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Salivary markers of oxidative stress and their relation to periodontal and dental status in children

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Abstract. *Background*: Previous studies have shown that salivary thiobarbituric acid reactive substances are related to the periodontal status in adults. Such an analysis has not been done on children yet. The aim of our study was to analyze salivary markers of oxidative stress in relation to periodontal and dental status in children.
Methods: The periodontal and dental status of 82 consecutive pediatric dental patients was assessed. The oral hygiene index (OHI), the papillary bleeding index (PBI) and the caries index (CI) were assessed as clinical parameters. Markers of oxidative stress and antioxidant status were measured in whole saliva samples.
Results: Multivariate analysis of covariance showed that the variability of PBI explains 10.9% of the variance of salivary thiobarbituric acid reacting substances (TBARS). Advanced oxidation protein products (AOPP) were related to CI (eta 8.6%). Measures of antioxidant status (total antioxidant capacity and ferric reducing ability of saliva) were partially determined by OHI (13.6% and 7.2%) and PBI (16.9% and 7.9%).
Conclusions: Antioxidant status in saliva is related to oral hygiene and periodontal status. Salivary TBARS are a potential sensitive marker of periodontitis in children, similarly to adults, at least on a population level. Salivary AOPP are related to caries. Potential diagnostic value of the analyzed markers should be analyzed in further interventional studies.

Keywords: Salivary TBARS, oxidative stress, saliva, periodontal status, dental caries

1. Introduction

Oxidative stress is a dysbalance between the production of free radicals and antioxidant status leading to oxidative damage of macromolecules including lipids and proteins. Markers of oxidative stress were found in saliva and were related to both, systemic and local oral diseases, the latter including inflammatory diseases such as gingivitis [1] and periodontitis [2], caries [3] and oral cancer [4]. The variability of the concentrations prevents the use in individual diagnostics, but the markers still have informational value on a population level [5]. This makes them useful in the investigation of the pathogenesis of oral diseases. The palette of oxidative stress markers is wide, but only few of them were studied in saliva and in relation to the status of oral tissues.

Thiobarbituric acid reacting substances (TBARS) are a marker of lipid peroxidation widely used in experimental research as well as in clinical studies. Although the specificity of the spectrophotometric or spectrofluorometric assay has been questioned in the past, TBARS are still measured, especially in studies focusing on inflammatory disorders [6,7]. TBARS are measurable in saliva with concentrations one to two logs lower than in

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Table 1
Characteristics of the study population

	girls ($n = 47$)	boys ($n = 35$)
Age (years)	14.1 ± 3.3	12.3 ± 3.8
OHI	0.62 ± 0.71	0.74 ± 0.71
PBI	0.47 ± 0.58	0.56 ± 0.70
CI	1.27 ± 0.84	1.24 ± 0.82

OHI – Oral Hygiene Index, PBI – Papillary Bleeding Index, CI – Caries Index.

plasma [5]. In contrast to other markers in saliva, salivary TBARS do not correlate with plasma levels [8]. The origin of salivary TBARS remains unknown, although a microbial production of free radicals might be responsible and was recently hypothesized as a cause of intraoral TBARS production [9].

In a previous clinical study we have shown that salivary TBARS are related to the periodontal status [8]. Although the association was relatively weak and the causality is far from being clear, salivary TBARS could prove useful in the research of periodontitis or in the monitoring of the disease. Oxidative stress might be both, a cause and a consequence of the periodontal inflammation. Biochemical detection of salivary TBARS could, thus, be used in the development of new therapies of periodontitis or in the non-invasive evaluation of its efficacy.

Saliva is a biological fluid with great potential in biomedical research, especially in dentistry [10]. The non-invasive sampling makes saliva particularly useful in the research on children, mentally disabled people or in experiments where repeated sampling is needed. Most of the studies focusing on salivary markers of oxidative stress were conducted in adult patients [2,11]. Whether the findings can be extrapolated to children is currently unknown, although previous studies found salivary measures of antioxidant status in relation to periodontal or dental status [12–15].

The aim of our study was to characterize salivary markers of oxidative stress in pediatric dental patients and their relation to clinical parameters of periodontal and dental status.

2. Methods

2.1. Subjects and sampling

Saliva samples were collected from children routinely examined in a dental ambulance ($n = 82$). The children were between 4 and 18 years old (13.4 ± 3.6 years). The basic characteristics of the patients according to their gender are summarized in Table 1. Whole saliva (2 ml) was collected by spitting into sterile tubes. Salivation was not stimulated. The duration of sampling varied. The final volume of 2 ml of liquid was achieved by all patients. The samples were stored frozen at -20°C. The samples were processed as soon as possible to minimize the effects of storage. AT least for salivary transcriptome the effect of storage temperature is minimal [16]. The clinical examination followed the sample collection. All procedures were carried out by the same dentist (V.C.) using standardized protocols with modified scoring systems. The oral health status of subjects was assessed using the modified oral hygiene index (OHI: 0 – no plaque present, 1 – plaque covers less than one third of tooth surface, 2 – plaque covers more than one third of tooth surface), papillary bleeding index (PBI: 0 – no bleeding on probing, 1 – subtle bleeding on probing, 2 – moderate to severe bleeding on probing) and caries index (CI: 0 – no caries, 1 – superficial lesion, 2 – lesion affecting the dentin). For all dental indices the highest score found was assign to the particular patient for further analysis. This research was approved by Ethical Committee of the Institute of Molecular Biomedicine, Comenius University in Bratislava, Slovakia. Parental informed written consent was obtained for all children before they were examined and samples collected.

2.2. Biochemical analysis

Whole saliva samples were centrifuged (10,000 $\times$g, 10 min, 4°C) to remove bacteria and cellular debris. Markers of oxidative stress analyzed in the samples included thiobarbituric acid reacting substances (TBARS), advanced oxidation protein products (AOPP) and advanced glycation end products (AGEs). Measures of antioxidant status assessed in this study were total antioxidant capacity (TAC) and ferric reducing activity of saliva (FRAS). Saphire II instrument (Tecan, Grödig, Austria) was used for all spectrophotometric and spectrofluorometric measurements. The protocols for the determination of the particular markers in saliva have been published previously [17]. Briefly, TBARS are measured at 553 nm after boiling with the thiobarbituric acid and derivatisation with n-butanol, AOPP after addition of glacial acetic acid at 340 nm, AGEs at the excitation wavelength of 370 nm and an emission wavelength of 430 nm. TAC was measured as TROLOX equivalents at 660 nm, FRAS after reaction with 2,4,6-tripyridyl-s-triazine in hydrochloric acid, acetate buffer and ferric chloride at 593 nm. As normal ranges for these markers have not been published yet, the results could not be used to discriminate healthy and diseased patients.

Table 2
General linear model analysis of the associations between clinical and biochemical parameters. Variance components are quantified as eta. The higher the eta value the higher the variance of the measured parameter explained by the corresponding independent factor. P value less than 0.05 indicates statistical significance

		TBARS	AOPP	AGEs	TAC	FRAS
Age						
	F	2,53	0,10	0,59	4,89	1,97
	p	0,12	0,75	0,45	0,03	0,17
	eta	4,8%	0,2%	1,2%	8,9%	3,8%
Gender						
	F	7,18	0,07	1,55	0,89	1,04
	p	0,01	0,79	0,22	0,35	0,31
	eta	12,5%	0,1%	3,0%	1,7%	2,0%
OHI						
	F	0,08	1,97	0,00	7,89	3,90
	p	0,78	0,17	0,95	0,01	0,05
	eta	0,2%	3,8%	0,0%	13,6%	7,2%
PBI						
	F	6,11	1,06	0,21	10,14	4,29
	p	0,02	0,31	0,65	0,00	0,04
	eta	10,9%	2,1%	0,4%	16,9%	7,9%
CI						
	F	0,34	4,70	1,60	0,12	1,83
	p	0,56	0,03	0,21	0,73	0,18
	eta	0,7%	8,6%	3,1%	0,2%	3,5%

OHI – Oral Hygiene Index, PBI – Papillary Bleeding Index, CI – Caries Index, TBARS – Thiobarbituric Acid Reacting Substances, AOPP – Advanced Oxidation Protein Products, AGEs – Advanced Glycation End Products, TAC – Total Antioxidant Capacity, FRAS – Ferric Reducing Ability of Saliva.

2.3. Statistical analysis

IBM SPSS 20.0 software and the general linear model command were used for the multivariate analysis of covariance. One-way ANOVA and Scheffe post hoc test were used to assess relationships between individual parameters. P-values less than 0.05 were considered significant. Data are presented as mean + standard deviation.

3. Results

The obtained data revealed that the model composed of age, gender and the analyzed clinical parameters describes 35.9% of the variance of TBARS, 13% for AOPP, 8.6% for AGEs, 20% for TAC and 14.5% for FRAS. The individual association between clinical and biochemical parameters are summarized in Table 2 showing eta values as a measure of the explained variability and tightness of the association, as well as p-values showing the statistical significance of the corresponding associations. Multivariate analysis using the general linear model showed that age is a significant variance component of TAC (eta 9.5%). Gender significantly effects salivary TBARS concentrations, with boys having higher TBARS by 35% in comparison to girls (eta 12.5%). OHI determines a significant portion of the variance of TAC and FRAS (eta 11.7% vs. 7.4%, respectively). The association between TAC and OHI was also confirmed using ANOVA ($F = 3.5$, $p = 0.035$) with significant differences according to post hoc Scheffe test between children with OHI score 1 and 2 ($p = 0.036$). PBI as a marker of periodontal health was a significant determinant of TAC (eta 16.9%) and FRAS (eta 7.9%). PBI was found to be a significant contributor to the variance of salivary TBARS (eta 10.9%). Children divided according to PBI differ in their salivary TBARS concentrations according to one way ANOVA ($F = 9.2$, $p < 0.001$). Post hoc Scheffe test shows significantly higher salivary TBARS in children with highest PBI in comparison to children with PBI score 0 ($p = 0.001$) and 1 ($p < 0.025$). CI as a marker of the dental status was found to be a significant determinant of salivary AOPP (eta 8.6%). Other associations were not significant (Fig. 1).

4. Discussion

Our results show that clinical parameters OHI and PBI are related to both analyzed markers of antioxida-

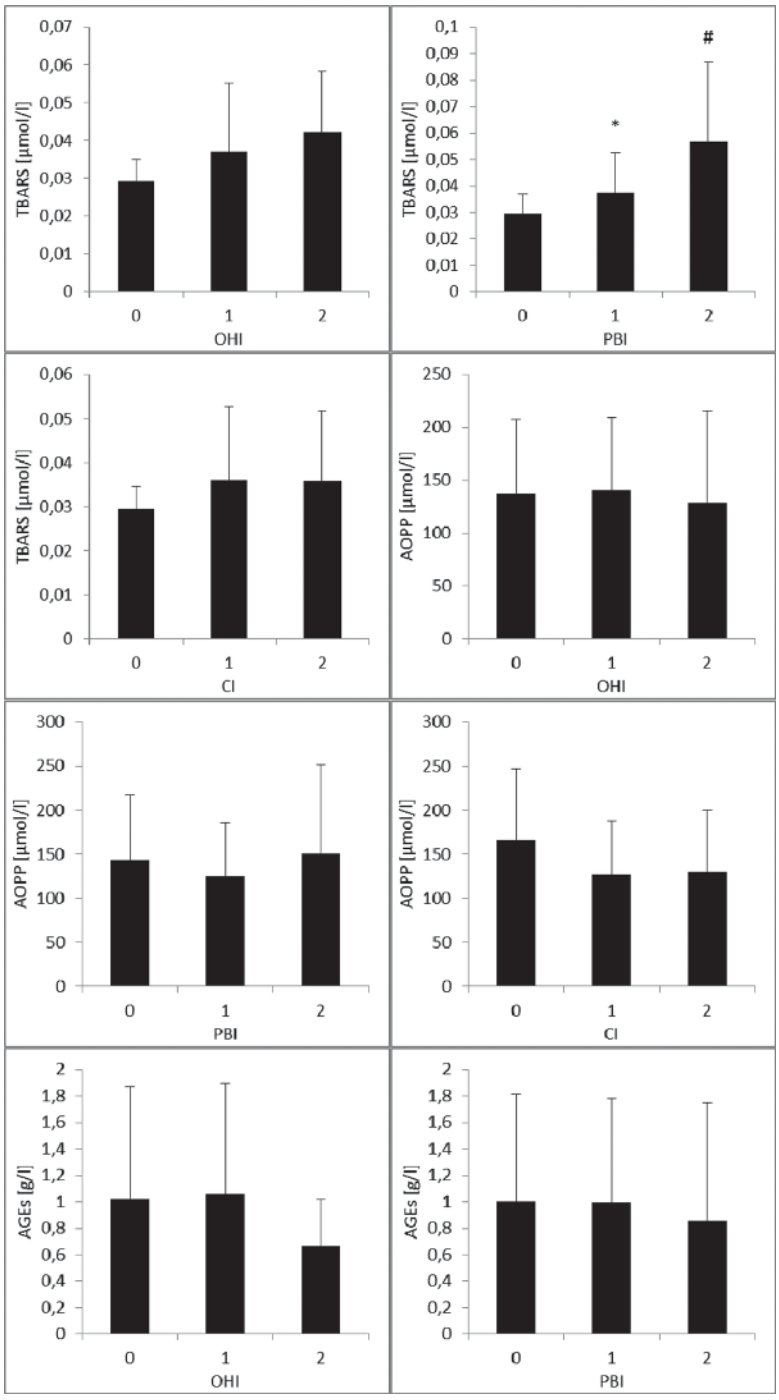

Fig. 1A. Associations between individual clinical and biochemical parameters. * $-$ $p < 0.05$ vs 0, # $-p < 0.05$ vs 1.

tive status – FRAS and TAC. In addition to markers of antioxidant status, PBI determines also a significant portion of the variance of salivary TBARS. This confirms previously published findings showing that salivary TBARS concentrations are tightly related to PBI in adults [8]. Whether this parameter could be used to discriminate between patients with bad oral hygiene with or without periodontitis requires further research. But more importantly, this finding supports the role of oxidative stress and specifically lipid peroxidation

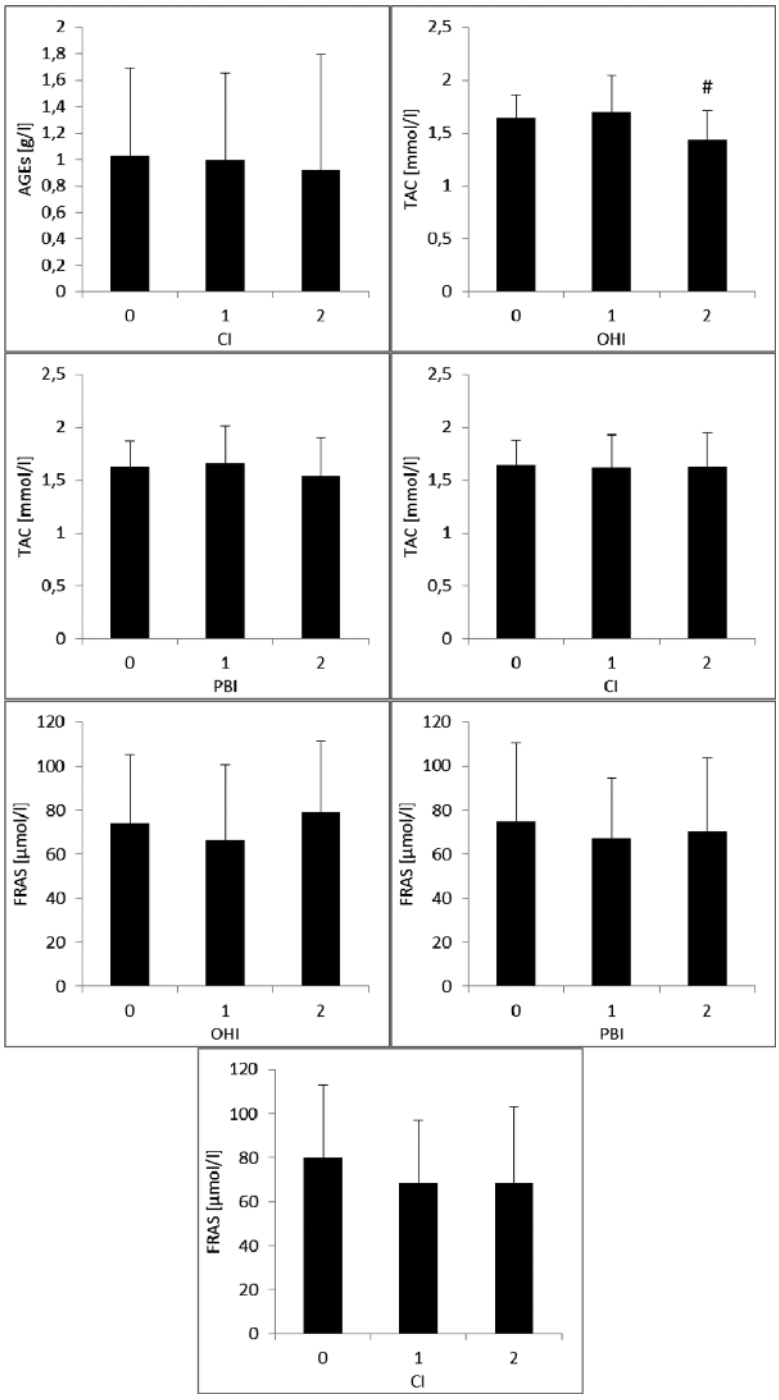

Fig. 1B. continued.

in periodontal diseases [18,19]. It also indicates the need for research on the origin of salivary TBARS, as it might shed light on the pathogenesis of periodontitis. The models for AOPP, AGEs and FRAS described less than 15% of their variability, which means that other factors not analyzed in the study might contribute to the variability. The search for these unknown factors should be the aim of further studies.

TBARS are a marker of lipid peroxidation and lipids, especially as part of the cell membrane are a major

component of the periodontal tissue. In contrast, dental tissues are beyond the anorganic salts composed to a major part of proteins and not lipids. In line with this, salivary TBARS are not associated with CI. The finding that AOPP – a marker of protein oxidation is related to CI according to the general linear model requires further research, as ANOVA revealed no significant individual association between CI and AOPP. Previous studies indicated that antioxidant status in saliva might be related to caries risk, especially in children [12,13, 15]. Protein oxidation and antioxidant status could be interconnected. Recently, it was hypothesized that AOPP might even be a part of the non-enzymatic antioxidant system [20]. Only interventional experiments will prove the role of AOPP in caries pathogenesis.

Assessment of oxidative stress markers in saliva has been shown to be particularly useful in ginigivitis and periodontitis [1,8,21]. However, most of the published results come from case control studies. Our present study has a cross-sectional design. This enables us to do statistical modeling that can shed light on the associations between clinical and biochemical parameters. In addition, our study is unique in the focus on children. According to our knowledge, this is the first study dealing with salivary oxidative stress markers in children. The association between oral health and salivary markers of oxidative stress might differ in children and adults due to differences in routine oral hygiene, differences in hormonal milieu and other physiological parameters. In comparison to our previously published similar study on adults no significant effect of age on salivary TBARS has been found. Of course, a long-term observational study analyzing the effects of aging would be valuable. The effect of aging on oxidative stress seems to be limited to adults. Salivary TBARS are also the only measured parameter that showed a gender difference with higher values in boys. This has already been shown by our previous research on adults [5].

Previous studies have found that salivary levels of immunoglobulins, especially immunoglobulin G against periodontal pathogens are associated with periodontitis [22]. Interestingly, patients with generalized aggressive periodontitis tend to have lower than normal levels of salivary immunoglobulins against periodontal pathogens [23]. We have not analyzed immunoglobulins in our samples. However, it would be interesting to see whether immunoglobulins are associated with oxidative stress markers and whether manipulation of the inflammatory status using antibiotics or immunomodulatory drugs affects salivary markers of oxidative and carbonyl stress.

In conclusion, our results show that at least in children particular salivary markers of oxidative stress are related to oral hygiene, periodontal status and dental status. The origin of salivary markers of oxidative stress and their potential role in the pathogenesis of oral diseases should be analyzed and proved in experimental studies.

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