equation composed on the basis of the curve the concentrations of SIgA are calculated in mg/ml.

Fig. 2. Standard curve- result of the examination

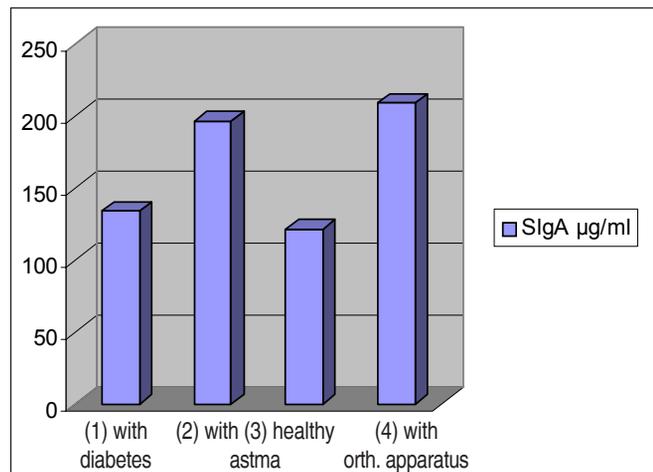


Statistic processing is made with the help of a computer program SPSS 16.

RESULTS AND DISCUSSION

1. Average values of SIgA in the three groups in comparison to the healthy children group are shown in the following diagram:

Fig. 3. Average values of IgA-S in saliva of examined children ($\mu\text{g/ml}$)



The average values of SIgA in healthy children's saliva and diabetic children are as follows: $121,34 \pm 15,027 \mu\text{g/ml}$ and $133,953 \pm 160,504 \mu\text{g/ml}$. Those values are lower than the average in asthmatic children ($196,456 \pm 145,308 \mu\text{g/ml}$) and those with orthodontic appliances ($208,874 \pm 125,953 \mu\text{g/ml}$). To draw a conclusion we can say that secretory immunity is stimulated in greater aspect by local immune factors and is less influenced by systemic diseases.

2. Evaluating periodontal status in examined children

2.1. PSR evaluation:

Results are shown in the next table № 1.

Tabl. 1. Evaluating periodontal status in examined children with PSR

Groups of children	n	PSR		
		0	1	2
(1) Diabetes	30	13	7	10
(2) Asthma	25	21	4	0
(3) Healthy	34	30	4	0
(4) Orthodontic treatment	27	23	3	4

Fig. 4. K. D. 14 years old, with diabetes



We noticed that in 13 (56,7%) of the children with diabetes index values of 1 and 2 are found. This suggests mild and average forms of gingival inflammation. Only in 4 of the asthmatic children and the healthy ones the PSR index shows 1 (mild gingival inflammation). In the last group (orthodontic treatment group) 7 children suffer gingivitis and half of them show PSR value 2. PSR values 3 or 4 were not found in the examined children.

2.2. Evaluating papilla bleeding using PBI:

Results are shown in the next table № 2.

Tabl. 2. Evaluating papilla bleeding using PBI (%)

Groups of children	n	Average% papillae	$\pm\text{SD}$	Δ	min- max %
(1)Diabetes	30	31,19	$\pm 37,99$	6,93	0- 100
(2)Asthma	25	1,71	$\pm 5,92$	1,18	0 - 21,41
(3)Healthy	34	2,29	$\pm 6,57$	1,12	0 - 25
(4)Orthodontic treatment	27	7,66	$\pm 12,19$	3,34	0 - 42,85
T P	$T_{1,3} = 4,367$ $P < 0,05$	$T_{1,4} = 3,076$ $P < 0,05$	$T_{3,4} = -2,202$ $P < 0,05$		

Fig. 5. K.D. 14 years old,with diabetes



The highest percent of bleeding papillae is again in the group of the diabetic children. 1/3 of the papillae are inflamed in a matter that provokes bleeding during probe examination. Gingival inflammation is generalized in this group. Affected papillae are between 0- 100%. This may be influenced by the systemic disease. In the rest of the groups the gingival inflammation takes up to 42,85% of the papillae due to local irritating factors.

Fig. 6. M.G.bad oral hygiene and orthodontic treatment



2.3. Evaluation of the periodontal status according to the diagnosis. Chronic plaque induced gingivitis were found in part of the children. In those with diabetes they were modified by the main disease. No periodontal lesions were found during the examination. The next table shows the results from the gingival status examination:

Tabl. 3. Frequency and influence of gingivitis in children according to the diagnosis

Gingivitis	1. Diabetes		2. Asthma		3. Healthy		4. Orthodontic treatment	
	n	%	n	%	n	%	n	%
with gingivitis	19	63,33	4	16,00	4	11,76	7	25,9
without gingivitis	11	36,66	21	84,00	30	88,25	16	74,1
Total	30	100	25	100	34	100	27	100
χ^2 P	$\chi^2_{1,3}=21,457$ (P<0,05)				$\chi^2_{2,3}=0,220$ (P>0,05)			
	$\chi^2_{3,4} = 10,597$ (P<0,05)							

Results show that in more of the diabetic children(63.33%) and in j of the orthodontically treated children there is plaque-induced gingivitis. The other two groups have a lower number of gingival inflammation (P < 0,05). There are different degrees of gingival inflammation. Diabetic children suffer the most severe degrees.

Used indexes (PBI and PSR) confirm clinical diagnosis

and disease degree.

3.The dependency of SIgA of oral hygiene and periodontal status in children with different diseases which change the oral medium

3.1. OHI S&L evaluation in examined children:

Results are shown in the next table.

Tabl. 4. OHI S&L in examined children

Groups of children	n	OHI S- L	±SD	Δ
(1)Diabetes	30	2,23	0,842	0,153
(2)Asthma	25	1,38	0,575	0,115
(3)Healthy	34	1,04	1,156	0,198
(4)Orthodontically treated	27	1,73	0,69	0,134
T P		$T_{3,4}=-2,72$ P=0,008*	$T_{2,3}=1,357$ P=0,180	$T_{1,2}=4,282$ P=0,0001*

Person correlation *P<0,05 **P<0,01	OHI/ SIgA PC=0,027	OHI/PSR PC=0,413**	OHI/PBI PC=0,459**	OHI/diagnosis PC=0,435**
---	-------------------------------------	-------------------------------------	-------------------------------------	---

More plaque and bad oral hygiene is seen in children with diabetes and orthodontic appliances.

There is no correlation between OHI and SIgA in examined children's saliva. This confirms the statement that SIgA cannot control subgingival plaque that is known to

be the the main risk factor for the registered periodontal pathology.

3.2. Evaluation of the correlation between SIgA and periodontal status of examined children. The study shows that there is no reliable difference between SIgA in children suffering gingivitis and the healthy ones.

Tabl. №5 SIgA in children with and without gingivitis

Children	n	SIgA (mean)	±SD	Δ
With gingivitis	78	154,02	131,61	14,90
Without gingivitis	38	155,78	144,51	23,44
T P				
Person correlation	T=0,948	P>0,05	PC=-0,033	

There is no correlation between SIgA and gingival status. This confirms the statement of weak influence by the secretory immunity on periodontal health in children.

range of standard and normal values. It is increased by local immunogenic stimuli. Orthodontic appliances are such stimuli;

2. Secretory immunity does not influence plaque accumulation and periodontal health in children.

CONCLUSIONS:

1. Secretory immunity (SIgA) in the mouth is in the

REFERENCES:

- Ainamo J & Bay, Problems and proposals for recording gingivitis and plaque. *Inter D J* 1975, 25, 229- 235.
- Bernimoulin JP. Recent concepts in plaque formation. *J Clin Peri-odontol* 2003; 30, (1), 7-9.
- Bokor-Bratiж M. Clinical significance of analysis of immunoglobulin A levels in saliva, *Med Pregl.* 53, (3-4), 164-8.
- Brandtzaeg P. Do salivary antibodies reliably reflect both mucosal and systemic immunity? *Ann N Y Acad Sci.* 2007, 1098, (3), 288-311.
- Brandtzaeg, P. Molecular and cellular aspects of the secretory immunoglobulin system. *Acta Pathol. Microbiol. Immunol. Scand.* 1995, 103, (1), 1-19.
- Childers, N. K., M. G. Bruce, and J. R. McGhee. Molecular mechanisms of immunoglobulin A defense. *Annu. Rev. Microbiol.* 1989, 43, (5), 503-536.
- Ciancio SG, Current status of indices of gingivitis. *J Clin Period* 1986, 13, 375-378.
- Fischman SL, Current status of indices of plaque. *J Clin Per*, 1986, 13, 371-374.
- Malamud Daniel. Salivary diagnostics The future is now. *J Am Dent Assoc.* 2006, 137, (3), 284-286.
- Nauntofte B, Tenevuo JO, Lagerluf F. Secretion and composition of saliva. In: Fejerskov O and Kidd E, eds. *Dental Caries. The disease and its clinical management.* Oxford. Blackwell Munksgard; 2003. p. 7-29.
- Piazzini L F, Periodontal screening & records (PSR) application in children and adolescent. *J Clin Ped Dent* 1994, 18(3)65-71.
- Rashkova M., M. Peneva, M. Baleva, N. Toneva, M. Belcheva, Study of oral biomarkers and candida in the oral ecosystem in childhood. *Project № 53/ 2007* supported by GRANT – Medical University, Sofia, 96p.
- www. Salimetrics.com- Salivary secretory IgA indirect enzyme immunoassay Kit Catalog) 1-1602.

Address for correspondence:

Maya Rashkova, Associate professor, DDS, Ph.D
Department of Pediatric Dentistry,
Faculty of Dental medicine, Medical University - Sofia,
1, G. Sofiyski Str., Sofia 1000, Bulgaria.
Mobile: 0359 888 215 033
e-mail: mayarashkova@mail.bg