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Periodontal Disease and Nuclear and Oxidative DNA Damage

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Abstract

Oral health is an important aspect of the overall health status of an individual. DNA damage has been associated with oral health and dental factors due to the increased of oxidative stress (OxS). DNA damage can produce a wide range of effects on human health. These effects could appear immediately, but others do not become evident much later. Chronic diseases have been study to understand their mechanisms, clinical implications, and the development of secondary disease such as cancer. Periodontitis is one of the most common oral diseases. It is an inflammatory chronic infectious disease, which is characterized by the loss of supporting tissues and tooth loss caused by periodontopathogens and long-term release of reactive oxygen species (ROS); thus, oxidative stress is increased during periodontitis. Oxidative stress can produce DNA damage, including the oxidation of nucleosides, which could cause DNA strand break. This oxidative damage leads the formation of micronuclei (MN) a marker of nuclear damage. Also, oxidative stress increased 8-hydroxy-2'-deoxyguanosine levels which are the most common stable product of oxidative DNA damage.

Keywords: periodontal disease, buccal mucosa, DNA damage, oxidative stress, saliva.

1. Introduction

DNA damage can generate many effects on human health and is the prime mechanism during carcinogenesis. Many of these effects could emerge directly, but others do not become

evident until much later. Chronic diseases have been studied to understand their mechanisms of perpetuation of clinical complications and the development of secondary diseases such as cancer [1]. Oxidative stress (OxS) and, therefore, DNA damage has an important impact on the pathogenesis of chronic disease [2]. Periodontal diseases are inflammatory disorders characterized by gingival inflammation in which periodontopathic bacteria generate immunological inflammatory responses [3]. The OxS plays an important role in the pathogenesis of periodontitis, which can lead damage to genetic material [4]. Since periodontal disease is an example of the excess effects locally and systemically of OxS over production, the effect of periodontal disease over oxidative and nuclear DNA damage is the topic attended in this chapter.

2. Periodontitis

Moreover, dental caries, periodontal disease is one of the most prevalent oral diseases in the world and includes the major conditions gingivitis and periodontitis, which is a group of conditions affecting the supporting tissues of the teeth—the gingiva, periodontal ligament, cementum, and alveolar bone [1]. The reversible form of the disease, gingivitis, comprises inflammation of the gingival tissue without loss of alveolar bone. It is plaque induced and can be reversed with improved oral hygiene. In disease-susceptible individuals, gingivitis may develop into periodontitis, which is a chronic inflammatory condition of the gingiva causing destruction of connective tissue as well as of alveolar bone resulting in reduced support for the teeth and, ultimately, tooth loss (**Figure 1**) [5].

Periodontitis initiated by the complex interaction between the presence of periodontal pathogens (e.g., *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Fusobacterium nucleatum*) and host response [6]. Besides, the presence of periodontal pathogens associated with the progressive form of the disease, microbial by-products, the host immune response, environmental and behavior factors, and genetics may contribute the risk for developing periodontal disease [7].

Two main forms of periodontitis have been identified, chronic or aggressive, and are characterized by gingival inflammation and bleeding, periodontal pocket formation, destruction of



Figure 1. Characteristics of periodontitis. Healthy periodontal tissue (right) and periodontitis (left). Periodontitis is characterized by progressive and irreversible alveolar bone loss, and, ultimately, loosening and loss of teeth.

connected tissues attachment, and alveolar bone loss. But, loss of connective tissue attachment is faster in aggressive periodontitis than in chronic periodontitis [8].

Moreover, the pathogenesis of periodontal diseases is mediated by the inflammatory response to bacteria in the dental biofilm. The inflammatory reaction that characterizes periodontitis is the complex interaction between periodontopathic bacteria and the host defense system, and its purpose is to protect the tissues from bacterial attack [6]. In periodontitis, neutrophil plays an important role in the initial host inflammation. Exaggerated neutrophil activity is the biological features characteristic of the periodontitis phenotype in agreement with a hyper-inflammatory host response. Thus, reactive oxygen species (ROS) produced from neutrophil are implicated in the destruction of periodontal tissue; therefore, oxidative stress (OxS) is enhanced during periodontitis [9].

3. Free radicals (FR) and oxidative stress

A free radical (FR) is any molecular species that contains at least one unpaired electron. The unpaired electron increased the chemical reactivity of an atom or molecule that generates a high instability. Due to the increase in FR, oxidative stress (OxS) arises. The OxS has been defined as a disturbance in the balance between the production of reactive oxygen/nitrogen species (ROS/RNS; FR) and the antioxidant defense system capacity to counteract their action [10]. The OxS occurs from an improved ROS/RNS generation or from a deterioration of the antioxidant protective ability. This process leads to the oxidation of biomolecules with consequent loss of its biological functions, whose manifestation is the potential oxidative damage to cells and tissues. Accumulation of ROS/RNS can result in several adverse effects such as lipid peroxidation, protein oxidation, and DNA damage (Figure 2) [11].

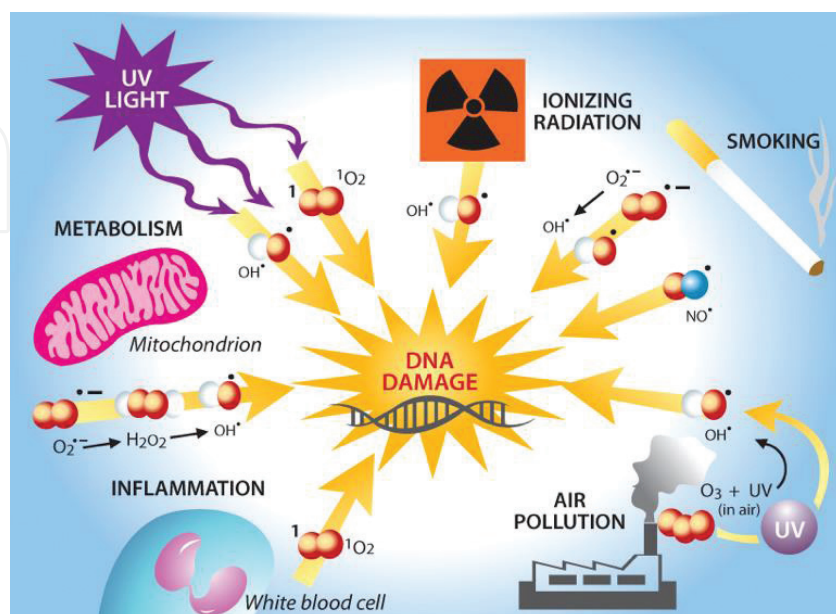


Figure 2. Sources of free radicals that arise oxidative stress.

4. Nuclear and oxidative DNA damage

DNA is chemically unstable and vulnerable to oxidation, due to its susceptibility to endogenous and exogenous damage. The endogenous genotoxic agents are mainly produced by cellular metabolism and composed of ROS and RNS, estrogen metabolites, and aldehydes produced by lipid peroxidation [12, 13]. The OxS leads to different lesions in DNA, including direct modification of nucleotide bases, training sites a purinic/a pyrimidinic, single strand break, the oxidation of nucleosides, which could cause DNA strand breaks; this type of damage could have teratogenic or carcinogenic consequences [14].

One method for measuring DNA nuclear damage is the micronuclei (MN) assay [15–17]. MN are extranuclear bodies originated from chromosome fragments or whole chromosome that spontaneously or because of clastogenic (agents who broke chromosome) or aneuploidogenic (disrupted the spindle apparatus) agents that were not incorporated into the nucleus after cell division (**Figure 3**) [18]. The MN formation leads to cell death, genomic instability, or cancer development. Therefore, the increased in MN frequency is linking to environmental and occupational exposure to genotoxic agents, lifestyle, genetic profile cancer, and occurrence of other diseases, and MN screening is considered as a biomarker of DNA nuclear damage [19].

On the other hand, different markers of oxidative DNA damage have been identified. The most popular markers were designed for lipid peroxidation, such as malondialdehyde (MDA), oxidized low-density lipoprotein (LDL), MDA-modified LDL, among other. In recent years, 8-hydroxy-2'-deoxyguanosine (8-OHdG or 8-oxodG) has appeared as a marker of oxidative stress in body fluids [20]. The 8-OHdG is the most common stable product of oxidative DNA damage caused by ROS. Among all purine and pyridine bases, guanine is most susceptible to oxidation. Hydroxyl radical addition to the eighth position of the molecule leads to the formation of guanine-modified product 8-OHdG (**Figure 4**) [21]. Oxidative-modified DNA in the form of 8-OHdG can be quantified to indicate the extent damage to genetic material is the most frequent and most mutagenic lesion in nuclear DNA and is important in mutagenesis and carcinogenesis processes [22].

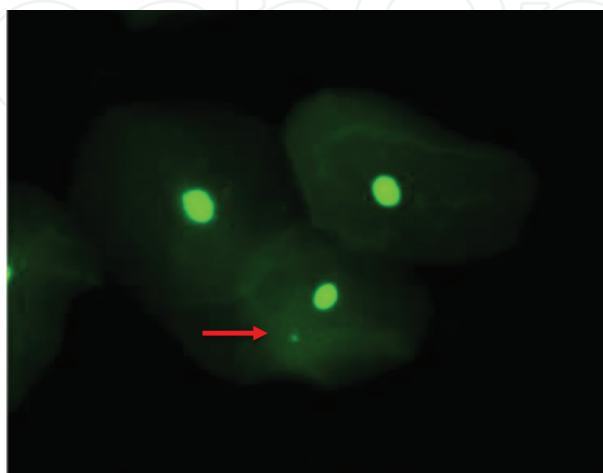


Figure 3. MN in buccal mucosa cells (oil-immersion objective 60x, acridine orange stain).

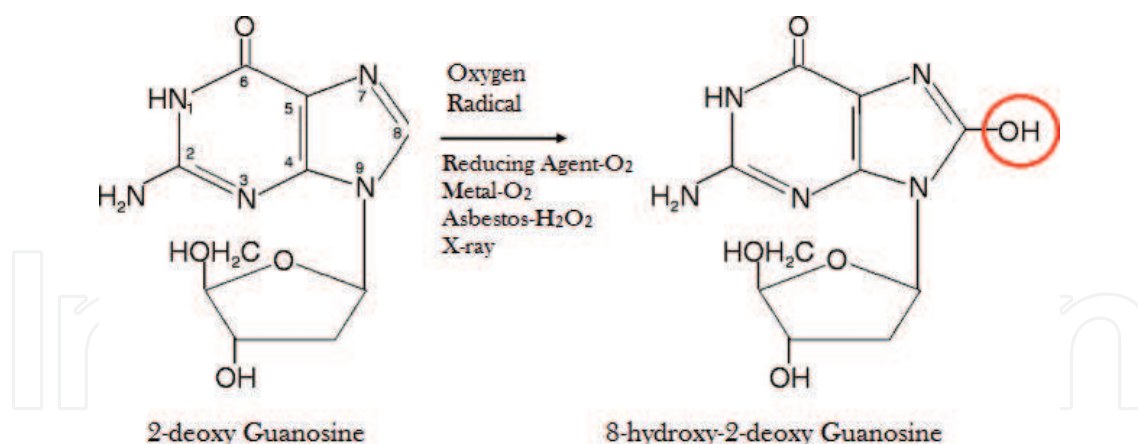


Figure 4. 8-OHdG formation by oxygen radicals.

5. Periodontitis and OxS: nuclear and oxidative DNA damage

The OxS plays an important role in the pathology of several diseases, including arthritis, Alzheimer’s disease, diabetes, Parkinson’s disease, and more recently periodontitis. The OxS is a phenomenon that occurs within the periodontal disease and has been linked with both onset of periodontal tissue destruction [23] and systemic inflammation [24]. Inflammatory periodontal disease resulting in tissue damage is mediated by ROS which are formed during the phagocytosis of periodontopathic bacteria by polymorphonuclear leukocytes. ROS generation can occur through different mechanisms such as protein disruption, lipid peroxidation, induction of proinflammatory cytokines, and DNA damage [22]. Therefore, periodontitis is associated with OxS which in turn can lead to nuclear and oxidative DNA damage and thus the formation of MN and 8-OHdG [25].

Some authors have been demonstrated that individual with periodontal disease exhibited an increase in the frequency of MN, which is directly related to DNA damage [25–27]. Similarly, elevated MN frequency has been reported in patients with cancer [28], rheumatoid arthritis [29], autoimmune diseases [30], and premature aging syndrome [31]. The presence of MN in a cell is an indicator of DNA damage and genetic instability, and it could be associated with the collateral complications in these patients and with future risk of cancer development in humans.

On the other hand, 8-OHdG level has been studied in oral pathologies, including periodontal disease [25, 32–35] and oral cancer [36]. As described above, in periodontitis OxS because of the formation of ROS, which is stimulated by neutrophils, produce damage of the bone-supporting tissues. The exceeds of ROS levels, the reduction of antioxidant enzyme activity, and defects in DNA repair mechanism led to increased oxidative DNA damage [35].

8-OHdG is used as a standard biomarker of oxidative-induced DNA damage mainly because of its reliable detectability. Elevated levels of 8-OHdG from cancer patients compared with healthy subjects have been observed in lung cancer [37], basal cell carcinoma [38], colorectal cancer [39], bladder cancer [40], and renal cell carcinoma [41]. With respect to periodontitis, published data on oxidative damage to DNA have been reported by many authors around

the world who investigated 8-OHdG levels in the saliva of periodontitis patients [4, 25, 33, 34]. These studies demonstrated that salivary levels of 8-OHdG in samples from periodontitis patients were significantly higher than those from periodontally healthy controls and indicated that salivary 8-OHdG levels may be a useful marker for disease activity and may be indirectly reflect disease severity parameters [4, 25, 33, 34].

Also, a study report significant positive correlation between MN frequency (a marker of nuclear damage) and 8-OHdG enzyme levels in subjects with periodontitis. This finding could suggest that these variables are associated and can be hypothesized that they could be linked in the development of periodontal disease [25]. The augmented 8-OHdG levels might indicate early oxidative mitochondrial DNA damage [33], and mitochondrial DNA undertakes OxS early than nuclear DNA [42].

6. Conclusion

Periodontal diseases are prevalent in human populations and represent a significant public health problem [43], and oxidative damage plays an important function in the pathogeny of the disease [9]. Nuclear and oxidative DNA damage area increased in subjects with periodontal disease, and genetic damage is a critical event not only in the initiation phase but also in the promotion and progression phases, which could be related to carcinogenesis events. Moreover, recent studies have associated periodontitis with some cancer including head and neck cancer [44], pancreatic cancer [45], colon cancer [46], and orodigestive cancers [47], which are relevant to the control of this disease and to promote the importance of good oral health.

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References

- [1] Yfjord JE, Bodvarsdottir SK. Genomic instability and cancer: Networks involved in response to DNA damage. *Mutation Research*. 2005;**592**:18-28. DOI:10.1016/j.mrfmmm.2005.05.010
- [2] Wu LL, Chiou CC, Chang PY, Wu JT. Urinary 8-OHdG: A marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. *Clinical Chimica Acta*. 2004;**339**:1-9. DOI: 10.1016/j.cccn.2003.09.010
- [3] Papapanou PN. Epidemiology of periodontal diseases: An update. *International Academy of Periodontology*. 1999;**1**:110-116.
- [4] Sezer U, Çiçek Y, Canakçi CF. Increased salivary levels of 8-hydroxydeoxyguanosine may be a marker for disease activity for periodontitis. *Disease Markers*. 2012;**32**:165-172. DOI:10.3233/DMA-2011-0876
- [5] Yucel-Lindberg T, Båge T. Inflammatory mediators in the pathogenesis of periodontitis. *Expert Reviews in Molecular Medicine*. 2013;**5**:15:e7. DOI:10.1017/erm.2013.8
- [6] Kornman KS. Mapping the pathogenesis of periodontitis: A new look. *Journal of Periodontology*. 2008;**79**:1560-1568. DOI: 10.1902/jop.2008.080213
- [7] Seymour GJ. Possible mechanisms involved in the immunoregulation of chronic inflammatory periodontal disease. *Journal of Dental Research*. 1987;**66**:2-9. DOI: 10.1177/00220345870660010401
- [8] Savage A, Eaton KA, Moles DR, Needleman I. A systematic review of definitions of periodontitis and methods that have been used to identify this disease. *Journal of Clinical Periodontology*. 2009;**36**:458-467. DOI: 10.1111/j.1600-051X.2009.01408.x
- [9] Chapple IL, Matthews JB. The role of reactive oxygen and antioxidant species in periodontal tissue destruction. *Periodontology*. 2000. 2007;**43**:160-232. DOI: 10.1111/j.1600-0757.2006.00178.x
- [10] Betteridge DJ. What is oxidative stress? *Metabolism*. 2000;**49**:3-8.
- [11] Lafleur MV, Retel J. Contrasting effects of SH-compounds on oxidative DNA damage: Repair and increase of damage. *Mutation Research*. 1993;**295**:1-10. DOI:10.1016/0921-8734(93)90006-O
- [12] De Bont R, van Larebeke N. Endogenous DNA damage in humans: A review of quantitative data. *Mutagenesis*. 2004;**19**:169-185.
- [13] Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry* 2015;**97**:55-74. DOI: 10.1016/j.ejmech.2015.04.040
- [14] Yfjord JE, Bodvarsdottir SK. Genomic instability and cancer: Networks involved in response to DNA damage. *Mutation Research*. 2005;**592**:18-28. DOI:10.1016/j.mrfmmm.2005.05.010

- [15] Torres-Bugarín O, Covarrubias-Bugarín R, Zamora-Perez AL, Torres-Mendoza BM, García-Ulloa M, Martínez-Sandoval FG. Anabolic androgenic steroids induce micronuclei in buccal mucosa cells of bodybuilders. *British Journal of Sports Medicine*. 2007;**41**:592-596. DOI: 10.1136/bjism.2006.032474
- [16] Zúñiga-González GM, Batista-González CM, Gómez-Meda BC, Zamora-Perez AL, Muñoz-Magallanes T, Ramos-Valdés C, Gallegos-Arreola MP. Micronuclei in diabetes: Folate supplementation diminishes micronuclei in diabetic patients but not in an animal model. *Mutation Research*. 2007;**634**:126-134. DOI:10.1016/j.mrgentox.2007.06.006
- [17] Zamora-Perez AL, Mariaud-Schmidt RP, Fuentes-Lerma MG, Guerrero-Velázquez C, Gómez-Meda BC, López-Verdín S, Zúñiga-González GM. Increased number of micronuclei and nuclear anomalies in buccal mucosa cells from people exposed to alcohol-containing mouthwash. *Drug and Chemical Toxicology*. 2013;**36**:255-260. DOI: 10.3109/01480545.2012.737804
- [18] Heddle JA, Cimino MC, Hayashi M, Romagna F, Shelby MD, Tucker JD, Vanparrys P, MacGregor JT. Micronuclei as an index of cytogenetic damage: Past, present and future. *Environmental and Molecular Mutagenesis*. 1991;**18**:227-291.
- [19] Bonassi S, Ugolini D, Kirsch-Volders M, Strömberg U, Vermeulen R, Tucker JD. Human population studies with cytogenetic biomarkers: Review of the literature and future perspectives. *Environmental and Molecular Mutagenesis*. 2005;**45**:258-270.
- [20] Sosa V, Moline T, Somoza R, Paciucci R, Kondoh H, LLeonart ME. Oxidative stress and cancer: An overview. *Ageing Research Reviews*. 2013;**12**:376-390. DOI: 10.1016/j.arr.2012.10.004
- [21] Di Minno A, Turnu L, Porro B, Squellerio I, Cavalca V, Tremoli E, Di Minno MN. 8-Hydroxy-2-deoxyguanosine levels and heart failure: A systematic review and meta-analysis of the literature. *Nutrition, Metabolism & Cardiovascular Diseases*. 2016;**16**:30194-30196. DOI: 10.1016/j.numecd.2016.10.009
- [22] Evans MD, Cooke MS. Factors contributing to the outcome of oxidative damage to nucleic acids. *Bioessays*. 2004;**26**:533-542. DOI: 10.1002/bies.20027
- [23] Chapple IL, Matthews JB. The role of reactive oxygen and antioxidant species in periodontal tissue destruction. *Periodontology*. 2000. 2007;**43**:160-232. DOI: 10.1111/j.1600-0757.2006.00178.x
- [24] Lamster IB, Pagan M. Periodontal disease and the metabolic syndrome. *International Dental Journal*. 2016;**67**:67-77. DOI: 10.1111/idj.12264
- [25] Zamora-Perez AL, Ortiz-García YM, Lazalde-Ramos BP, Guerrero-Velázquez C, Gómez-Meda BC, Ramírez-Aguilar MÁ, Zúñiga-González GM. Increased micronuclei and nuclear abnormalities in buccal mucosa and oxidative damage in saliva from patients with chronic and aggressive periodontal diseases. *Journal of Periodontology Research*. 2015;**50**:28-36. DOI: 10.1111/jre.12175
- [26] Bastos-Aires D, Azevedo A, Pereira ML, Perez-Mongiovi D, Teixeira A. Preliminary study of micronuclei levels in oral exfoliated cells from patients with periodontitis. *Journal of Dental Sciences*. 2013;**8**:200-204. DOI:10.1016/j.jds.2012.12.007

- [27] D'Agostini F, Calcagno E, Micale RT, La Maestra S, De Flora S, Cingano L. Cytogenetic analysis of gingival epithelial cells, as related to smoking habits and occurrence of periodontal disease. *International Journal of Hygiene and Environmental Health*. 2013;**216**:71-75. DOI: 10.1016/j.ijheh.2012.01.005
- [28] Duffaud F, Orsière T, Villani P, Pelissier AL, Volot F, Favre R, Botta A. Comparison between micronucleated lymphocyte rates observed in healthy subjects and cancer patients. *Mutagenesis*. 1997;**12**:227-231.
- [29] Ramos-Remus C, Dorazco-Barragan G, Aceves-Avila FJ, Alcaraz-Lopez F, Fuentes-Ramirez F, Michel-Diaz J, Torres-Bugarin O, Ventura-Aguilar A, Zuñiga-González G. Genotoxicity assessment using micronuclei assay in rheumatoid arthritis patients. *Clinical and Experimental Rheumatology*. 2002;**20**:208-212.
- [30] Milenkova M, Milanov I, Kmetska K, Deleva S, Popova L, Hadjidekova V, Groudeva V, Hadjidekova S, Domínguez I. Chromosomal radiosensitivity in patients with multiple sclerosis. *Mutation Research*. 2013;**749**:3-8. DOI: 10.1016/j.mrfmmm.2013.08.004
- [31] Weirich-Schwaiger H, Weirich HG, Gruber B, Schweiger M, Hirsch-Kauffmann M. Correlation between senescence and DNA repair in cells from young and old individuals and in premature aging syndromes. *Mutation Research*. 1994;**316**:37-48. DOI:10.1016/0921-8734(94)90006-X
- [32] Akalın FA, Baltacıoğlu E, Alver A, Karabulut E. Lipid peroxidation levels and total oxidant status in serum, saliva and gingival crevicular fluid in patients with chronic periodontitis. *Journal of Clinical Periodontology*. 2007;**34**:558-565. DOI: 10.1111/j.1600-051X.2007.01091.x
- [33] Takane M, Sugano N, Iwasaki H. New biomarker evidence of oxidative DNA damage in whole saliva from clinically healthy and periodontally diseased individuals. *Journal of Periodontology*. 2002;**73**:551-554. DOI: 10.1902/jop.2002.73.5.551
- [34] Canakçı CF, Canakçı V, Tatar A, Eltas A, Sezer U, Çiçek Y, Oztas S. Increased salivary level of 8-hydroxydeoxyguanosine is a marker of premature oxidative mitochondrial DNA damage in gingival tissue of patients with periodontitis. *Archivum Immunologiae et Therapiae Experimentalis (Warsz)*. 2009;**57**:205-211. DOI: 10.1007/s00005-009-0026-9
- [35] Su H, Gornitsky M, Velly AM, Yu H, Benarroch M, Schipper HM. Salivary DNA, lipid, and protein oxidation in nonsmokers with periodontal disease. *Free Radical Biology and Medicine*. 2009;**46**:914-921.
- [36] Bahar G, Feinmesser R, Shpitzer T, Popovtzer A, Nagler RM. Salivary analysis in oral cancer patients: DNA and protein oxidation, reactive nitrogen species, and antioxidant profile. *Cancer*. 2007;**109**:54-59. DOI: 10.1002/cncr.22386
- [37] Erhola M, Toyokuni S, Okada K, Tanaka T, Hiai H, Ochi H, Uchida K, Osawa T, Nieminen MM, Alho H, Kellokumpu-Lehtinen P. Biomarker evidence of DNA oxidation in lung cancer patients: Association of urinary 8-hydroxy-2'-deoxyguanosine excretion with radiotherapy, chemotherapy, and response to treatment. *FEBS Letter*. 1997;**409**:287-291. DOI: 10.1016/S0014-5793(97)00523-1

- [38] Kinoshita A, Wanibuchi H, Morimura K, Wei M, Shen J, Imaoka S, Funae Y, Fukushima S. Phenobarbital at low dose exerts hormesis in rat hepatocarcinogenesis by reducing oxidative DNA damage, altering cell proliferation, apoptosis and gene expression. *Carcinogenesis*. 2003;**24**:1389-1399. DOI: 10.1093/carcin/bgg079
- [39] Sato T, Takeda H, Otake S, Yokozawa J, Nishise S, Fujishima S, Orii T, Fukui T, Takano J, Sasaki Y, Nagino K, Iwano D, Yaoita T, Kawata S. Increased plasma levels of 8-hydroxydeoxyguanosine are associated with development of colorectal tumors. *Journal of Clinical Biochemistry and Nutrition*. 2010;**47**:59-63. DOI: 10.3164/jcbrn.10-12
- [40] Chiou CC, Chang PY, Chan EC, Wu TL, Tsao KC, Wu JT. Urinary 8-hydroxydeoxyguanosine and its analogs as DNA marker of oxidative stress: Development of an ELISA and measurement in both bladder and prostate cancers. *Clinical Chimica Acta*. 2003;**334**:87-94. DOI: 10.1016/S0009-8981(03)00191-8
- [41] Wada T, Tanji N, Ozawa A, Wang J, Shimamoto K, Sakayama K, Yokoyama M. Mitochondrial DNA mutations and 8-hydroxy-2'-deoxyguanosine Content in Japanese patients with urinary bladder and renal cancers. *Anticancer Research*. 2006;**26**:3403-3408
- [42] Nomoto K, Tsuneyama K, Takahashi H, Murai Y, Takano Y. Cytoplasmic fine granular expression of 8-hydroxydeoxyguanosine reflects early mitochondrial oxidative DNA damage in nonalcoholic fatty liver disease. *Applied Immunohistochemistry & Molecular Morphology*. 2008;**16**:71-75. DOI: 10.1097/PAI.0b013e31803156d5
- [43] Bloching M, Reich W, Schubert J, Grummt T, Sandner A. The influence of oral hygiene on salivary quality in the Ames Test, as a marker for genotoxic effects. *Oral Oncology* 2007;**43**:933-939. DOI: 10.1016/j.oraloncology.2006.11.006
- [44] Gondivkar SM, Gondivkar RS, Gadbail AR, Chole R, Mankar M, Yuwanati M. Chronic periodontitis and the risk of head and neck squamous cell carcinoma: Facts and figures. *Experimental Oncology*. 2013;**35**:163-167.
- [45] Michaud DS, Joshipura K, Giovannucci E, Fuchs CS. A prospective study of periodontal disease and pancreatic cancer in US male health professionals. *Journal of National Cancer Institute*. 2007;**99**:171-175. DOI: 10.1093/jnci/djk021
- [46] Kostic AD, Gevers D, Pedamallu CS, Michaud M, Duke F, Earl AM, Ojesina AI, Jung J, Bass AJ, Tabernero J, Baselga J, Liu C, Shivdasani RA, Ogino S, Birren BW, Huttenhower C, Garrett WS, Meyerson M. Genomic analysis identifies association of *Fusobacterium* with colorectal carcinoma. *Genome Research*. 2012;**22**:292-298. DOI: 10.1101/gr.126573.111
- [47] Ahn J, Segers S, Hayes RB. Periodontal disease, porphyromonas gingivalis serum antibody levels and orodigestive cancer mortality. *Carcinogenesis*. 2012;**33**:1055-1058. DOI: 10.1093/carcin/bgs112