ASSESSMENT AND COMPARISON OF 8-HYDROXY-2-DEOXYGUANOSINE AS A BIOMARKER FOR DETECTION OF PERIODONTITIS: A PILOT STUDY.

Aiswarya Achari[1], Girish Suragimath[2]*, Siddhartha Varma[3], Sameer Zope[4], Ashwinirani SR[5]

Abstract

Introduction: Periodontitis is a multifactorial and infective oral disease leading to the destruction of the periodontium. Detecting periodontal disease with biomarkers will help in the diagnosis and assessment of prognosis. Reactive oxygen species (ROS) are produced by polymorphonuclear neutrophils (PMNs), during phagocytosis and digestion of periodontal pathogens. The reactive nature of ROS leads to oxidative stress causing host tissue damage locally and systemically. 8-Hydroxy-2-deoxyguanosine (8-OHdG), an oxidative stress biomarker, is implicated in inflammatory disease pathogenesis. The present study evaluated the 8-OHdG concentrations in saliva and serum of healthy subjects and periodontitis patients.

Methods: In this cross-sectional study, a total of 50 patients, 25 in each group i.e., healthy subjects and periodontitis patients were enrolled. Clinical and radiographic examinations were carried out to confirm the diagnosis of periodontitis. Blood and saliva samples were collected and subjected to enzyme-linked immunosorbent assay (ELISA) for evaluation of 8-OHdG. The obtained data were analysed and compared statistically.

Result: Periodontal pocket depth (PPD) and clinical attachment loss (CAL) were significantly higher in periodontitis patients ($p<0.001$). Serum and salivary 8-OHdG levels were significantly higher in periodontitis patients ($p<0.001$).

Conclusion: Salivary and serum 8-OHdG can be used as potential biomarkers for the diagnosis and prognosis of periodontal diseases.

Keywords: *Periodontitis, Oxidative Stress, Saliva, Serum, 8-Hydroxy-2-Deoxyguanosine, Reactive Oxygen Species*

[1]Post Graduate Student, Department of Periodontology, School of Dental Sciences, Krishna Vishwa Vidyapeeth Deemed to be University, Karad, Maharashtra, India-415110.
[2*]Professor and Head, Department of Periodontology, School of Dental Sciences, Krishna Vishwa Vidyapeeth Deemed to be University, Karad, Maharashtra, India-415110. E-Mail address: drgirishsuragimath@gmail.com
[3]Associate Professor, Department of Periodontology, School of Dental Sciences, Krishna Vishwa Vidyapeeth Deemed to be University, Karad, Maharashtra, India-415110.
[4]Associate Professor, Department of Periodontology, School of Dental Sciences, Krishna Vishwa Vidyapeeth Deemed to be University, Karad, Maharashtra, India-415110.
[5]Assistant Professor, Department of Oral Medicine Diagnosis and Radiology, School of Dental Sciences, Krishna Vishwa Vidyapeeth Deemed to be University, Karad, Maharashtra, India-415110.

***Corresponding author:** Girish Suragimath
*Department of Periodontology, School of Dental Sciences, Krishna Vishwa Vidyapeeth Deemed to be University, Karad, Maharashtra, India-415110. E-Mail address: drgirishsuragimath@gmail.com

DOI: -10.48047/ecb/2023.12.si5a.0582

Introduction

Periodontitis is a chronic inflammatory disease that affects the supporting tissues of the dental elements, resulting in loss of periodontal attachment and resorption of alveolar bone, which if left untreated can lead to tooth loss.[1] Polymorphonuclear neutrophils (PMNs) take part in the first immune phase.[2] PMNs produce antimicrobial factors called reactive oxygen species (ROS) during the phagocytosis and digestion of periodontal bacteria. ROS generation occurs through various mechanisms like protein disruption, lipid peroxidation, induction of proinflammatory cytokines and DNA damage.[3] Once the function of ROS is completed, antioxidants are produced to suppress their high reactivity.[4]

Homeostasis is a complex process in all living systems that controls and regulates system functions in response to the outside environment changes.[5] In the homeostasis system, the reactive oxygen species (ROS) regulate the balance between health and illness in a living system. During the correct equilibrium of oxidative stress, ROS behave as the cellular messenger, stimulate the production of molecules for the correct function of the cells, and stimulate the immune system to react against pathogens. When the infection persists, the balance of homeostasis is broken, and the levels of ROS increase, causing circulating oxidative stress, further resulting in oxidative damage and destruction of the host tissues locally and systemically.[6]

It was observed that in an oxidative stress environment, different elements of the periodontium such as collagen, elastin, proteoglycans, and glycosaminoglycans (hyaluronic acid) started to degrade.[7]

8-hydroxy-2-deoxyguanosine (8-OHdG), a modified base product of DNA represents one of the major products generated by the reaction of hydrogen peroxide and has been used as a marker of oxidative DNA damage.[8] 8-OHdG, one of more than 20 oxidative bases, was first reported by Kasai and Nishimura (1984).[9] 8-OHdG is a sensitive parameter for DNA damage and the most studied of base damage products.[10] Studies demonstrated that 8-OHdG in body fluids can act as a biomarker of oxidative stress and is implicated in the pathogenesis of malignancy, inflammatory and autoimmune disorders.[11]

Saliva is emerging as a highly popular diagnostic fluid due to its cost-effectiveness, bio-availability, non-invasive accessibility, minimally technique-sensitive collection standards and also due to the relative stability of salivary analytes for storage.[12]

Serum high sensitivity levels have been strongly associated with clinical periodontal parameters, and particularly with bleeding on probing, a marker of periodontal inflammation.[13] Biomarker determination in saliva and serum is becoming an important part of laboratory diagnosis and the prediction of periodontal diseases. There is dearth of knowledge regarding the role of 8-OHdG in periodontal diseases and its estimation through saliva. With this background the present study evaluated and compared the 8-OHdG concentrations in saliva and serum in healthy subjects and periodontitis patients.

Materials And Methods

Study Ethics

This cross-sectional, observational study was conducted in the department of Periodontology, School of Dental Sciences, Krishna Vishwa Vidyapeeth (KVV), Karad, Maharashtra, India.

The approval from the Institutional Ethical Committee of KVV (Protocol number-KVV/IEC/02/2021) was obtained prior initiation of the study.

Sample Size

Fifty patients reporting to the department of Periodontology were enrolled for the study. The sample size was calculated using the statistical formula $N= 2[Z\alpha+Z\beta]^2 / (\mu1-\mu2/ \delta)^2$, where $\mu1-\mu2/ \delta$ is defined as effect size (Es), $Z\alpha = 1.6448$, $Z\beta= 0.8416$ with level of significance 5% and power of 80%.

The selected patients were divided into two groups each consisting of 25 patients as healthy subjects (Group A) and Periodontitis patients (Group B).

Inclusion and exclusion criteria

Non-smoker patients in the age group of 30-55 years, willing to participate in the study were included. A non-smoker was considered according to Buduneli et al. as those who never smoked or reported having quit smoking at least two years.[14] Patients with any systemic diseases like diabetes mellites, cardiovascular diseases, pregnant and lactating females, patients on medication affecting the periodontal health or with history of periodontal therapy within three months were excluded from the study.

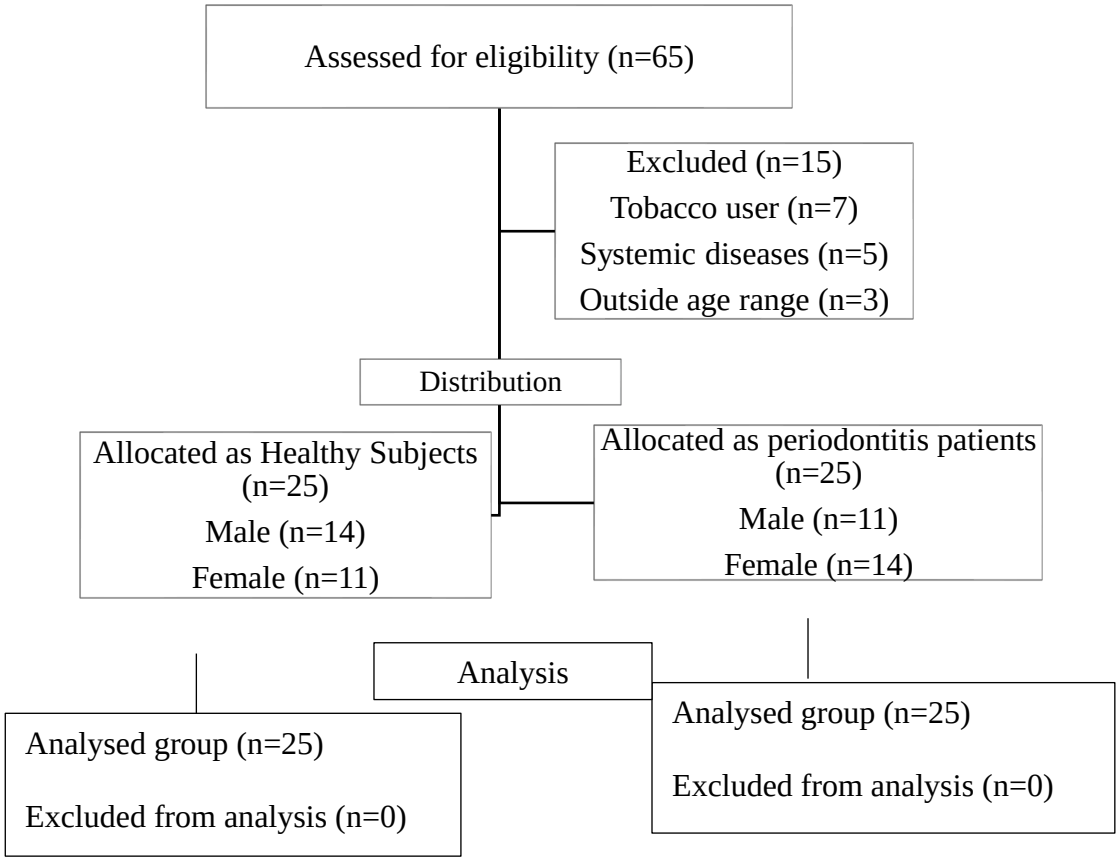
Selection Criteria for healthy subjects

Subjects who maintained good oral hygiene with plaque index (PI) and gingival Index (GI) score less than one. Subjects with less than ten percent of the sites with bleeding on probing (BOP), periodontal probing depth (PPD) less than or equal to three millimetres with absence of clinical attachment loss (CAL) were considered.

Selection Criteria for periodontitis patients

Staging and grading of periodontitis patients was arrived, based on the clinical and radiographic evaluation, according to Tonetti MS et al. 2018, and stage II grade B patients were recruited for the study.[15] Stage II included moderate periodontitis with interdental CAL of three to four millimetres and maximum probing pocket depth of less than or equal to five millimetres and radiographic horizontal bone loss of 15 to 33% at the coronal third. Grade B comprised of direct evidence of progression less than or equal to two millimetres over five years. Indirect evidence of disease progression (% bone loss/age) of up to one percent and destruction that commensurate with the biofilm deposits. The flow chart describes the distribution of the sample size in Figure 1.

Figure 1: Flow diagram of the selection of study subjects.



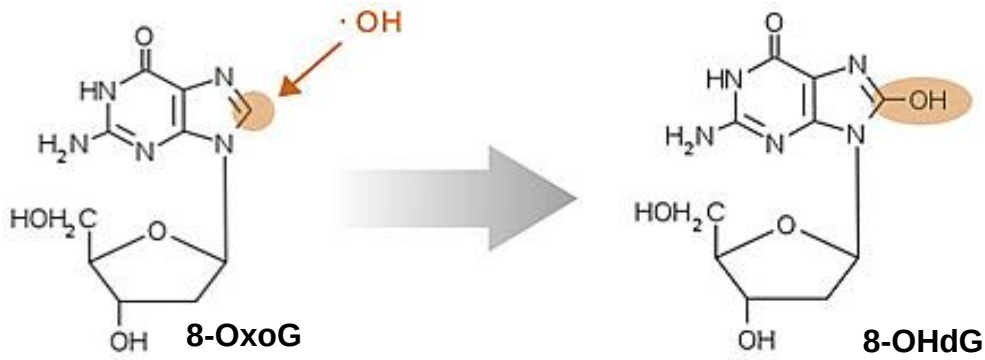


Figure 2: **Hydroxylation of 8-OxoG by the addition of an OH group at the eighth position leads to 8-OHdG formation**

Data Collection
Patient information sheet was provided and an informed consent was obtained from all the patient prior enrolling into the study. A thorough case history was documented in a specifically designed case proforma as per the inclusion criteria of the study.

Evaluation of the Periodontal Status
All study participants were evaluated clinically at their first visit in the Departments of Periodontology. Clinical parameters recorded were PI, GI, periodontal disease index (PDI), PPD and CAL using a Williams periodontal probe at six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual) of all teeth present, except third molars.

Sample Collection
Saliva and blood samples were collected from each patient.

Collection of saliva
Unstimulated two millilitres (ml) of whole saliva sample was collected in morning 10 am to 11 am, two hours after the last meal to standardize the collection according to the circadian rhythm. Prior collection of saliva samples, the subjects were asked to rinse thoroughly using distilled water. The subjects were asked to look downwards and saliva was collected by modified drain method. The desired volume (two millilitres) of saliva was pipetted out in a test tube. Saliva samples were centrifuged at 4000 rotations per minute (rpm) for ten minutes to remove the cell debris and half millilitres of the supernatant was stored in one and half millilitres plastic vial at -80°C, having a tracking number until analysis.

Collection of Blood
Under aseptic conditions, two millilitres of blood were collected from the antecubital fossa by venepuncture method using a 20-guage needle in plain vacutainer. The blood sample were allowed to clot at room temperature for one hour, serum was extracted from blood by centrifuging at 3000rpm for five minutes. The 0.5 ml of extracted serum was immediately transferred to a plastic vial and stored at -80ºC having a tracking number till the time of assay.

Estimation of salivary and serum 8-OHdG levels
Levels of serum and salivary 8-OHdG levels were determined using commercially available kit (8-OHdG ELISA kit, KTE60839, Abbkine Inc, Wuhan, China). The assay was carried out according to manufacturer's instruction and directions by using Elisa reader (LisaQuant TS, Tulip diagnostics Pvt. Ltd. Goa, India).

Statistical Analysis
The salivary and serum levels of 8-OHdG were calculated using quadratic regression equation. The data thus obtained was compiled and arranged in Microsoft Excel sheet (version 2019). All analysis were performed using SPSS (Statistical Package for the Social Sciences) software version 26. Mean PDI, PPD, CAL, saliva and serum 8-OHdg was obtained by using one-way ANOVA test. Pairwise comparison was done using Turkey's post hoc t-test.

Result
The current study was conducted to examine the serum and salivary levels of 8-OHdG in healthy subjects (Group A), periodontitis patients (Group B). The patients included in the study were subjected to clinical evaluation, and blood and saliva samples were collected and subjected to analysis for 8-OHdG levels.
Among the 50 patients, males were 25 (50%) and females were 25 (50%). Patients within the age range of 30-65 years were included in the study. In Group A the mean age was 30.8±4.42 years. In Group B the mean age was 43.92±11.66 years. The gender and age distribution is presented in Table 1. Comparison of periodontal parameters i.e., PDI, PPD and CAL shown in Table 2 were highly significant ($p<0.001$) between Group A and Group B.
On pairwise comparison of PDI scores, PPD and CAL, the mean difference between Group A and B were highly significant presented in Table 3.
Mean serum and salivary 8-OHdG levels were highly significant with both the groups (Table 4).
The mean difference of serum 8-OHdG between Group A and B were highly significant (Table 5).

Table 1: Comparison of gender and age distribution

Groups	Male N (%)	Female N (%)	Mean age
Group A (Periodontal healthy) (n=25)	14 (56%)	11 (44%)	30.8±4.42 years
Group B (Stage II Grade B periodontitis) (n=25)	11 (44%)	14 (56%)	43.92±11.66 years

Table 2: Comparison of PDI score, PPD and CAL

PDI	Mean	SD	One-way Anova F test	p-value, Significance
Group A (Periodontal healthy)	0.0	0.0	F = 408.64	p<0.001**
Group B (Stage II Grade B periodontitis)	5.44	0.91		
PPD	Mean	SD	One-way Anova F test	p-value, Significance
Group A (Periodontal healthy)	1.0	0.0	F = 395.79	p<0.001**
Group B (Stage II Grade B periodontitis)	6.08	0.95		
CAL	Mean	SD	One-way Anova F test	p-value, Significance
Group A (Periodontal healthy)	1.0	0.0	F =779.05	p<0.001**
Group B (Stage II Grade B periodontitis)	8.72	1.06		

**p<0.001 – highly significant

Table 3: Comparison of mean PDI score, PPD and CAL between healthy subjects (Group A), periodontitis patients (Group B)

Tukey's post hoc test for pairwise comparison			
Group	Parameter	Mean Difference	p-value, Significance
Group A (Periodontal healthy) vs Group B (Stage II Grade periodontitis)	PDI Score	5.44	p<0.001**
	PPD	5.08	p<0.001**
	CAL	7.72	p<0.001**

**p<0.001 – highly significant

Table 4: Comparison of serum and salivary 8-OHdG levels

Serum 8-OHdG	Mean	SD	One-way Anova F test	p-value, Significance
Group A (Periodontal healthy)	0.2949	0.596	F =16.038	p<0.001**
Group B (Stage II Grade B periodontitis)	0.3456	0.138		
Salivary 8-OHdG	Mean	SD	One-way Anova F test	p-value, Significance
Group A (Periodontal healthy)	1.6715	0.37	F = 3.507	p<0.001**
Group B (Stage II Grade B periodontitis)	1.9375	0.418		

**p<0.001 – highly significant

Table 5: Comparison of serum and salivary 8-OHdG between healthy subjects (Group A), periodontitis patients (Group B)

Tukey's post hoc test for pairwise comparison			
Group	Sample	Mean Difference	p-value, Significance
Group A (Periodontal healthy) vs Group B (Stage II Grade B periodontitis)	Serum	0.0507	p<0.001**
	Saliva	0.0266	p<0.001**

**p<0.001 – highly significant

Discussion

Living organisms have progressed various defence mechanisms in order to provide a balance between the formation and elimination of ROS in enduring oxygen-rich cellular environments. The imbalance between the systemic production of ROS and the ability of cells to detoxify the ROS or to restore the impairment is termed as "oxidative stress".[16] Periodontitis results due to the destruction of supporting structures of the teeth in response to a variety of agents both of host and bacterial origin. The progression of periodontitis depends on the interaction between the host and the dental plaque.[17] 8-OHdG is formed when a hydroxyl group gets added to the eighth position of 8-hydroxyguanine (8-OxoG) on oxidation (Figure 2).[18] During periodontal inflammation, high level of saliva 8-OHdG and low level of saliva antioxidant, creates increased oxygen radical activity. A correlation was reported between 8-OHdG and periodontopathogenic bacteria and the determination of 8-OHdG level in saliva is a useful biomarker to evaluate the effectiveness of periodontal state and periodontal treatment.[17] Numerous DNA damage products are produced during oxidative damage by DNA base modifications, covalent crosslinks or single or double-stranded breaks. Among DNA nucleobases, guanine has the lowest oxidation potential and is the most prone to oxidative damage.[19] 8-OHdG being water soluble is secreted out of the cell into the extracellular space after being removed from the DNA helix. Therefore, monitoring extracellular 8-OHdG will provide insight into oxidative DNA damage.[20]

In our study the gender predilection was 50% with age range from 30 to 43 years, which is similar to the study by Villa-Correa et al., 2015, who noted no statistical differences in age and gender.[21]

In our study, the mean PPD and CAL was significantly higher in patients with periodontitis as compared to healthy subjects. Our results were similar to the study conducted by U. Sezer et al in 2012, who proved that both PPD and CAL were highest in chronic periodontitis patients followed by chronic gingivitis patients and healthy subjects.[22]

Fenotglu et al in 2015 conducted a study to determine the serum levels of malondialdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG) in chronic periodontitis (CP) and hyperlipidaemia patients. The authors concluded that increased levels of MDA and 8-OHdG in hyperlipidaemia patients may be a result of a harmful oxidative status in association with hyperlipidaemia and periodontitis.[23] Our study showed similar results implying that the serum 8-OHdG levels were significantly higher in patients with periodontitis as compared to healthy subjects.

In our study, salivary 8-OHdG levels were significantly higher in patients with periodontitis compared to healthy subjects. The results were in accordance to a study conducted by S. Merve Altıngoz et al in 2021, investigating the association between 8-OHdG, glycation and inflammation markers and periodontal clinical parameters in periodontitis and periodontally healthy patients with type 2 diabetes and corresponding systemically healthy controls. Salivary 8-OHdG levels were significantly higher in periodontitis compared to periodontally healthy patients, regardless of systemic status.[13]

Kurgan et al 2015 conducted a similar study and proved that salivary and serum levels of 8-OHdG levels significantly higher in the chronic periodontitis group in comparison with the control group. Serum levels of 8-OHdG were lower than the salivary levels; increased dilution of the molecule in blood and low sensitivity of the ELISA may be accountable for lower concentration of 8-OHdG levels in serum. Salivary 8-OHdG may be associated with local impact of periodontal disease.[24]

Serum and salivary 8-OHdG levels were elevated in periodontitis patients, denoting that the levels of both serum and salivary 8-OHdG can be used as a diagnostic and prognostic biomarker for locally induced oxidative stress in periodontitis patients. Dental chairside diagnostic kit assessing the salivary 8-OHdG levels, should be created for diagnosis of periodontitis and assessment of prognosis during treatment of periodontal diseases.

Acknowledgements

We would like to thank all study subjects for participating in this study.

Declaration of interest statement

N/A

References

1. Van Dyke TE, Bartold PM, Reynolds EC. The Nexus Between Periodontal Inflammation and Dysbiosis. Front Immunol. 2020 Mar 31; 11:511. doi: 10.3389/fimmu.2020.00511.
2. Slots J. Periodontitis: facts, fallacies and the future. Periodontol 2000. 2017 Oct;75(1):7-23. doi: 10.1111/ prd.12221.
3. Arunachalam, R., Reshma, A. P., Rajeev, V., Kurra, S. B., Prince, M. R. J., & Syam, N. (2015). Salivary 8-Hydroxydeoxyguanosine – a valuable indicator for oxidative DNA damage in periodontal disease. The Saudi Journal for Dental Research, 6(1), 15–20. doi: 10.1016/j.sjdr.2014.04.002
4. Tretter V, Hochreiter B, Zach ML, Krenn K, Klein KU. Understanding Cellular Redox Homeostasis: A Challenge for Precision Medicine. Int J Mol Sci. 2021 Dec 22;23(1):106. doi: 10.3390/ijms23010106.
5. Billman GE. Homeostasis: The Underappreciated and Far Too Often Ignored Central Organizing Principle of Physiology. Front Physiol. 2020 Mar 10; 11:200. doi: 10.3389/fphys.2020.00200.
6. Dahiya P, Kamal R, Gupta R, Bhardwaj R, Chaudhary K, Kaur S. Reactive oxygen species in periodontitis. J Indian Soc Periodontol. 2013 Jul;17(4):411-6. doi: 10.4103/0972-124X.118306.
7. Nessa, N., Kobara, M., Toba, H., Adachi, T., Yamamoto, T., Kanamura, N., … Nakata, T. (2021). Febuxostat Attenuates the Progression of Periodontitis in Rats. Pharmacology, 106(5-6), 294–304. doi:10. 1159/000513034
8. Ock CY, Kim EH, Choi DJ, Lee HJ, Hahm KB, Chung MH. 8-Hydroxydeoxyguanosine: not mere biomarker for oxidative stress, but remedy for oxidative stress-implicated gastrointestinal diseases. World J Gastroenterol. 2012 Jan 28;18(4):302-8. doi: 10.3748/wjg.v18.i4.302.
9. Kasai H, Nishimura S. DNA damage induced by asbestos in the presence of hydrogen peroxide. Gan. 1984 Oct;75(10):841-4.

10. Cardin R, Piciocchi M, Bortolami M, Kotsafti A, Barzon L, Lavezzo E, Sinigaglia A, Rodriguez-Castro KI, Rugge M, Farinati F. Oxidative damage in the progression of chronic liver disease to hepatocellular carcinoma: an intricate pathway. World J Gastroenterol. 2014 Mar 28;20(12):3078-86. doi: 10.3748/wjg. v20.i12.3078.
11. Pilger A, Rüdiger HW. 8-Hydroxy-2'-deoxyguanosine as a marker of oxidative DNA damage related to occupational and environmental exposures. Int Arch Occup Environ Health. 2006 Oct;80(1):1-15. doi: 10.1007/s00420-006-0106-7.
12. Kc S, Wang XZ, Gallagher JE. Diagnostic sensitivity and specificity of host-derived salivary biomarkers in periodontal disease amongst adults: Systematic review. J Clin Periodontol. 2020 Mar;47(3):289-308. doi: 10.1111/jcpe.13218.
13. Altıngöz SM, Kurgan Ş, Önder C, Serdar MA, Ünlütürk U, Uyanık M, Başkal N, Tatakis DN, Günhan M. Salivary and serum oxidative stress biomarkers and advanced glycation end products in periodontitis patients with or without diabetes: A cross-sectional study. J Periodontol. 2021 Sep;92(9):1274-1285. doi: 10.1002/JPER.20-0406.
14. Buduneli N, Kardeşler L, Işik H, Willis CS 3rd, Hawkins SI, Kinane DF, Scott DA. Effects of smoking and gingival inflammation on salivary antioxidant capacity. J Clin Periodontol. 2006 Mar;33(3):159-64. doi: 10.1111/j.1600-051X.2006.00892. x.
15. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. J Periodontol. 2018 Jun;89 Suppl 1: S159-S172. doi: 10.1002/JPER.18-0006.
16. Bardaweel SK, Gul M, Alzweiri M, Ishaqat A, ALSalamat HA, Bashatwah RM. Reactive Oxygen Species: the Dual Role in Physiological and Pathological Conditions of the Human Body. Eurasian J Med. 2018 Oct;50(3):193-201.
doi: 10.5152/ eurasianjmed. 2018.
17. Hendek MK, Erdemir EO, Kisa U, Ozcan G. Effect of initial periodontal therapy on oxidative stress markers in gingival crevicular fluid, saliva, and serum in smokers and non-smokers with chronic periodontitis. J Periodontol. 2015 Feb;86(2):273-82. doi: 10. 1902/jop.2014.140338.
18. Floyd RA, Watson JJ, Wong PK, Altmiller DH, Rickard RC. Hydroxyl free radical adduct of deoxyguanosine: sensitive detection and mechanisms of formation. Free Radic Res Commun. 1986;1(3):163-72.
doi: 10.3109/ 10715768609083148.
19. Sriram Kanvah and Gary B. Schuster. Oxidative damage to DNA: Inhibition of guanine damage. Pure Appl. Chem. Pure Appl. Chem., Vol. 78, No. 12, pp. 2297–2304, 2006; Vol. 78, No. 12, pp. 2297–2304.
20. Zhang Y, Newcomb PA, Egan KM, Titus-Ernstoff L, Chanock S, Welch R, Brinton LA, Lissowska J, Bardin-Mikolajczak A, Peplonska B, Szeszenia-Dabrowska N, Zatonski W, Garcia-Closas M. Genetic polymorphisms in base-excision repair pathway genes and risk of breast cancer. Cancer Epidemiol Biomarkers Prev. 2006 Feb;15(2):353-8. doi: 10.1158/1055-9965.
21. Villa-Correa YA, Isaza-Guzmán DM, Tobón-Arroyave SI. Prognostic Value of 8-Hydroxy-2'-Deoxyguanosine and Human Neutrophil Elastase/α1-Proteinase Inhibitor Complex as Salivary Biomarkers of Oxidative Stress in Chronic Periodontitis. J Periodontol. 2015 Nov;86(11):1260-7. doi: 10.1902/jop .2015. 150293.
22. Sezer U, Ciçek Y, Canakçi CF. Increased salivary levels of 8-hydroxydeoxyguanosine may be a marker for disease activity for periodontitis. Dis Markers. 2012;32(3):165-72. doi: 10.3233/DMA-2011-0876.
23. Fentoğlu Ö, Kırzıoğlu FY, Bulut MT, Kumbul Doğuç D, Kulaç E, Önder C, Günhan M. Evaluation of lipid peroxidation and oxidative DNA damage in patients with periodontitis and hyperlipidemia. J Periodontol. 2015 May;86(5):682-8. doi: 10.1902/jop. 2015. 140561.
24. Kurgan Ş, Önder C, Altıngöz SM, Bağış N, Uyanık M, Serdar MA, Kantarcı A. High sensitivity detection of salivary 8-hydroxy deoxyguanosine levels in patients with chronic periodontitis. J Periodontal Res. 2015 Dec;50(6):766-74. doi: 10.1111/jre.12263.