OXIDATIVE STRESS MARKER IN THE AGGRESSIVE PERIODONTAL DISEASE - SALIVARY 8-HIDROXYDEOXYGUANOSINE

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ABSTRACT

Aim of the study In this study we aimed to identify if there is a correlation between the oxidative stress marker 8-hidroxydeoxyguanosine (8-OHdG) from saliva and the orodental clinical status, evaluated by periodontal pocket depth, in aggressive periodontitis. **Material and methods** The study group was formed by 54 subjects (healthy control, patients with aggressive periodontitis (AgP) and periodontal pocket depth (PPD) between 4 to 5 mm and patients with AgP and PPD between 6-9 mm), which were clinical oro-dental evaluated. We measured the salivary 8-OHdG biomarker by using ELISA method, competitive technique. **Results** We identified statistically significant differences between the amount of salivary 8-OHdG quantified in healthy group versus AgP patients with PPD 4-5 (p=0.039) and also versus patients with PPD 6-9 (p=0.0001). In our study we also identified a high statistical correlation between the level of salivary 8-OHdG and the PPD of AgP patients (r=0.0446, p=0.005). **Conclusions** The results of our study demonstrate that salivary 8-OHdG oxidative stress biomarker can be used in the diagnosis and monitoring of aggressive periodontal disease.

Keywords: oxidative stress, 8-OHdG, saliva, aggressive periodontitis

INTRODUCTION

Periodontal disease has been shown to be known and treated for over 5000 years, and in our country, the first evidence of treating gums are dated 1828 and belong to Selingher (1, 2). After Carranza (3) periodontal disease is an "inflammatory disease of the tissues supporting the teeth caused by specific microorganisms or groups of specific microorganisms which results in progressive destruction of the periodontal ligament and alveolar bone, while periodontal pocket depth increases."

Aggressive periodontal disease (AgP) is

characterized by a loss of the tooth attachment structures accompanied by progressive destruction of the alveolar bone, all of which occurs in young adults (4).

The last report of the Platform for Oral Health in Europe in September 2012 (5) shows that are very few epidemiological data on periodontal disease, of very poor quality and even absent in some European countries. It is estimated that more than 50% of Europeans suffer from some form of periodontal disease and that more than 10% have a severe form of the disease, while worldwide, the percentage is between 5% and 20%. By using modern methods of diagnosis were identified as main pillars of AgP pathogenesis the presence of periodontal bacteria and exacerbated immune response, this disease being currently listed as a "complex multifactorial etiology" disease (6).

One of the phenomena that generate AgP is oxidative stress (7). As defined by Sies, oxidative stress is the "altered physiological balance between oxidants and antioxidants in favor of the former" (fig. 1, after Halliwell et al., 2011 (9)) leading to lesions in the body (8).

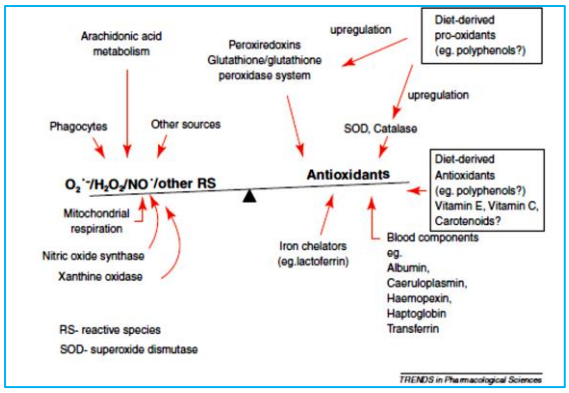

Figure 1. Balance between antioxidants and ROS

These lesions were called "oxidative damage" and appear either after exposure to an increased concentration of reactive oxygen species (ROS) and/or when the amount of antioxidants is reduced (8). ROS are not only involved in oxidative stress, but also have their role as mediators between internal and external stressors and intra- and extracellular environment (10).

In the AgP, by the presence of bacterial antigens, a process of stimulating polymorphonuclear leukocytes takes place by producing and releasing an increased amount of ROS, culminating with oxidative lesions in the gingival tissue, periodontal ligament and alveolar bone (11). ROS do an initial depolymerization of extracellular matrix components, followed by the action of lipid peroxidation, oxidation of the enzymes and the induction of cytokines formation with the cellular DNA damage (8, 10, 11, 12). Hydrogen peroxide, although it is not considered to be a potent ROS, can pass the nuclear membrane and produce changes in DNA, including nucleotide oxidation (12). In the aggressive periodontal disease, the most studied oxidative DNA damage is the formation of 8-Hidroxydeoxyguanosine (8-OHdG) (8), which is a change that occurs in about one in 105 of the guanosine bases in normal human cells (13) (fig. 2, after Badea et al., 2010 (14)).

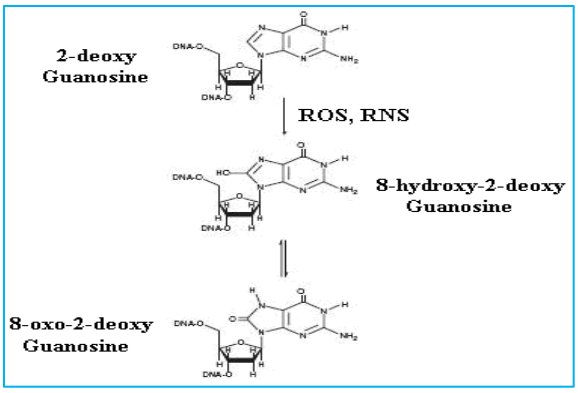

Figure 2. 8-OHdG and 8-oxodG synthesis

In the nuclear and mitochondrial DNA, 8-OHdG and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) is the predominant form found in lesions due to oxidative stress and therefore were imposed as biomarkers of oxidative stress (15).

Also, recent studies show that this biomarker value increases before the appearance of clinical signs of periodontal disease (14). The purpose of the study is to evaluate the possible correlation between salivary 8-OHdG oxidative stress biomarker and orodental clinical status, expressed by periodontal pocket depth, in aggressive periodontal disease.

MATERIAL AND METHODS

1. Study group

The study group consisted of 54 subjects who were divided after clinical examination as follows: healthy - 17 subjects and patients with AgP - 37 subjects. Clinical examination of the oral cavity was performed by inspection, palpation and percussion, with the record of CPITN index (Community Periodontal Index of Treatment Need). The group of patients with aggressive periodontal disease has been divided into two groups, depending on the depth of the periodontal pocket as follows: 20 patients with average periodontal pockets deep between 4 and 5 mm (PPD 4-5) and 17 patients with periodontal pocket depth between 6 and 9 mm (PPD 6-9).

2. Saliva sampling and markers analyzing

Saliva sampling was done after orodental clinical examination, in the same time slot for all subjects (10:00-12:00 hours). The obtained saliva samples were centrifuged at 8000 rpm for 10 minutes (16).

In order to measure 8-OHdG biomarker we used ELISA, competitive technique, as described by the manufacturer (Cayman Chemical, USA). This assay is based on competition of 8-OHdG from the sample and 8-OH-dG-acetylcholinesterase conjugate for a limited amount of 8-OHdG monoclonal antibodies; the product of this enzymatic reaction has a distinct yellow color and is read at 412 nm (16).

3. Statistical analyses

We statistically analyzed our results by using SPSS 19.0 for Windows and MedCalc 11.0. When we compared the results obtained for 8-OHdG levels in our study groups we used Student's t-test and for a $p<0.05$ was considered to have a statistic significance.

For correlations between the 8-OHdG values with the depth of gingival sulcus or PPD we used Person test.

4. Ethical permission

We obtained the University Ovidius from Constanta Ethics Committee agreement to comply with the ethical principles for medical research involving human subjects, under the auspices of the International Medical Association Declaration of Helsinki. Subjects were informed about the purpose of investigations. The subjects participated in the study on the basis of informed consent which was signed by them.

RESULTS

Our results show that there are highly significant statistical differences between the values of 8-OHdG identified at healthy group compared with those from patients with AgP ($p = 0.003$) (fig. 3).

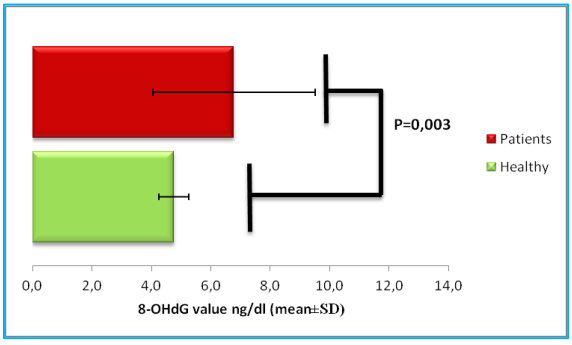

Figure 3. Graphic representations of the 8-OHdG mean values for healthy vs. AgP patients group (Student's test)

The results show that there are highly significant statistical differences between the 8-OHdG levels at healthy and patients with 6-9 mm PPD groups ($p=0.0001$) and between

the two groups of patients with AgP (p=0.001). There are also statistical significant diferences between the 8-OHdG levels at healthy and patients with 4-5 mm PPD (p = 0.039) (fig. 4).

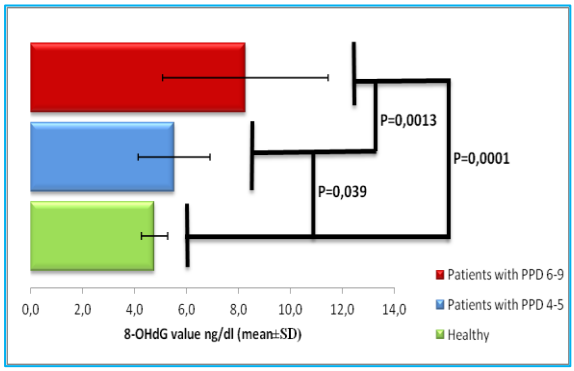

Figure 4. Graphic representations of the 8-OHdG mean values for healthy vs. PPD 4-5 and PPD 6-9 AgP groups (Student's test)

We found that there is a high statistical correlation between periodontal pocket depth and amount of 8-OHdG oxidative stress biomarker levels in patients with AgP (r=0.446, p=0.005).

There is moderate correlation between 4-5 mm pocket depth at AgP patients with and 8-OHdG biomarker value (r=0.448, p=0.047), according to Pearson test. On the other hand, there is no statistically correlation between gingival sulcus depth and 8-OHdG biomarker value in healthy group (r=0.020, p=0.930). Also, we found no correlation between the oxidative stress marker values and 6-9 mm periodontal pocket depth in the group of patients (r=0.066, p=0.802).

DISCUSSIONS

In our study we examined 54 subjects, from which 31.48% were clinically orodental healthy and 68.52% presented clinical signs of aggressive periodontitis. The values of salivary 8-OHdG oxidative stress marker were higher at the patients compared to the healthy subjects, as a consequence of oxidative DNA damage in the periodontal tissue.

Also, the results of our study indicate that there are correlations between the salivary 8-OHdG levels and the depth of gingival sulcus or periodontal pocket. This leads to the ideea that this biomarker's value is an indicator for the oxidative stress processes and that it can be used in the early diagnosis of the aggressive periodontal disease.

There are studies that support the idea that ROS can damage DNA in many ways, and that the guanosine lesions are the most encountered ones. These leasions are due to the fact that this DNA base has the least oxidation potential and it is the most attacked by the oxidative species (17).

Our results regarding the correlation between salivary 8-OHdG and periodontal disease are similar to those obtained by Takane and Arunachalam (18, 19), with the specification that their studies were conducted on patients with chronic periodontal disease. Also, similar results targeting the association between 8-OHdG and AgP were obtained Sezer et al. (20) showing that there is a highly significant statistical correlation between the values of salivary 8-OHdG and clinical modifications.

The results of our study and of those cited in the speciality literature (18, 19, and 20) prove that this biomarker can be successfully used in the diagnosis and monitoring of patients with periodontal disease.

More than this, our results, similar with the ones from other studies cited in the literature (14, 18, 19, 21) show that this biomarker may be used early in the diagnosis of periodontal disease, and in perspective, it may be used as a monitoring tool for periodontal disease treatment efficiency. This is based on high statistical significance between orodental clinical status and 8-OHdG oxidative stress biomarker value.

CONCLUSIONS

1. Our study results demonstrates that

salivary 8-OHdG biomarker of oxidative stress can be used in the diagnosis and monitoring of aggressive periodontal disease.

2. The evaluation of salivary 8-OHdG oxidative stress biomarker will allow timely establishment of a therapeutic plan to prevent early loss of teeth.

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