

# Salivary thiobarbituric acid reacting substances and malondialdehyde – Their relationship to reported smoking and to parodontal status described by the papillary bleeding index

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**Abstract.** *Background.* Thiobarbituric reacting substances (TBARS) are markers of lipoperoxidation. The best-known specific TBARS is malondialdehyde (MDA). Results from our previous studies have shown that TBARS can be measured in saliva and are increased in patients with gingivitis. Whether MDA is the main TBARS in saliva from patients with altered parodontal status is unknown.

*Aim.* To observe the relationship between the parodontal status and TBARS, MDA and the number of epithelial cells in saliva.

*Subjects & Methods.* In Study I saliva and plasma samples of 15 patients (8F, 7M) suffering from inflammatory periodontal diseases were gathered and TBARS levels were measured in these samples. In Study II saliva samples from 217 consecutive stomatologic patients were collected and analysed for TBARS spectrofluorometrically, MDA by high-performance liquid chromatography and epithelial cell count by light microscopy. Papillary bleeding index (PBI) was determined in standard stomatologic examination.

*Results.* In Study I results from our previous studies showing no correlation between salivary and plasma TBARS levels were confirmed. This indicates that the local salivary level of TBARS is unlikely to be directly affected by systemic oxidative stress. In Study II higher PBI was associated independently (adjusted for age and sex) tightly with higher TBARS ( $p < 0.001$ ) and with lower number of epithelial cells in saliva ( $p < 0.05$ ). Smokers had higher salivary MDA levels ( $p < 0.003$ ) and lower number of epithelial cells in saliva ( $p < 0.01$ ).

*Conclusion.* Salivary TBARS are a simple parameter that partially reflects the parodontal status with a potential usefulness in the clinical stomatology. We show herein that salivary MDA is dependent on age and smoking, but there is no correlation between MDA and PBI. Further studies should uncover the main salivary TBARS compound in patients with altered parodontal status and trace the origin of these salivary lipoperoxidation markers.

Keywords: Malondialdehyde, thiobarbituric acid reacting substances, oxidative stress, saliva, gingivitis

## 1. Introduction

Thiobarbituric acid reacting substances (TBARS) are produced during lipoperoxidation – oxidative stress-

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induced damage of lipids and are, thus, a widely used marker of oxidative stress [9,29]. However, they represent a heterogeneous group of compounds – best-known is malondialdehyde (MDA). MDA is a product of breaking long carbon strings of fatty acids. Some time ago TBARS were thought to be equal to MDA, however, since doubts were published regarding this equation [16], the term MDA should be only used in association with the use of high performance liquid chromatography (HPLC) and other specific measurement methods.

TBARS is associated with parodontopathies when measured directly in the injured gingival tissue [38]. In our previous studies we have shown that TBARS can be found in measurable concentrations in saliva and that these levels are higher in patients with parodontopathies and their origin is unlikely to be plasma [8, 24]. Whether the difference in patients is caused by a rise of MDA and which other factors influence salivary TBARS levels is unknown. The aim of this study was to uncover these questions and, moreover, to find out how tight is the correlation between salivary TBARS and the severity of the parodontopathy with regard to the potential clinical usefulness of this parameter.

## 2. Subjects & methods

### 2.1. Subjects & sampling

In Study I saliva and plasma samples of 15 patients (8 females and 7 males) with an average age of  $46.3 \pm 16.3$  yr. suffering from inflammatory periodontal diseases were gathered. In Study II saliva samples from 217 consecutive stomatologic patients (143 females and 74 males) with an average age of  $31.6 \pm 13.9$  yr. were collected. Samples in both studies were taken in the morning before eating or washing the teeth without the use of any stimulants [24]. Patients with known systemic diseases were excluded. Self-reported smoking (defined as current smoking of more than one cigarette per day) was involved in the analysis as a known producer of local oxidative stress. In study II patients underwent an examination of their parodontal status using an adapted Papillary Bleeding Index (PBI; with bleeding scores 0, 1, 2, 3; [11]).

### 2.2. TBARS determination

Collected plasma and saliva samples were frozen until measurement. Salivary and plasmatic TBARS

were gauged by spectrofluorometric method ( $\lambda_{ex.} = 515$  nm,  $\lambda_{em.} = 535$  nm) after derivatization with 0,67% thiobarbituric acid in acidic medium of phosphoric acid (100°C, 45 min.; [21]). After derivatization, the coloured product was extracted to n-butanol [22], centrifuged (3000 rpm, 10 min.) and measured against the standard (1,1,3,3-tetramethoxypropan). TBARS concentration was expressed in  $\mu\text{mol/l}$  on the basis of the calibration curve.

### 2.3. MDA determination

Salivary and plasmatic levels of MDA were determined by high performance liquid chromatography [5, 17]. Samples were prepared as for TBARS determination. As mobile phase was used methanol:buffer saline solution (2:3), which was prepared as follows: 120 ml of phosphate buffer saline was filled to 600 ml with distilled water and 400 ml of methanol was added [6]. Mobile phase was corrected to pH 6,3 by phosphoric acid. Flow rate was set to 0,75 ml/min using one 307 Piston pump model. Separations were made with a column Sepharon SGX C<sub>18</sub> 3 × 150 mm, 7  $\mu\text{m}$  particles (Tessek, Ltd., Czech republic). The effluent (100  $\mu\text{l}$ ) was monitored with LKB Broma 2151 photometer detector set to the wavelength of 532 nm. Retention time of MDA was about 4 minutes.

### 2.4. Epithelial cells count

Large whole round or oval shaped epithelial cells in 10  $\mu\text{l}$  of diluted saliva (200  $\mu\text{l}$  vortexed saliva without centrifugation and 800  $\mu\text{l}$  of distilled water) were counted. The examined volume of 10  $\mu\text{l}$  matches with one view field (10x objective and 15x ocular) in light microscopy. The examiner had no information about the reference of the samples.

### 2.5. Statistical analysis

Multifactorial ANOVA, independent t-test, standard nonparametric Wilcoxon test for two related samples and Pearson correlation analysis were used. Analysis of the results was performed with Microsoft Excel<sup>®</sup> software, XLStatistics 5.51 and SPSS 11.0.

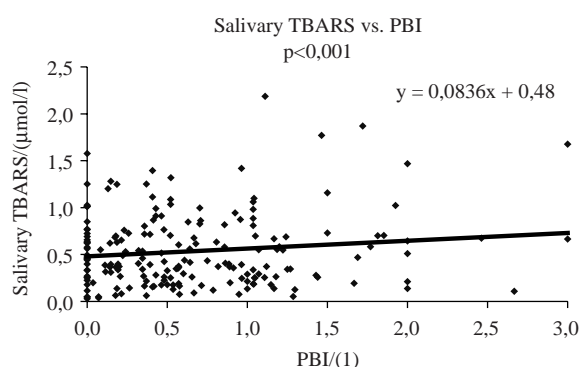


Fig. 1. Salivary TBARS correlate tightly with PBI. Patients with higher PBI had higher levels of salivary TBARS ( $p < 0.001$ ).

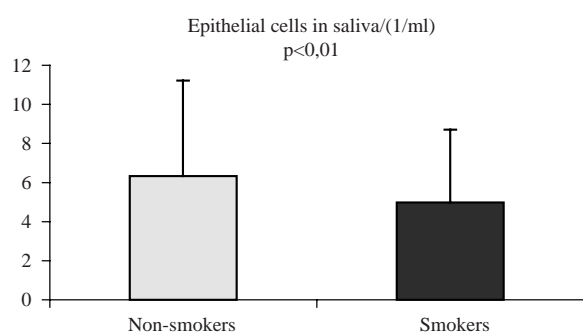


Fig. 2. Epithelial cells in saliva were higher in non-smokers than in smokers ( $p < 0.01$ ).

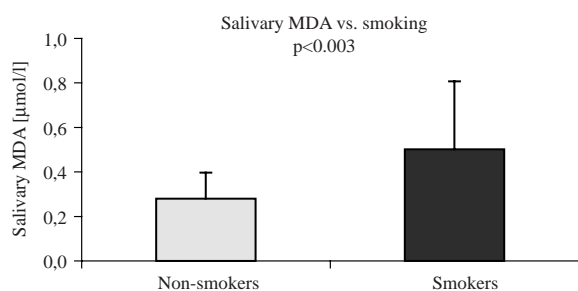


Fig. 3. Salivary MDA levels were higher in smokers than in non-smokers ( $p < 0.003$ ).

### 3. Results & discussion

No significant correlations were found in Study I between salivary and plasma TBARS in patients with parodontopathies ( $p = 0.95$ ) confirming our results from previous studies and indicating that an influence of plasma TBARS on salivary levels is unlikely. The origin of TBARS in saliva is still unknown [27], however, a local intraoral production is very probable [31]. This result also underlines that systemic oxidative stress

does not alter salivary TBARS directly [28,34,35]. We, thus, assume, that salivary TBARS may reflect the local oral oxidative stress, although the producer is still hidden [25]. Some indices point towards oral microbial flora as possible source or cause of oxidative stress [37]. Whether the overproduction of reactive oxygen species is involved in the pathogenesis of parodontopathies or it is just a consequence of the local inflammation is unknown [36], although some hypotheses were already published [39]. Although PBI – a standard marker of the parodontal status was higher in the older age ( $p < 0.014$ ), we found that even after adjustment for age the level of salivary TBARS is under tight influence of PBI ( $p < 0.001$ ; Fig. 1) and the interaction of age and PBI ( $p < 0.016$ ). Surprisingly, there is no significant relationship between PBI and MDA ( $p = 0.12$ ). It can be concluded, that TBARS other than MDA must be associated with higher PBI and, thus, with worse parodontal status. Sialic acid belongs to the agents that are described to imitate MDA in the determination using thiobarbituric acid [26]. Moreover, sialic acid can be found in great amounts at the surface of most cells and is therefore a suitable candidate [4,18]. Further research should concentrate on the salivary levels of free sialic acid [19,33] and their relationship to the parodontal status [7,10,23]. On the other hand, the number of epithelial cells in saliva – a possible source of sialic acid [41] was found to be higher in patients with lower PBI ( $p < 0.05$ ) and in non-smokers ( $p < 0.01$ ; Fig. 2).

We suppose two possible hypotheses for the explanation of these relations. Defective mucosal tissue has an altered regeneration capacity [13], especially in chronic inflammatory diseases like chronic gingivitis [12] or due to smoking [30]. Therefore, the turnover of the epithelial cells is diminished and the number of epithelial cells in saliva is, thus, reduced. Another possibility is that the difference is caused by cell counting criteria [2]. Only definite large round or oval shaped epithelial cells were included, not vague fragments or cell grit [40]. It is possible that the overall cell material amount in saliva is higher in patients with parodontopathies [20], but the number of whole large cells is reduced due to the inflammation associated tissue injury [31]. MDA as a specific marker of oxidative stress was higher in older patients ( $p < 0.03$ ). Oxidative stress is increased in the older age, particularly due to the locally reduced activity of cellular antioxidative enzymes [1,37]. Besides, MDA was also significantly higher in smokers than in non-smokers ( $p < 0.003$ ; Fig. 3), however, this influence disappeared after the adjustment for age.

Smoking is known for inducing oxidative stress and, hence, this difference can be explained by a local over-

production of reactive oxygen species and a subsequent lipoperoxidation due to smoking [15,32,42]. On the other hand, reported smoking was not associated with worse parodontal status ( $p = 0.84$ ). A detailed analysis of the effects of smoking on salivary TBARS requires a further study concentrating on smoking habits, which has not been the aim of this study.

The main finding of this study is that TBARS are a simple and suitable parameter that reflects the parodontal status in stomatologic patients. Its usefulness in the clinic should be further evaluated. However, more than a tool for the first diagnosis the TBARS dynamics could be used for the evaluation and monitoring of the treatment procedure and healing progress.

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