SALIVARY INTERLEUKIN-1 AND 8-HIDROXYDEOXYGUANOSINE AND THEIR RELATION WITH THE PERIODONTAL STATUS

Aureliana Caraiane¹, Victoria Badea², Florin Ciprian Badea³*, Mircea Grigorian⁴

¹Ovidius University from Constanta, Romania, Faculty of Dental Medicine, Department of Oral Rehabilitation
²Ovidius University from Constanta, Romania, Faculty of Dental Medicine, Department of Oral Microbiology and Immunology
³Ovidius University from Constanta, Romania, Faculty of Dental Medicine
⁴Ovidius University from Constanta, Romania, Faculty of Dental Medicine, Department of Physiology

*Corresponding author: Florin Ciprian Badea, PhD Student
Ovidius University
Constanta, Romania
e-mail: Ciprian_md@yahoo.com

ABSTRACT

Aim of the study This study aims to quantify interleukin-1 (IL-1) and 8-hidroxydeoxyguanosine (8-OHdG) from saliva and to identify a possible correlation between the values of these biomarkers and the periodontal status. Material and methods We made clinical oro-dental evaluation for 40 subjects which were divided in 4 groups: healthy control, gingivitis (G), chronic periodontitis (CP) and aggressive periodontitis (AP). We collected saliva according to the recommendations of the World Health Organization and measured the quantity of the two biomarkers by using ELISA method. Results The values of both biomarkers increased progressively from healthy oro-dental status to the status of CP and AP. There are highly statistically significant differences between the amount of salivary IL-1 quantified in healthy group versus AP patients and between CP and AP (p<0.0001). There are also highly statistically significant differences between the level of 8-OHdG quantified in the saliva of healthy group compared to those with chronic and aggressive periodontitis (p<0.0001). We identified highly statistically significant differences between the level of IL-1 and 8-OHdG quantified in patients with AP (p<0.0001). Our study shows a high correlation between periodontal pocket depth and the level of 8-OHdG identified in patients with CP and AP (r=0.854, p=0.0004 for CP and r=0.708, p=0.021 for AP). Conclusions Our study demonstrates that these two biomarkers may be useful in assessing the health of the periodontium.

Keywords: interleukin-1, 8-OHdG, saliva, periodontal status

INTRODUCTION

As defined by the vast majority of authors, periodontitis is a disease resulting from the interaction of periodontopathogenic bacterial species and the host response [1, 2, 3]. Importance of periodontal disease is demonstrated by its high incidence (15-20%) as presented in WHO report [4].

In periodontal disease, the production of reactive oxygen species (ROS) is a consequence of cell destruction resulting from the presence of periodontal bacteria species [5, 6]. Defensive reaction of the organism in
condition of ROS hyperproduction cause oxidative modification of host cells, expressed by the dysfunction of DNA, lipid peroxidation and protein oxidation [7, 8].

In time, the use of classical parameters of periodontal health assessment or periodontal disease, including Periodontal Pocket Depth (PPD), Bleeding On Probing (BOP), Clinical Attachment Loss (CAL) indexes became insufficient for achieving a correct diagnosis, for treatment monitoring and most important, for establishing an early diagnosis of periodontal disease.

Modern technology has allowed the materialization of the concept regarding the identification of salivary biomarkers, so the correct assessment of the disease phenomenon and early identification of the first signs of disease have created the premises of new treatment protocols [9, 10, 11, 12].

In this context, 8-OHdG is the most important product of DNA oxidative destruction caused by ROS; it can be identified at an elevated level in all bodily fluids and tissues, where an inflammatory process exists. [13]

Cytokines are a group of pro-inflammatory mediators among which IL-1, tumor necrosis factor and IL-6, have been shown to be particularly important in the periodontal disease. [14, 15]

Our study aims to show the possibility to use 8-OHdG and IL-1, quantified in saliva, as biomarkers for assessing the health or disease of the periodontium.

**MATERIAL AND METHODS**

1. **STUDY GROUP**

We comprised in our study 40 subjects, 18 females and 22 males, aged between 30 to 40 years old, from Constanta, Romania. The clinical oro-dental examination was performed by two calibrated examiners in the Department of Preventive Dentistry, Ovidius University Constanta, Faculty of Dental Medicine. The clinical examination included the following indicators: PPD, BOP, gingival index (GI) and Community Periodontal Index for Treatment Needs (CPITN).

Inclusion criteria were: non smoking persons, without chronic diseases and any antibiotic treatment in the last 6 month.

At the end of clinical examination, the study group was divided as follows: clinically healthy - 10 persons (25%), gingivitis - 8 patients (20%), chronic periodontitis - 12 patients (30%) and aggressive periodontitis - 10 patients (25%).

2. **SALIVA SAMPLING AND MARKERS ANALYZING**

Saliva samples were collected without stimulation after clinical examination by using the passive collection in sterile containers. The salivary samples were centrifuged 8000 rpm/10min for 8-OHdG and at 10000 rpm/10min for IL-1 and then analyzed. Identification of the two biomarkers was done by using Enzyme-Linked ImmunoSorbent Assay (ELISA) method, as follows: for 8-OHdG we used a kit from Cayman Chemicals (ELISA kit-competitive method, Cayman Chemical, USA) [16] and for IL-1 a kit from PromoKine (Human IL-1 beta ELISA kit) [17].

3. **STATISTICAL ANALYSES**

The data were statistically analyzed by using SPSS 19.0 for Windows and MedCalc
For establishing the correlation between the clinical parameters and the values of the two biomarkers we used Pearson’s correlation coefficient.

The statistical highlights between the values of IL-1 and 8-OHdG from the 4 study groups were evaluated by using Student’s t-test, p value less than 0.05 was considered to be statistically significant.

4. ETHICAL PERMISSION

We obtained the ethical permission for this medical research study involving human subject from the Professional Ethical Committee of Ovidius University from Constanta, according to the World Medical Association Declaration of Helsinki.

RESULTS

Descriptive analysis of study groups, depending on the evaluated clinical parameters are presented in Table 1.

Table 1. Distribution of clinical signs in the study groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>G</th>
<th>CP</th>
<th>AP</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPD</td>
<td>1.40±0.70</td>
<td>3.04±0.33</td>
<td>4.46±0.45</td>
<td>7.19±0.80</td>
</tr>
<tr>
<td>BOP</td>
<td>16.54±2.14</td>
<td>18.16±3.60</td>
<td>43.10±2.30</td>
<td>56.16±11.43</td>
</tr>
<tr>
<td>GI</td>
<td>0.64±0.48</td>
<td>1.02±0.56</td>
<td>2.08±0.22</td>
<td>2.65±0.61</td>
</tr>
<tr>
<td>CPITN</td>
<td>0.94±0.76</td>
<td>1.20±0.82</td>
<td>3.46±0.52</td>
<td>5.72±1.17</td>
</tr>
</tbody>
</table>

Quantitative results on 8-OHdG expressed in ng/mL in the 4 study groups are as follows: healthy 1.16±1.01, gingivitis 3.80±0.12, chronic periodontitis 4.51±0.35, and aggressive periodontitis 8.81±2.64.

Regarding IL-1 values, expressed in pg/mL we obtained: healthy 9.84±7.15, gingivitis 40.19±18.21, chronic periodontitis 46.55±40.34, and aggressive periodontitis 584.34±242.17.

Figures from 1 to 6 show the differences between average values of IL-1 and 8-OHdG for each of the four groups of subjects from this study.

Figure 1. Graphic representations of the IL-1 and 8-OHdG mean values for healthy vs. gingivitis groups

Figure 2. Graphic representations of the IL-1 and 8-OHdG mean values for healthy vs. chronic periodontitis groups

Figure 3. Graphic representations of the IL-1 and 8-OHdG mean values for healthy vs. aggressive periodontitis groups
The results show that there are statistically significant differences on the levels of IL-1 identified in the saliva of healthy individuals compared with that of patients with AP and between the levels of IL-1 quantified in the saliva of patients with CP compared with AP (p<0.0001). Also, regarding the amount of 8-OHdG quantified in the studied groups, we found statistically significant differences between all four groups studied (p<0.05).

The highest statistical differences were recorded between the healthy group and the one with gingivitis (p=0.0002); also we found highly statistically significant differences on the amount of 8-OHdG identified in saliva of the control group and those with CP and AP (p<0.0001).

Studying statistical differences between mean values of IL-1 and 8-OHdG in the 4 studied groups we found that there are statistical differences (p<0.05), having higher statistically significant differences between the values of the two biomarkers at the patients with AP (p<0.0001).

Regarding the association between 8-OHdG and periodontal pocket depth we showed that there is a high correlation between these two parameters in patients with CP (p=0.0004, r=0.854) and those with AP (p=0.0218, r=0.708).

The analysis on the association between IL-1 and periodontal pocket depth showed no correlation between these two parameters. Also no significant differences was found between the values of the two biomarkers and gender in all 4 groups taken into study (p>0.05).

DISCUSSIONS

There are numerous studies suggesting an association between the salivary 8-OHdG levels and the degree of periodontal damage. [18, 19] Thus, Sezer U. et al. [18] demonstrated that there is a significant correlation between the salivary 8-OHdG level and PPD, CAL indexes; Takane et. al. [20] showed in his studies that salivary 8-OHdG is highly correlated with periodontal

Figure 4. Graphic representations of the IL-1 and 8-OHdG mean values for gingivitis vs. chronic periodontitis groups

Figure 5. Graphic representations of the IL-1 and 8-OHdG mean values for gingivitis vs. aggressive periodontitis groups

Figure 6. Graphic representations of the IL-1 and 8-OHdG mean values for chronic vs. aggressive periodontitis groups
pocket depth, concluding that this biomarker can be used for periodontitis diagnosis and monitoring. Moreover, in our previous studies [21] we showed that 8-OHdG is a sensitive biomarker that becomes positive before the appearance of clinical signs, which demonstrates its practical utility in early diagnosis of periodontal disease.

Studies also demonstrate that this biomarker can be used successfully in monitoring the treatment of periodontal disease [20, 22, 23, 24].

There are studies in which is presented the correlation between the amount of salivary levels of IL-1 beta and the degree of periodontal destruction, suggesting the usefulness of this biomarker in the diagnosis and monitoring of periodontal disease. Thus, we can differentiate active from inactive lesions within periodontal disease by quantification of this biomarker, as shown in studies of Chaudhari A.U. et al. [25] and Al-Ghurab B.H. et al. [26].

These studies have been expanded in recent years through the research of molecular biology, which led, at least for now, to conflicting results on the relationship between IL-1 gene polymorphism and susceptibility for developing aggressive periodontal disease [21].

In the literature there are few data regarding the association between IL-1 and 8-OHdG quantified from saliva in periodontal disease. A part of this study was conducted in this direction and the results showed that there are statistically significant differences between the values of them quantified in all four study groups, demonstrating their utility as biomarkers in periodontal disease.

The results of our research, within the limits of the study group sizes, proves that this biomarkers accurately reflect both, the state of periodontal health and also, its varying degrees of damage, as found in the periodontal disease.

CONCLUSIONS

According to our results, we can conclude that salivary 8-OHdG and IL-1 can be useful tools for monitoring the health or disease status of the periodontium.

REFERENCES
5 Takaaki T., Daisuke E., Manabu M., Effects of periodontal therapy on circulating oxidative stress, Oxidative Stress in Applied Basic Research and Clinical Practice, 2014, 147-156.


18 Sezer U., Cicek Y., Canakci C.F., Increased salivary levels of 8-hydroxydeoxyguanosine may be a marker for disease activity for periodontitis, Dis Markers, 2012, 32:3, 165-172.

19 Canakci C.F., Canakci V., Tatar A., Eltas A., Sezer U., et al., Increased salivary levels of 8-hydroxydeoxyguanosine is a marker of premature oxidative mitochondrial DNA damage in gingival tissue of patients with periodontitis, Arch.Immunol.Ther.Exp., 2009, 57, 205-211.


25 Chaudhari AU, Byakod GN, Waghmare PF, Karhadkar VM. Correlation of Levels of Interleukin-1b in Gingival Crevicular Fluid to the Clinical Parameters of Chronic Periodontitis. The Journal of Contemporary Dental Practice, January-February 2011, 12:1,52-59