

SALIVARY 8-HIDROXY-2-DEOXY GUANOSINE AS OXIDATIVE STRESS BIOMARKER FOR THE DIAGNOSIS OF PERIODONTAL DISEASE

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Abstract

Many studies that have been conducted highlight the existence of a correlation between markers of oxidative stress and various diseases, among which periodontitis. The group of patients studied consisted of 85 patients with periodontitis and 49 healthy subjects selected according to their oro-dental status. The evaluation of the salivary level of oxidative stress was performed by assessing the salivary 8-hidroxy-2-deoxy guanosine (8-OHdG) by an ELISA competitive method. The level of 8-OHdG in healthy oro-dental patients was between 1.85-1.20ng/mL; in patients with periodontitis the values before the treatment were 7.50-3ng/mL, and 4.50-1.80ng/mL after the treatment.

The study demonstrates the existence of a correlation between the values of this biomarker and the status of periodontal tissue, allowing us to appreciate, in conclusion, that the level of 8-OHdG may be used for the diagnosis and treatment of the periodontal disease.

Rezumat

În literatura de specialitate se evidențiază faptul că există o corelație între markerii de stres oxidativ și diferite afecțiuni, printre care și boala parodontală. Lotul de subiecți luați în studiu a fost constituit din 85 de pacienți cu parodontoză și 49 subiecți sănătoși selectați în funcție de statusul clinic oro-dentar. Identificarea nivelului de stres oxidativ salivar a fost realizat prin evaluarea 8-hidroxi-2-deoxi guanozinei (8-OHdG) salivare folosind metoda ELISA competitivă. Valoarea 8-OHdG la subiecții sănătoși din punct de vedere oro-dentar a fost cuprinsă între 1.85-1.20 ng/mL; la pacienții cu parodontită, nivelul 8-OHdG înainte de tratament a fost între 7.50-3 ng/mL, iar după tratament între 4.50-1.80 ng/mL.

Studiul demonstrează existența unei strânse corelații între valorile biomarkerului și starea parodonțiului, ceea ce ne permite să apreciem în concluzie că determinarea biomarkerului 8-OHdG oferă posibilitatea utilizării acestuia în diagnosticul și evaluarea tratamentului bolii parodontale.

Keywords: 8-hidroxy-2-deoxy guanosine, oxidative stress, periodontitis.

Introduction

Oxidative stress is defined as the result of an imbalance between oxidant factors and protective antioxidant systems; it may occur due to an excess of free radicals, or by the diminishing of the antioxidant systems. In the presence of a minor oxidative system it may be adapted with the stimulation of the antioxidant system and with the partial/complete compensation of the oxidative overload. For the situations where the oxidative stress is important, lesions occur at the level of the lipid, protein, or DNA components that could eventually cause the death of the cells. The complexity of modern life makes the sources of aggression of the human body to be varied and complex; some are related to the type of food, alcohol abuse, smoking, pollution, and UV radiation-each of these, or in association, represent the source of oxidative stress through the production of free radicals [3, 8, 10].

In recent years numerous studies that have been conducted highlight the existence of a correlation between markers of oxidative stress and various diseases, namely it is estimated that over 200 diseases (atherosclerosis, cardiovascular diseases, cancer, degenerative brain diseases, periodontitis), are due to oxidative stress [6].

Free radicals are defined as molecules or fragments of molecules characterized by a great potential for chemical reactivity and containing one or more odd electrons. The presence of the odd electron gives these molecules a very high chemical reactivity; free radicals also react with the majority of the molecules in the organism and at the end resulting other free radicals. The most important free radicals are: free radicals of oxygen and of nitrogen [10, 13].

Defined as a perfect host of oxidation reduction reactions, the human body constantly creates free radicals in the physiological conditions, situation that does not influence the health if the endogenous antioxidant systems are working efficiently (superoxide-dismutase, catalase, glutathione-peroxidase, glutathione-transpherase). The imbalance between the antioxidant systems and the excess production of free radicals leads to the oxidative stress installation [2, 6].

The harmful role of free radicals is immense-the cellular targets mostly affected are cell membranes and DNA in which intimate destructive

mechanisms are represented by the oxidation of lipids (especially internal mitochondrial membrane lipids), oxidation of proteins with enzymatic activity and DNA damage (Figure 1) by altering nitrogen bases and the breakage of DNA chains [13].

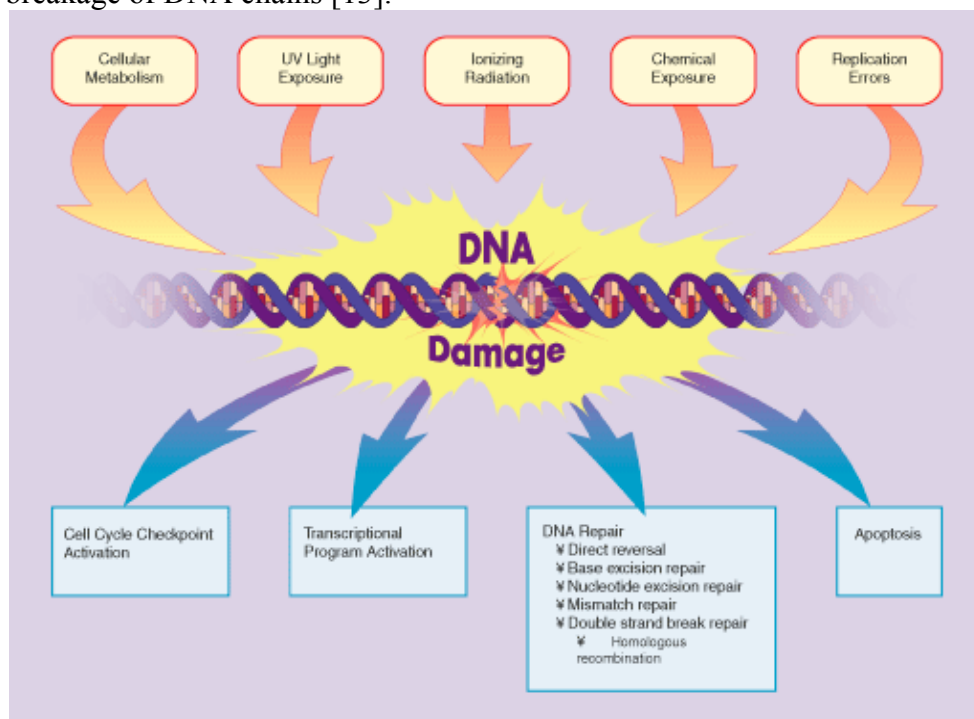
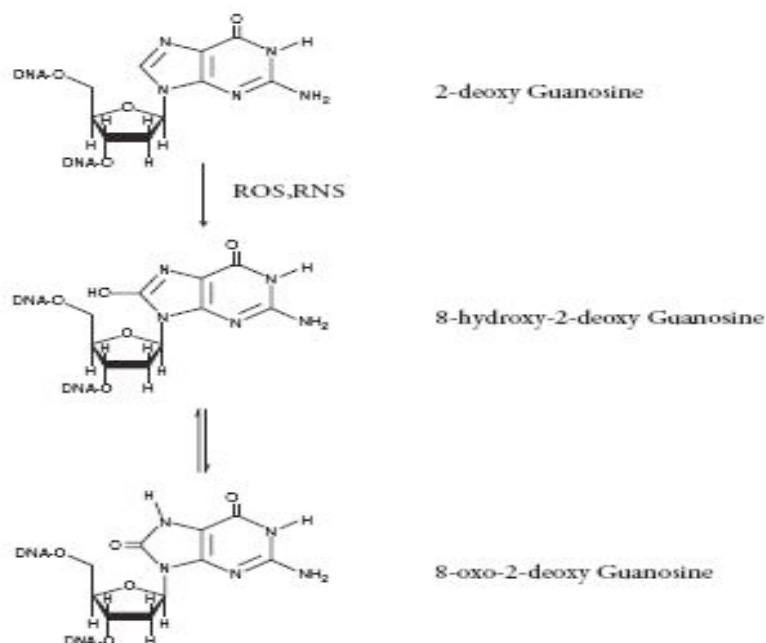


Figure 1.
DNA damage
(Friedberg E.C., 1995)

Reactive oxygen species can attack the DNA, especially at the guanine, thymine and deoxyribose level with consequence over the replication process. One of the first modifications that the DNA suffers through the radical attack is the transformation by oxidation of deoxyguanosine (constituent of the DNA) and the formation of the 8-hydroxy-2-deoxyguanosine (8-OHdG). The cells possess enzymes that could remove the radicals of 8-OHdG, and so repairing the DNA (Figure 2). The continuous attack of the reactive oxygen species cause the permanent modification of the DNA with severe consequences on the physiology or even the death of the target cells.

**Figure 2.**

The formation of 8-OHdG

(ROS=reactive oxygen species; RNS=reactive nitrogen species)

Periodontal disease is defined as an infection of the support structures of the tooth and is caused by bacterial species present in the subgingival space [1, 9, 14].

The invasion of periodontal tissue by bacterial species is provided by the action of bacterial toxins and enzymes; all these bacterial products induce inflammatory reactions in the presence of reactive oxygen species (ROS) in the subgingival space and by the destruction of the nucleic acid and ending with the occurrence of 8-OHdG [4, 5].

As provided in a World Health Organisation (WHO) study, periodontal disease is identified at present between 5-20% of middle-aged adults worldwide. Based on current trends set out by WHO [12], it is estimated that by 2020 half the world's population with average age will be affected by the periodontal disease. In these terms - "The Strategy for prevention and control of chronic disease" recommends the implementation of rapid diagnostic methods and a screening program for the early diagnosis of periodontal disease. The use of saliva and other oral fluids (gingival fluid) was recently introduced in modern medicine; the similarity with the blood regarding many of its components, the non-invasive collection

methods of saliva, made possible the replacement of blood with saliva as biological product explored for the diagnosis of many diseases [7, 11, 15].

Our study on the quantitative determination of the salivary 8-OHdG in the periodontal disease was performed for the first time in Romania, framing in the modern medical research on the periodontal disease and is aligned to the modern European studies on exploratory and scientific fundamental research.

Materials and methods

The group of patients studied consisted of 85 patients non-smokers and without a history of chronic diseases, selected according to oro-dental clinical examination (degree of gum retraction, dental mobility, depth of periodontal pockets, bleeding on palpation) and Community Periodontal Index of Treatment Need (CPITN index) assessment which was systematized as follows: chronic marginal deep periodontitis (n=39), chronic marginal surface periodontitis (n=40), periodontitis dystrophy (n=6).

In Table 1 there are presented the criteria used in the evaluation of the studied patients.

Table I.
Clinical periodontal profile of patients

	Number	%
Men	52	61.17%
Women	33	38.83%

	Mean±SD
Medium age	42.88±1.21
Visible plaque (%)	59.56%±6.6
Bleeding on probing (%)	42.12±4.6
Periodontal pockets (mm)	4.30±0.50
CPITN -index	3.32±0.76

For a proper interpretation of the results we used a control group composed of 49 oro-dental healthy subjects. Informed consent was obtained from the subjects for the saliva collection, and during the clinical examination, the saliva was collected and stored at -80°C until analyzed. Clinical examination was followed by the salivary 8-OHdG determination (ELISA kit-competitive method, Cayman Chemical, USA). After this first evaluation patients were subjected to conventional periodontal treatment:

supra and subgingival scaling, subgingival curettage, local anti-inflammatory treatment; 6 weeks later patients were reassessed clinically and biochemically for the salivary 8-OHdG determination.

In periodontitis the neutrophils play an important role in the inflammatory response to the periodontal pathogens; therefore we estimated the inflammatory process by microscopic examination of the saliva and depending on the leukocyte frequency in each microscopic field; it was noted a qualitative leukocyte score marked with +, ++, +++, +++++.

Differences in salivary 8-OHdG levels were analyzed by Student's t-test; statistical significance was defined as $p < 0.05$.

Results and discussion

The group of patients with chronic periodontitis that were investigated before the treatment recorded 8-OHdG levels between 7.50-3.00 ng/mL (Table II and Figure 3) and were correlated with CPITN index as follows:

- patients with chronic marginal deep periodontitis (39) and dystrophic (6) maximum CPITN score 3, had 8-OHdG values ranging between 7.50-5.25 ng / mL; the microscopic examination showed the leukocyte score noted with +++ and +++++.
- patients with chronic marginal surface periodontitis and maximum CPITN score 2, had 8-OHdG values between 5.00-3.00 ng/mL; microscopic examination of saliva showed an inflammation score + and ++ (Table II and Figure 3).

Table II.
Concentration of 8-OHdG before the treatment

Concentration of 8-OHdG	Number of Patients with periodontitis
7.50 ng/mL	8
7.00 ng/mL	10
6.40 ng/mL	12
5.50 ng/mL	9
5.25 ng/mL	6
5.00 ng/mL	9
4.40 ng/mL	8
3.90 ng/mL	7
3.50 ng/mL	6
3.00 ng/mL	10

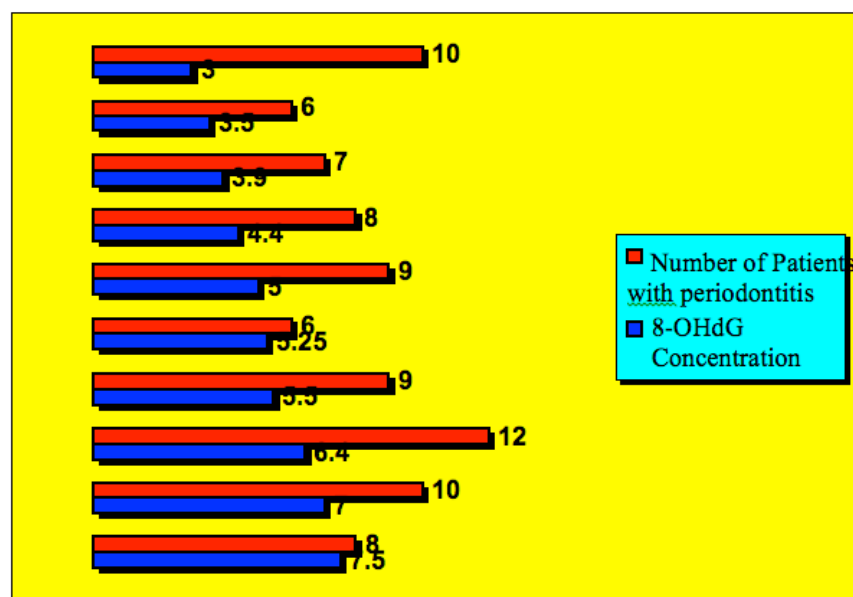


Figure 3.
Concentration of 8-OHdG before the treatment

8-OHdG values in the 49 oro-dental healthy subjects are presented in Table III and Figure 4. The biomarker values were between 1.85-1.20ng/mL.

Table III.
Concentration of 8-OHdG in the healthy subjects

Concentration of 8-OHdG	Number of healthy subjects
1.85 ng/mL	11
1.70 ng/mL	10
1.65 ng/mL	10
1.50 ng/mL	7
1.40 ng/mL	4
1.50 ng/mL	4
1.20 ng/mL	3

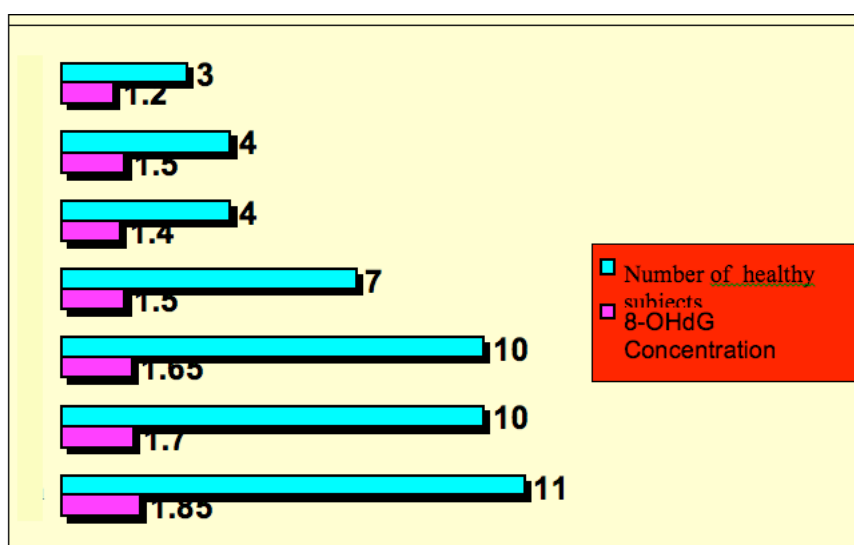


Figure 4.
Concentration of 8-OHdG in the healthy subjects

The patients were treated for 6 weeks and were biochemically evaluated for the determination of salivary 8-OHdG; the obtained results are shown in Table IV and Figure 5.

Table IV.
Concentration of 8-OHdG after the treatment

Concentration of 8-OHdG	Number of Patients with periodontitis
4.50 ng/mL	8
4.30 ng/mL	10
4.15 ng/mL	12
4. ng/mL	9
3.80 ng/mL	6
3 ng/mL	9
2.40 ng/mL	8
1.90 ng/mL	7
1.85 ng/mL	6
1.70 ng/mL	10

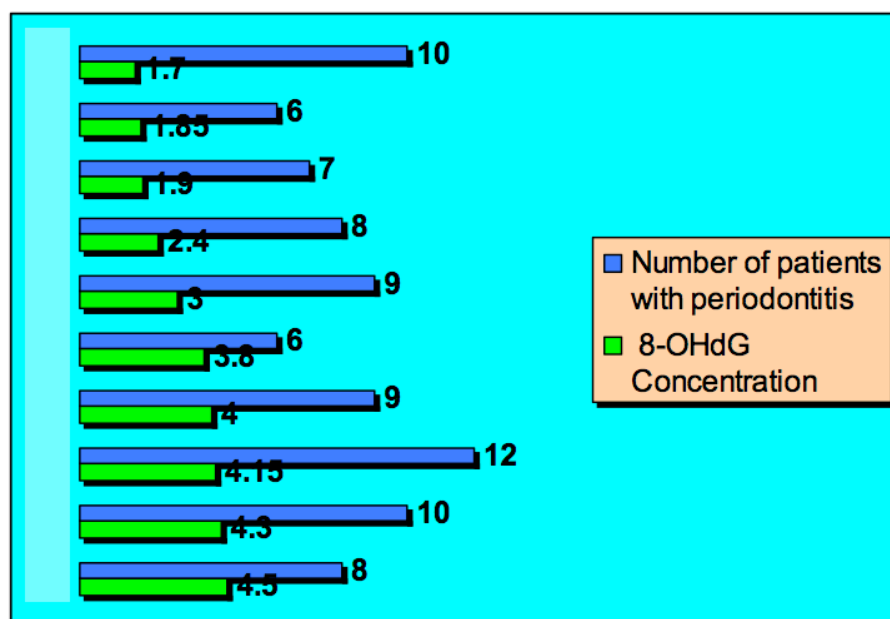


Figure 5.
Concentration of 8-OHdG after the treatment

Patients with chronic marginal deep periodontitis (39) and dystrophic periodontitis (6) after the treatment had 8-OHdG levels between 4.50-3.80ng/mL; the microscopic examination shows a decrease of inflammatory reaction which was noted with the salivary score +++ and ++; CPITN index 2. Patients with superficial chronic marginal periodontitis (40) showed after the treatment 8-OHdG levels lower than 3.00 ng/mL; the microscopic examination showed a decrease of inflammatory reaction which was noted with the salivary score +; CPITN index 1.

In the present study we found representative the decreased level of 8-OHdG in saliva of periodontal patients after the treatment-statistical processing of the data showed a statistical significant correlation between 8-OHdG levels after and before the treatment ($p=0.0045$); also, we observed a positive correlation between 8-OHdG levels in saliva of periodontal patients as compared with healthy subjects ($p=0.0003$). Our results are similar with those reported in the literature [4, 7].

For 10 patients with the 1.70 ng/mL 8-OHdG level and microscopic leukocyte score +, the oro-dental examination showed that they are clinically cured; 6 patients with the 8-OHdG value of 1.85 ng/mL, presented at the oro-dental exam only slight bleeding gums, which shows that the biomarker level decreases before the disappearance of the clinical signs. There is a very high sensitivity of the biomarker 8-OHdG, in the sense that

it decreases and reaches normal levels before the disappearance of clinical signs; this allows us to assume hypothetically the real and possible reverse situation, that this biomarker may increase before the appearance of the clinical signs of periodontal disease, and so, 8-OHdG can be used in the early diagnosis of periodontitis.

Further studies will examine the oro-dental status in healthy subjects simultaneously with the determination of the 8-OHdG levels, and so we will continue to verify this hypothesis.

Conclusions

- The inflammatory reaction estimated in saliva samples of patients with periodontitis demonstrates a direct proportionality relationship between the inflammatory process shown by the leukocyte score and the aggressiveness of periodontal disease expressed by the levels of 8-OHdG.
- There is a direct proportionality between the oro-dental status evaluated by the CPITN index and the 8-OHdG level; CPITN index decreased after the treatment in parallel with the decrease of the 8-OHdG level.
- Our study allows us to conclude that saliva is a biological product that can be easily collected and may be used for the quantification of 8-OHdG as an oxidative stress biomarker in the diagnosis and monitorisation of the treatment in periodontitis; moreover, salivary 8-OHdG analysis for periodontal diagnosis may prove a cost-effective method for screening the population.

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References

1. Armitage G., Parodontal diagnosis and classification of periodontal disease, *Periodontology*, 2004, vol. 3, 9-21;
2. Angela A., Mariana M., Serum oxidative stress parameters and homocysteine levels in patients with renal transplant, *Farmacia*, 2008, nr. 3, 352-358;
3. Anna Orient, Ágnes Donkó, Attila Szabó, Thomas L. Leto, Miklós Geiszt, Novel sources of reactive oxygen species in the human body, *Nephrology Dialysis Transplantation*, 2007, 22 (5): 1281-1288;
4. Balwant Rai, Simmi Kharb, Effect of scaling and root planning on salivary 8-hidroxydeoxyguanosine levels: Periodontitis, *The Journal of Dental Science*, 2007, vol. 5, no 2, 1-10;

5. Cenk Fatih Canakci, Yasin Cicek, Abdulkadir Yildirim, Increased levels of 8-hidroxydeoxyguanosine and malondialdehyde and its relationship with antioxidant enzymes in saliva of periodontitis patients, *European Journal of Dentistry*, April 2009, vol. 3(2), 100-106;
6. H.M. Alessia, Oxidative stress exercise and aging, *Ed. World Scientific*, march 2006, vol. 1, 1-19;
7. Masatoshi Takane, Naoyuki Sugano, Tsunehiro Ezawa, Toshio Uchiyama, A marker of oxidative stress in saliva-association with periodontally-involved teeth of a hopeless prognosis, *Journal of Oral Science*, 2005, no. 1, vol. 47, 53-57;
8. Maria M., Maria G., Alexandra T, Redox-sensitive signalling factors and antioxidants, *Farmacia*, 2009, 4, 399-411;
9. Masatoshi Takane, Naoyuki Sugano, Iwasaki H, Iwano Y, Schimizu N, New biomarker evidence of oxidative DNA damage in whole saliva from clinically healthy and periodontal diseased individuals, *J. Periodontology*, may 2002, 73 (5): 551-555;
10. Olinescu R., Radicali liberi in fiziopatologia umana, Seria "Medicina", Editura Tehnica, Bucuresti, 1994, 132-156;
11. Taba M Jr, Kinney J, Kim AS, Giannobile, Diagnostic biomarkers for oral and periodontal diseases, *Dent Clin North Am.*, 2005 Jul; 49(3): 551-71;
12. W.H.O. Strategy for prevention and control of chronic diseases; Oral Health Surveys: basic method-4th.ed. W.H.O. Library Cataloguing in Publication Data, Geneva, Switzerland, 1997;
13. Yinsheng Wang, Bulky, DNA Lesions Induced by Reactive Oxygen Species, *Chem. Res. Toxicol.*, 2008, 21 (2), 276-281;
14. Y. Sawamoto, N. Sugano, H. Tanaka, Detection of periodontopathic bacteria and an oxidative stress marker in saliva from periodontitis patients, *Oral Microbiology and Immunology*, aug. 2005, vol. 20, 216-220;
15. Zhang Lei, Xiao Hua, Wong David T, Salivary Biomarkers for Clinical Applications, *Molecular Diagnosis & Therapy*, 2009 vol. 13, 245-259.

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