

The Importance of Oxidative Stress and Antioxidants in Periodontal Diseases: Review

Periodontal Hastalıklarda Oksidatif Stres ve Antioksidanların Önemi

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ABSTRACT Periodontal diseases are inflammatory diseases due to some complex interactions between the host immuno-inflammatory responses and periodontopathogenic bacteria. The two important events that affect the pathophysiologic process of periodontal diseases are the immun system activation and the production of reactive oxygen species (ROS). The increased production of ROS and the decreased antioxidant levels led to oxidative stress that has an important role in pathogenesis of periodontal diseases. The purpose of this review is to give information about the destructive mechanism of oxidative stress in periodontal diseases and the defensive effect of antioxidants against this destruction.

Key Words: Periodontal diseases; reactive oxygen species (ROS); antioxidants; oxidative stress

ÖZET Periodontal hastalıklar, konak immün inflamatuvar yanıtlar ve periodontopatogenik bakteriler arasındaki kompleks etkileşimlerin sonucu meydana gelen inflamatuvar hastalıklardır. Periodontal hastalıkların patofizyolojik sürecini etkileyen iki önemli olay immün sistemin aktivasyonu ve reaktif oksijen türleri (ROS)'nin üretimidir. Dokularda ROS'un artan üretimi ve azalan antioksidan miktarı periodontal hastalıkların patogeneğinde önemli rol oynayan oksidatif strese yol açmaktadır. Bu derlemenin amacı, periodontal hastalık durumunda oksidatif stresin yıkım mekanizması ve antioksidanların bu yıkıma karşı oluşturdukları savunma etkisi hakkında bilgi vermektir.

Anahtar Kelimeler: Periodontal hastalıklar; reaktif oksijen türleri (ROS); antioksidanlar; oksidatif stres

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There is an oxidative balance between reactive oxygen species (ROS) which have occurred as a result of the reactions in body and their antioxidants. As long as this balance exists organism does not affect from ROS. If this balance changes in favour of ROS then tissue damage occurs because of oxidative stress.¹ It is shown that oxidative stress has a key role in pathogenesis of many diseases such as rheumatoid arthritis, chronic obstructive lung disease, acquired immune deficiency syndrome (AIDS), atherosclerosis and periodontal diseases.² Oxidative stress causes tissue damages in different mechanisms such as DNA injury, lipid peroxidation, pro-

tein damage, important enzymes' oxidation and pro-inflammatory cytokines' stimulation.²

ROS is a collective term used for free radical molecules which produced during oxygen (O_2) reduction [superoxide radical (O_2^-), hydroxyl radical (OH) and nitric oxide (NO)] and non-radical molecules which produced during oxygen production [hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCL)]. ROS serve as signal molecules in the regulation of some various cellular processes. The most important effect of ROS that cellular biomolecular damage during oxidative stress.

ROS can occur as endogen-based as a result of waste-product or as exogen-based as a result of exposure to environmental factors.³ Exogen based ROS occurs owing to air pollution, ozone, radiation, chemicals, toxins and pathogenic microorganisms. Nevertheless, endogen resources occur during respiratory explosion. This respiratory explosion happens when electron leakage which based on mitochondrial electron transport chain and inflammatory cells (macrophage, neutrophils) are trying to stop foreign, harmful species (Figure 1).⁴ Polymorphnuclear leukocyte (PMNL) activation causes increase in oxidation of glucose by way

of hexose monophosphate shunt. During respiratory explosion nicotinamide adenine dinucleotide phosphate (NADP) is used as an electron transmitter. When molecular oxygen (O_2) is reduced to superoxide radical (O_2^-) NADP production increases and get activated by way of hexose monophosphate shunt. This O_2^- radical gets free in extracellular and phagosome space. Meanwhile, NO is produced by macrophages by means of nitric oxide synthesis during/ in acute inflammation of vascular endothelial cells.⁵ Hydrogen peroxide (H_2O_2) is produced owing to dismutation of superoxidant. When this hydrogen peroxide reacts with Fenton in existence of Fe^{+2} , hydroxyl radical (OH) which is the most harmful and reactive free oxygen radical occurs.

The phagocytes pour their granules which contains myeloperoxidase, in the phagocytic vacuole in extracellular space. Myeloperoxidase produces hypochlorous acid with hydrogen peroxide (H_2O_2) by catalyzing chloride oxidation. These compounds create toxic agents by reacting with biologically important microorganisms.⁶ Although superoxide radical is a weak reactive radical and tied to hydroxyl radical, it can affect much of the biological target in periodontal relation.⁶

Hydroxyl radical stimulates a classical free radical chain reaction known as lipid peroxidation.⁶ Moreover, it causes destruction of the chondroitin sulfate, proteoglycans, proteins and glycosaminoglycan chains into alveolar bone.³

Hypochlorous acid is a strong antimicrobial agent. It is more toxic than superoxide radical or hydrogen peroxide. Moreover, it causes inactivation of important enzymes, damage of cell membrane functions, and reduction of some extracellular matrix components' adhesive features.⁷ (ROS production in PMNL activation is summarized in Figure 1).

Antioxidants are defense mechanisms that prevent ROS occurrence and negative effects of ROS. Normally, there is a balance between ROS and antioxidants. But in case of excessive ROS production or antioxidants reduction oxidative stress occurs, and harms tissues.¹

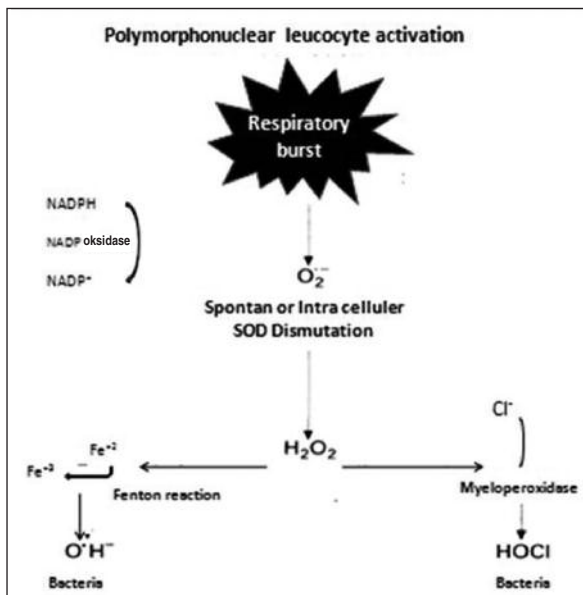


FIGURE 1: ROS production in PMNL activation⁹

EFFECTS OF OXIDATIVE STRESS ON PERIODONTAL DISEASES

Periodontal diseases are one group of the most common chronic diseases among adults.⁸ Periodontal diseases are inflammatory diseases that occur owing to complex reactions between host immune-inflammatory responses and periodontopathogenic bacteria.⁹ It is thought that periodontal tissue damage occurs because ROS and neutrophil enzymes get free for long time, and when there is an abnormal immune and inflammatory reaction on microbial plaque. In recent years like in many diseases, oxidative stress is defined as one of the important reasons of periodontal diseases.¹⁰

Recent studies have shown that in the pathogenesis of tissue damage occurred due to periodontal disease, importance of reactive oxygen types is stressed, and it is seen that their ROS amount increases during periodontal diseases.⁴ Oxidative stress is also important for pathogenesis of periodontal diseases and its complications.¹¹

Periodontal diseases effect the 5 to 20% of pregnant women and they are seen that oxidative stress increase at this population.¹² It has been postulated that preeclampsia is connected with periodontal diseases, increased oxidative stress and destructive antioxidant defense mechanisms.¹³

Periodontitis is accepted as the sixth complication of diabetes.¹⁴ The decrease in salivary reduced-antioxidant levels in patients with type 1 diabetes mellitus may have a role in periodontal tissue destruction by predisposing tissues to oxidative stress.¹⁵

It believed that a closed relationship exist between obesity and periodontitis.¹⁶ It has been postulated that increasing of serum reactive oxygen metabolites induce the gingival oxidative stress on obesity rats.¹⁷

The host immune reaction which is formed against bacterial colonization accumulated in subgingival areas is starting point of periodontal diseases. While host immune reactions provide protection and defense they also cause tissue dam-

age.¹⁸ Between responses given by the host for various bacterial activities, leukocytes (especially PMNL) serve as first host defense.⁴ Neutrophils are dominant inflammatory cells in gingival connective tissue, pocket epithelium and gingiva.¹⁹

Neutrophils stimulated by bacterial pathogens move into inflamed site and phagocytic bacteria. In case of oxygen availability phagocytic cells have oxidative killing mechanisms and during this process they produce free radicals. Production of ROS is a complementary feature of normal cellular metabolism and these free radicals create toxic effects on microorganisms.³ But, when these products exceed cells' antioxidant defense strength they harm normal host cells and affect pathogenesis of various diseases.³

In periodontal diseases reactive oxygen species increase due to either electron leakage occurred when oxygen directly moved into mitochondria is passing from respiratory chain or functionally production of oxygen radicals by phagocytes.³

ROS causes periodontal tissue injury by various mechanisms.⁶

1. LIPID PEROXIDATION (BY THE WAY OF LIPOOXYGENASES AND ACTIVATION OF CYCLOOXYGENASES

It is shown in many studies that lipid peroxidation (LPO) changes cell membrane's functions and structural unity; moreover, LPO levels in periodontitis increase.²⁰ High lipid peroxidation levels were found at periodontitis-induced atherosclerotic rats.²¹ It has been postulated that the lipid peroxides which produced by the result of periodontitis, can cause to the beginning phase of atherosclerotic disease with diffusing to blood at the inflammation space.²¹ Lipids are the most sensitive biomolecules for ROS. LPO process begins when polyunsaturated oil acids, membranes or lipoproteins react with ROS. As a result of LPO chain reaction, oil acids turn into lipid peroxide.²² Lipid peroxide occurred in an uncontrolled way is caused oxidative stress that harms cell integrity.²² Lipid peroxidation level can be measured by using thiobarbituric acid reactive substances (TBARS) to an-

alyze malondialdehyde (MDA). This malondialdehyde is produced by way of lipid destruction happened during oxidative stress.²³

2. DNA INJURY

ROS cause DNA injury on periodontal tissue cells.⁶ 8-hydroxydeoxyguanosine (8-OHdG) is an oxidized nucleoside that is excreted in the bodily fluids with DNA repair.²⁴ Therefore as an indicator of DNA injury related to ROS frequently 8-OHdG is used. 8-OHdG is one of the most commonly seen oxidative based damage product of ROS into DNA. Moreover, its mutagenity is well known.²⁵ It is composed when hydroxide radical (OH), most powerful reactive oxygen, added to 8th position of guanine molecule. Guanine has the lowest ionization capacity among DNA compounds. Thus, it is the major target of ROS. It is shown that saliva 8-OHdG levels increase in periodontitis cases.²⁶ Also it was showed that the increased salivary 8-OHdG levels and decreased salivary antioxidant activities project increased oxygen radical activity during periodontal inflammation by the use analyses of saliva.²⁷

3. PROTEIN INJURY (ESPECIALLY GINGIVAL HYALURONIC ACID AND PROTEOGLYCANS)

Hyaluronan is a non-sulfate glycosaminoglycan which is a component of extracellular matrix placed into many tissues like periodontal tissues. Sulfate glycosaminoglycans are more resistant than non-sulfate glycosaminoglycans to ROS destruction.²⁸ Hyaluronan has a key role on each of wound healing phases such as inflammation, granulation and re-epithelization.²⁹ ROS cause the destruction of extracellular matrix components such as collagen, proteoglycan and hyaluronan.³⁰ It is shown that ROS depolymerize gingival hyaluronic acid and proteoglycans.³¹ Protein modifications caused by ROS changes protein structure. Irreversible protein modifications cause inactivation of some proteins and permanent detrimental effects on the cell. Carbonylation is one of proteins' irreversible, non-enzymatic modifications.³² Protein carbonylation is clear indication of oxidative injury of proteins.¹⁰ Baltacıoğlu

et al. reported that, the serum and GCF PC levels were found high in the patients with chronic periodontitis than the healthy people. Also they declared that this levels as a sign of oxidative damage in periodontal tissues.⁶

4. STIMULATION OF PRO-INFLAMMATORY CYTOKINE FREED BY MONOCYTES AND MACROPHAGES, DECREASE OF INTRA-CELL THIOL COMPONENTS AND NUCLEAR FACTOR κ B(NF- κ B) ACTIVATION³³

In case of periodontal disease level of various cytokines and chemokines produced by normal cells and inflammatory cells in periodontal tissues increase. Some pro-inflammatory cytokines (TNF- α , granulocyte-macrophage colony stimulating factor, IL-1, IL-6, IL-8), growth factors (platelet activating factor) and lipopolysaccharides (LPS) have a stimulation effect on oxidative mechanisms of neutrophile.³⁴ Tumor necrotizing factor- α (TNF- α) is the main factor on ROS production by neutrophiles of people who are healthy or having chronic, aggressive periodontitis.³⁵ It is claimed that cytokines regulate oxidative activities of neutrophiles and they have a role on determination of oxidative stress in tissues (Figure 2).³

Nuclear factor- κ B and activator protein-1, which are the two redox sensitive transcription factors, are very important in pathogenesis of periodontal diseases. These factors can be activated with different stimulators such as bacterial products, viral proteins, cytokines (IL-2, IL-6, IL-8, β -interferon and TNF- α), growth factors, radiation, ischemia and oxidative stress.³⁶⁻³⁹ Arabacı et al. found that, NF- κ B had a high activity rate for the patients with chronic periodontitis than the compared control group. They also declared that this reason was significantly similar with the clinical measurements.⁴⁰ Via IL-1 and TNF- α , NF- κ B production will increase and cause increase in oxidative products of PMNL. Moreover this increase will affect periodontal inflammation, and more tissues damage. In in-vitro and in-vivo studies it was shown that in the induction of osteoclasts and osteoclastic activities NF κ B signal way has an important role (Figure 2).⁴¹

ANTIOXIDANTS INTO PERIODONTAL DISEASES

Antioxidants are effective mechanisms on prevention of ROS formation and its turning into less reactive molecule and cleaning, healing of ROS-based wounds and adjustment of suitable conditions for other antioxidants.⁵⁰

Antioxidant defence system is very dynamic and its components are sensitive against any difference occurred into body's redox balance. Normally, body has a multilateral antioxidant defence system against free radicals (Figure 3).⁴

Antioxidants are classified as chain breaker or cleaner antioxidants [Vitamin E (α -tocopherol), Vitamin C (ascorbic acid), Vitamin A (β -carotene), urate, bilirubin and thiol], preventive antioxidants (preventive Fenton reactions) and natural proteins (albumin, transferrin, lactoferrin, ceruloplasmin, haptoglobin and ascorbic acid) and enzymatic antioxidants (SOD, catalase and glutathione peroxidase).⁶

Superoxide dismutase (SOD), is one of the antioxidant enzymes that catalyzes $O_2^{\cdot -}$'s alternation into H_2O_2 and O_2^- , and protects the cell from ROS' harmful effects. SOD protects cell against harmful

effects such as $O_2^{\cdot -}$'s lipid peroxidation. It is also effective on intracellular termination of phagocytosed bacteria.³

It is shown that periodontal ligament has SOD enzyme that will provide biological protection against ROS, especially $O_2^{\cdot -}$, during inflammatory reaction. It was claimed that the bacterial LPS stimulate superoxide from the gingival fibroblasts and creates the importance defence mechanisms of fibroblasts during inflammation with SOD induction.⁵¹ Wei et al. reported at their study that, the gingival SOD activity levels were found high for the patients with chronic periodontitis. It was also said that, this increase of SOD activity levels sign the increase of the production of $O_2^{\cdot -}$ and this will cause with the oxidative stress.⁵²

Glutathione peroxidase (GSH-Px) is selenium-based enzyme which is responsible for detoxification of hydroperoxide. GSH-Px with other antioxidants prevent oxidative components from harming phagocyte cells.⁵³ In a study made among patients having periodontitis, it is claimed that periodontal treatment increases glutathione peroxidase level, and in relation, it decreases pocket depth and attachment loss.³

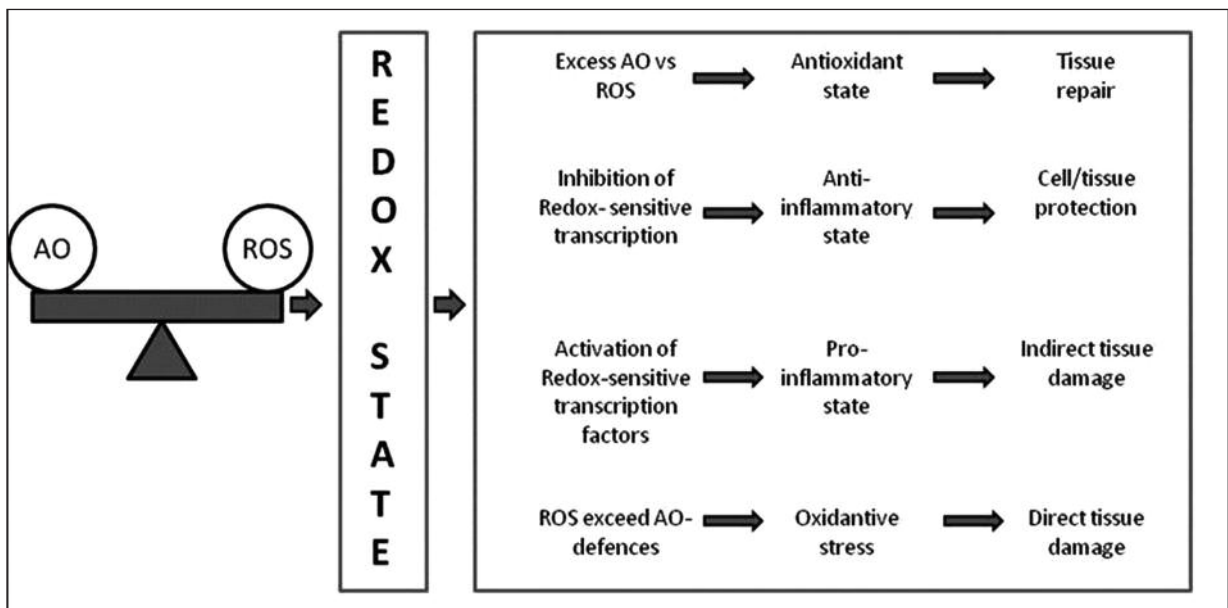


FIGURE 3: Biological effects of balance alterations between ROS and antioxidants (AO: Antioxidants, ROS: Reactive oxygen species).⁴

Catalase turns H_2O_2 into water and oxygen. In granulomatous cells catalase protects the cell against its own respiratory explosion. It decomposes H_2O_2 to prevent OH radical production.³ GSH-Px, catalase and SOD affect cell protection process together and synergistically.

Glutathione S-transferase (GST) directly affects neutralization of hydroperoxide occurred during lipoperoxidation, and it is shown that GST activities increase in periodontal inflammation process.⁵⁴

Vitamin C is also a powerful antioxidant due to its reductive features. It is a strong cleaner for O_2^- , OH^- and hypochlorous acid. In collagen synthesis it is necessary for lysine and proline's hydroxylation. It's effective for/ in immunity and wound healing processes. It is shown that there is a negative relation between plasma Vitamin C levels and periodontal attachment loss.⁵⁵ It is found that gingival crevicular fluid (GCF) concentrations of ascorbic acid are three times bigger than plasma's, and it is shown that it hampers neutrophil activation occurred due to collagenase in GCF.⁵⁶ Vitamin C protects lipids against lipid peroxidation in plasma, and it has an important role in α -tocopheral regeneration.⁵⁷

Vitamin E (α -tocopherol) is a strong chain breaker antioxidant. It is found in membrane phospholipids.⁵⁸ It composes first defence wall that protects polyunsaturated oil acids in cell membrane phospholipids from free radical effects. Vitamin E reduces superoxide and hydroxyl radicals, lipid peroxide radicals and other radicals.⁵⁹ Lipid peroxidation chain reaction is ended via Vitamin E.³ It is reported that there is a strong synergy between Vitamin E, C and glutathione, and with the addition of Vitamin C, plasma Vitamin E level increases.⁶⁰ In a study made on rats it is claimed that Vitamin E is an important protector against alveolar bone loss.⁶¹ Moreover, in another study it was found that Vitamin E and selenium combination is a protector against collagen destruction related to ROS.⁶² It was stated that Vitamin E is a prostaglandin inhibitor, so it is effective in the reduction of periodontal inflammation.⁶

Vitamin A (β -carotene) has an important role on reparation of cell destruction (especially DNA injury) occurred due to oxidative reasons. It is reported that among the rats having/ suffering from lack of Vitamin A, bone resorption decreases or reverses and epithelial keratosis and leukocyte's infiltration increase.⁶³

Melatonin has a strong antioxidant effect on oxidative injury and inflammatory processes. In recent studies it was reported that melatonin with type-1 collagen synthesis stimulate proliferation and increase bone formation.⁶⁴ In their study Srinath et al. showed that level of melatonin concentration in/within gingival sulcus liquid and saliva of a patient having periodontitis disease is lower than melatonin level of healthy people.⁶⁵

Coenzyme Q_{10} (KQ) is an important part of cell energy metabolism that naturally occupies into all tissues' mitochondria including gingiva. It is an antioxidant, and it has an important role in the inhibition of lipid and protein peroxidation. Among patients having periodontal disease it is claimed that gingival and serum KQ levels decrease and there can locally be lack of KQ in gingival tissues. It was stated that in a periodontal disease, KQ's locally or systemic application improves health and prevents periodontal inflammation.⁶⁶ Kuru et al. reported that application of systemic KQ in the early phases of periodontal disease treatment decreases gingival lipid peroxidation level more than any application made without it.⁶⁷

Uric acid is a strong cleansing antioxidant that cleans radicals such as hypochlorous acid. It is recognized as an important molecule in immune system because it regulates reduced glutathione (GSH), especially IL-2 based T-lymphocyte proliferation.⁶⁸ GSH has a great effect on periodontal diseases because it is effective in the regulation of pro-inflammatory cytokines.⁶⁹ GSH has many biological functions for/in prevention of oxidative injury such as removal of hydroperoxides, stabilization and detoxification of biologic membranes in prevention of oxidative injury.⁷⁰

Production of antioxidant thiols on epithelial surfaces is an important defence mechanism against unwanted ROS-caused injury (PMNL-based or exogenous-based).⁷¹

CONCLUSION

Reactive oxygen species produced by inflammatory and immune cells are related to tissue injury. To defence against such kind of injuries our body has an antioxidant defence system.

While ROS are secreted in small amounts they are important molecules of host defence mechanism, but if its secretion increases because of different stimuli it supports local tissue damage throughout inflammatory reaction. Stimulus such as developing technology, environmental pollution, smoking, UV and modern life stress cause so

much ROS production. Negative effects of ROS are generally seen on immune system and inflammatory reactions.

Periodontal disease is known as one of the commonly seen chronic diseases among adults. Treatment of the tissue loss occurred owing to periodontal diseases around teeth is both difficult and expensive. If it is not treated it becomes an entrance door for microorganisms and causes tooth loss. As well as understand the importance of ROS and antioxidants for periodontal diseases, practice about conservative treatments for gingival health of people with predisposed periodontal diseases can develop. It can also guide studies about increasing the antioxidant capacities of periodontal tissues which performs in addition to the mechanical treatments of periodontal diseases.

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