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

L’ubomíra Tóthová, Viera Celecová and Peter Celec

Institute of Molecular Biomedicine, Comenius University, Bratislava, Slovakia
Private Stomatological Praxis, Krupina, Slovakia
Department of Molecular Biology, Comenius University, Bratislava, Slovakia
Institute of Pathophysiology, Comenius University, Bratislava, Slovakia

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...
Whether the oxidative stress were conducted in adult patients [2,11]. Most of the studies focusing on salivary markers of oxidative stress are conducted in experiments where repeated sampling is needed. In the research on children, mentally disabled people and patients in need of special care, non-invasive sampling makes saliva particularly useful in biomedical research, especially in dentistry [10]. The salivary flow is minimal [16]. The clinical examination of the periodontal status was performed 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 assigned 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. 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.

|          | 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.

### 2.2. Biochemical analysis

Whole saliva samples were centrifuged (10,000 × 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-triprydyl-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.

| parameter | 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.

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
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
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.

**Source of funding**

This study was sponsored by the Slovak research and development agency, grant VMSP-II-0027-09.

**Acknowledgments**

The authors would like to thank all the patients for participating in the study.

**References**

[1] Buduneli, N., et al., Effects of smoking and gingival inflammation on salivary antioxidant capacity. J Clin Periodontol, 2006. 33(3): p. 159-64.
[2] Guentsch, A., et al., Lipid peroxidation and antioxidant activity in saliva of periodontitis patients: effect of smoking and periodontal treatment. Clin Oral Investig, 2008. 12(4): p. 345-52.
[3] Kumar, D., R.K. Pandey, and D. Agrawal, An estimation and evaluation of total antioxidant capacity of saliva in children with severe early childhood caries. Int J Paediatr Dent, 2011. 21(6): p. 459-64.
[4] Bahar, G., et al., Salivary analysis in oral cancer patients: DNA and protein oxidation, reactive nitrogen species, and antioxidant profile. Cancer, 2007. 109(1): p. 54-9.
[5] Behuliak, M., et al., Variability of thiobarbituric acid reacting substances in saliva. Dis Markers, 2009. 26(2): p. 49-53.
[6] Kurutas, E.B., et al., Effects of antioxidant therapy on leukocyte myeloperoxidase and Cu/Zn-superoxide dismutase and plasma malondialdehyde levels in experimental colitis. Mediators of Inflammation, 2005(6): p. 390-394.
[7] Bernardi, F., et al., Oxidative stress and inflammatory markers in normal pregnancy and preeclampsia. Journal of Obstetrics and Gynaecology Research, 2008. 34(6): p. 948-951.
[8] Celec, P., et al., Salivary thiobarbituric acid reacting substances and malondialdehyde – their relationship to reported smoking and to periodontal status described by the papillary bleeding index. Dis Markers, 2005. 21(3): p. 133-7.
[9] Vlkova, B. and P.Celec, Does Enterococcus faecalis contribute to salivary thiobarbituric acid-reacting substances? In Vivo, 2009. 23(2): p. 343-5.
[10] Kaufman, E. and I.B. Lamster, The diagnostic applications of salivary – a review. Crit Rev Oral Biol Med, 2002. 13(2): p. 197-212.
[11] Wei, D., et al., Lipid peroxidation levels, total oxidant status and superoxide dismutase in serum, saliva and gingival crevicular fluid in chronic periodontitis patients before and after periodontal therapy. Aust Dent J, 2010. 55(1): p. 70-8.
[12] Dodwad, R., A.V. Betigeri, and B.P. Preeti, Estimation of total antioxidant capacity levels in saliva of caries-free and caries-active children. Contemp Clin Dent, 2011. 2(1): p. 17-20.

[13] Hegde, A.M., K. Rai, and V. Padmanabhan, Total antioxidant capacity of saliva and its relation with early childhood caries and rampant caries. J Clin Pediatr Dent, 2009. 33(3): p. 231-4.

[14] Tulunoglu, O., S. Demirtas, and I. Tulunoglu, Total antioxidant levels of saliva in children related to caries, age, and gender. Int J Paediatr Dent, 2006. 16(3): p. 186-91.

[15] Uberos, J., et al., Influence of the antioxidant content of saliva on dental caries in an at-risk community. Br Dent J, 2008. 205(2): p. E5.

[16] Lee, Y.H., et al., Direct Saliva Transcriptome Analysis. Clinical Chemistry, 2011. 57(9): p. 1295-1302.

[17] Celec, P., et al., Oxidative and carbonyl stress in patients with obstructive sleep apnea treated with continuous positive airway pressure. Sleep Breath, 2011.

[18] Masi, S., et al., Oxidative stress, chronic inflammation, and telomere length in patients with periodontitis. Free Radic Biol Med, 2011. 50(6): p. 730-5.

[19] D’Aiuto, F., et al., Oxidative stress, systemic inflammation, and severe periodontitis. J Dent Res, 2010. 89(11): p. 1241-6.

[20] Selmechi, L., Advanced oxidation protein products (AOPP): novel uremic toxins, or components of the non-enzymatic antioxidant system of the plasma proteome? Free Radic Res, 2011. 45(10): p. 1115-23.

[21] Sculley, D.V. and S.C. Langley-Evans, Periodontal disease is associated with lower antioxidant capacity in whole saliva and evidence of increased protein oxidation. Clin Sci (Lond), 2003. 105(2): p. 167-72.

[22] Takeuchi, Y., et al., Immunoglobulin G subclass antibody profiles in Porphyromonas gingivalis-associated aggressive and chronic periodontitis patients. Oral Microbiology and Immunology, 2006. 21(5): p. 314-318.

[23] Casarin, R.C.V., et al., Levels of Aggregatibacter actinomycescomitans, Porphyromonas gingivalis, inflammatory cytokines and species-specific immunoglobulin G in generalized aggressive and chronic periodontitis. Journal of Periodontal Research, 2010. 45(5): p. 635-642.