Review Article

Antioxidant enzymes in periodontitis

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ABSTRACT

Periodontitis is basically an inflammatory disease initiated by the subgingival biofilm and modified by the individual's aberrant inflammatory/immune response. Various studies have pointed toward the role of oxidative stress in periodontitis. As the reactive oxygen species and antioxidants are in dynamic equilibrium, any disturbance in one would lead to changes in the other. As studying, individual antioxidants is a vast field, this review focuses on the role of antioxidant enzymes in periodontitis and in other related systemic conditions. It is highlighted that oxidative stress may be the missing link in these associations of periodontitis and other conditions. Also, the antioxidant enzymes may be considered a useful biomarker for periodontal diseases and antioxidant supplementation may be of help to reduce the burden of periodontal destruction without having much extra effort.

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1. Introduction

Reactive oxygen species (ROS) are continuously generated by most tissues as an integral part of normal cellular metabolism. ROS collectively describe oxygen free radicals and other non-radical oxygen derivatives involved in oxygen radical production. These include superoxide $O_2^{\bullet}$, hydroxyl $OH^{\bullet}$, hydroperoxyl $HOO^{\bullet}$, nitric oxide $NO^{\bullet}$, alkoxy $RO^{\bullet}$, singlet oxygen 1O_2 , ozone O_3 , hypochlorous acid $HOCl$ and hydrogen peroxide H_2O_2 .¹ Reactive oxygen species are actively involved in cell signaling and metabolic processes. ROS also play a role in pathogenic processes in a range of inflammatory disorders. Excessively produced ROS molecules are proficient enough of initializing the periodontal tissue destruction.² The various mechanisms of tissue destruction by ROS include lipid peroxidation, DNA damage, protein damage, oxidation of important enzymes, and the release of pro-inflammatory cytokines by monocytes and macrophages.^{3,4}

Antioxidants are defined as those substances which when present at low concentrations, compared to those of an oxidizable substrate, will significantly delay or inhibit oxidation of that substrate.⁵

Antioxidants are classified based on their mode of action as

- Preventive antioxidants: e.g. Enzymes Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), DNA repair enzymes.
- Scavenging (chain breaking antioxidants): Ascorbate (vitamin C), carotenoids (including retinol – vitamin A), uric acid, α -tocopherol (vitamin E), polyphenols (flavonoids).

The reactive oxygen species and antioxidants are in a dynamic equilibrium in normal physiology. Whenever there is a shift in this delicate equilibrium either by an augmented ROS release or activity or by a diminished antioxidant defense mechanism, oxidative stress results. Oxidative stress was defined by Sies as a disturbance in the pro-oxidant–antioxidant balance in favor of the former, leading to potential damage.⁶

It is believed that almost 1–3 billion reactive species are released by every cell per day.⁷ As these ROS may cause both direct and indirect tissue damage, these must be neutralized by the antioxidants. This clarifies the crucial role of antioxidants in the safeguarding of normal health.

Periodontal diseases affect 10–15% of the world population⁸ and are one of the leading reason of tooth loss. Periodontitis is basically an inflammatory disease, which is initiated by the subgingival biofilm and is modified by the individual's aberrant inflammatory/immune response. The polymorphonuclear leukocytes (PMNL) are the prime inflammatory cells in gingiva and periodontal tissues.⁹ In periodontitis, PMNLs which are naturally capable of producing ROS are thought to be functionally activated and thus lead to increased ROS production. Various recent studies

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have indicated that chronic periodontal diseases are associated with hyperreactive neutrophils with increased ROS production in response to Fc-gamma receptor stimulation.^{10,11} The role of ROS cannot be studied in isolation as there is a dynamic equilibrium between ROS and antioxidants.³ Any variation in the two will lead to an imbalance and thus interference with the homeostasis.

Studying the total antioxidant capacity (TAOC) provides an overview of combined effectiveness of the contributing antioxidants, but it does not highlight which individual antioxidant species is of importance for a pathogenic process.¹² Also, in interventional studies, the TAOC may change as a consequence of intervention but any specific antioxidant which is of crucial importance may vary differently.

As studying individual antioxidants in detail is a vast field, this review focuses primarily on the importance of antioxidant enzymes in periodontitis.

2. Interaction of ROS and antioxidants

The intracellular interaction of ROS and antioxidants is crucial as it regulates the transcription process at gene level. Transcription factors, such as nuclear factor- κ B and activating protein-1 are reported to be redox-sensitive. Subsequent to induction of pro-inflammation, the slight changes in the redox state activate these transcription factors, which ultimately leads to tissue destruction.

An upward shift of oxidants intracellularly may cause damage to vital cell structures and biomolecules, disruption of cell membrane and ultimately cell death by necrosis or apoptosis. An extracellular oxidants level increase may cause damage to the both mineralized and unmineralized extracellular matrices and their constituents.

The ROS cause tissue damage by various mechanisms, which include protein damage by causing reversible or irreversible protein folding or unfolding, protein fragmentation and polymerization reactions; and protease degradation of the modified protein. Another way is by causing lipid peroxidation which leads to production of various conjugated dienes, lipid peroxides, and aldehydes accumulation of which intracellularly, disrupt the cell membrane integrity and leads to collapse of cells. DNA damage by causing various mutations like base pair mutations, insertion, deletion and strand breaks are also reported to be induced by ROS like hydroxyl radicals.^{3,4}

Preventive antioxidants like SOD, catalase, glutathione peroxidase function by enzymatic removal of superoxide and hydrogen peroxide ions. SOD is found in all the tissues and cells of aerobic organisms. There are three types of SODs in mammalian cells, namely the cytosolic Cu Zn SOD, mitochondrial Mn-SOD, and extracellular Cu Zn SOD. SOD is a key antioxidant enzyme that efficiently and specifically scavenges superoxide anion (O_2^-) by catalyzing its dismutation to H_2O_2 and O_2 .⁵ Catalase, GR and GPx accelerate H_2O_2 reduction to water. Scavenging antioxidants like Vitamin C acts by scavenging various oxidants like superoxide, peroxyl, and perhydroxy radicals.

Various other properties also affect the mechanism of action of antioxidants and it is important to note that one antioxidant may act in more than one ways. For e.g. at the cell membrane level, the lipid soluble antioxidants like Vitamin E and carotenoids are more effective and offer protection against lipid peroxidation. Whereas within the extracellular tissue fluids, the water-soluble scavengers are more crucial and active.

3. Animal studies

In early nineties, Misaki et al.,¹³ demonstrated the beneficial results of superoxide dismutase (SOD) on *Porphyromonas*

gingivalis induced inflammation and periodontal wound healing in wistar rats. Subsequently, Petelin et al.¹⁴ studied the role of subgingival application of liposome-encapsulated SOD and/or CAT in experimentally induced periodontitis in beagle dogs by evaluating periodontal healing and regeneration process. Local delivery of liposome-encapsulated SOD was found to be useful as it decreased the periodontal inflammation, promoted periodontal healing and alveolar bone deposition; however, CAT did not offer observable benefits at the concentration used. The study pointed to the beneficial role of subgingival application of liposome encapsulated SOD for the management of inflammatory periodontal diseases.

Reduced activities of SOD, GR, GPx, and increased malondialdehyde (MDA) in blood serum in ligature-induced periodontitis in rats has also been revealed suggesting the role of oxidative stress in this inflammatory disorder.¹⁵

4. Role of antioxidants in periodontitis

The presence of SOD enzyme in periodontal ligament was demonstrated by Jacoby et al.,¹⁶ using both biochemical and immunohistochemical techniques. The activity of SOD in periodontal ligament was found to be considerably less than that in red blood cells and also decreased with age. Spontaneous production of superoxide in gingival crevicular fluid (GCF)¹⁷ and enhanced superoxide production by PMNs¹⁸ was also demonstrated in periodontitis patients in early nineties.

In an in vitro study involving gingival biopsies, the gingival tissue adjacent to 4 mm sulci demonstrated a significant and progressive reduction in catalase and SOD activity. The greatest reduction was evident within tissues adjacent to >6 mm sulci.¹⁹ It was hypothesized that the diminished SOD and catalase activity promote ROS production, which in turn causes tissue destruction.

Chapple et al.²⁰ suggested that both GCF and plasma total antioxidant capacity (TAOC) is significantly reduced in patients with periodontal disease in comparison to periodontally healthy controls. The concentrations of both reduced and oxidized glutathione in GCF were also found to be reduced in chronic periodontal disease. Diminished antioxidant capacity in saliva, GCF, serum has also been suggested by various other studies.^{21–23}

In a contrasting study, the enzymatic antioxidant activities have been found to be significantly higher in periodontitis patients compared to healthy controls in the plasma, erythrocytes, erythrocyte membranes and gingival tissues. It was speculated that the scavenging of disproportionately generated lipid peroxidation products at the inflammatory sites may be responsible for enhanced enzymatic antioxidant activity.²⁴

Canakci et al.²⁵ detected reduced antioxidant activities in saliva reflecting increased oxygen radical production or activity during periodontal inflammation. However, the increased oxidative stress was not correlated with the severity of periodontal destruction.

The changes in antioxidant enzymes activity as a result of scaling and root planing has also been demonstrated, which also supports the hypothesis about role of oxidative stress in periodontal destruction.²⁶ Novakovic et al.²⁷ concluded that salivary antioxidants like SOD and GPx credibly reflected periodontal response and the tissue response to treatment.

Positive correlation of antioxidant enzymes and the studied periodontal parameters has also been established in various studies. In a previous study, a highly significant negative correlation between the periodontal parameters studied and salivary antioxidant levels was established.²⁸ It was speculated that as the periodontal condition deteriorates due to ROS production, the antioxidants are utilized to maintain the balance and so the normal level decreases.

5. Antioxidant enzymes in smokers

Increased gingival SOD, GSH-Px, and CAT activities in smokers compared to periodontally matched non-smoker and former smoker patients has been shown which was attributed to the protective and adaptive mechanism developing in the tissue.²⁹ It has been suggested that although smoking induces selective increase of AO enzyme activity in the tissues as self-defense mechanism,³⁰ this increase is inadequate to defend the tissues from the harmful effects of smoking.³¹

A reduced SOD activity in saliva and GCF, which correlated with pocket depth and clinical attachment level has been reported in heavy and light smokers.³² A reduction in SOD and glutathione levels and elevation of catalase levels in gingival tissues³³ and a higher GSH-Px activity in saliva³⁴ was demonstrated in smokers with or without periodontitis compared to matched non-smoker controls. All these studies point to role of oxidative stress in amplified periodontal destruction in smokers.

6. Antioxidant enzymes in diabetics

In a study by Akalin et al.,³⁵ gingival SOD activity was found to be increased significantly in diabetic periodontitis and periodontally healthy groups in comparison to the matched non diabetic groups. It was suggested that diabetes increases gingival SOD activity while periodontitis decreases it. The increase in diabetes was attributed to protective and adaptive mechanism developing in tissue in response to oxidative stress.

In another study,³⁶ gene expression of SOD 1 and 2, CAT and GPx was evaluated in type 2 diabetics. SOD 2 was overexpressed in poorly controlled diabetics with chronic periodontitis (CP), GPx was upregulated in CP while CAT and SOD 1 were not found to be significantly influenced by either of the inflammatory conditions. Almost a three fold increase in catalase activity in saliva of pregnant women with diabetic complications was found compared to healthy controls but a correlation with the parameters of oral health could not be established.³⁷

On comparison of diabetics with CP and healthy individuals with CP, in saliva the activities of SOD and CAT were decreased while that of GR was found to be elevated.³⁸ It was reasoned that the capacity to detoxify oxygen radicals was diminished because of excessive oxygen radical production from auto-oxidation of glucose, glycated proteins, and glycation of antioxidant enzymes.³⁹

7. Antioxidant enzymes in pregnancy

Canakci et al. opined that both systemic and local antioxidant activities are influenced by periodontal diseases as well as preeclampsia in pregnancy.⁴⁰ It was hypothesized that the oxidative stress resulting from periodontal disease may be a risk factor for initiation, progression and severity of preeclampsia in pregnant women.

Another study⁴¹ demonstrated that SOD concentration in GCF and serum were lower in pregnant patients compared to periodontally matched non-pregnant group and were lower in the periodontitis groups than in the control groups. It was speculated that the decline in antioxidant defense may be the reason for this.

8. Role of supplementation

The role of supplementation of antioxidants locally and systemically is not very clear. Certain studies suggest that adjunctive supplementation of Vitamin E⁴² and lycopene⁴³ improves periodontal healing and antioxidant defense. Low dose doxycycline has been reported to have an effect on the antioxidant

enzymes levels in experimental periodontitis in rats. Subantimicrobial dose doxycycline inhibited both systemic and local oxidative stress, thus preventing periodontal tissue breakdown.⁴⁴

9. Conclusion

This review highlights the possible role of oxidative stress in periodontitis and associated systemic factors. On basis of the studies available, an inference cannot be drawn whether oxidative stress is the cause or result of periodontal inflammation, i.e., whether the oxidative stress state preceded periodontitis development or vice versa. In spite of the above fact, there is no doubt that severe periodontitis is autonomously related with increased oxidative stress and decreased anti-oxidant capacity.

The bidirectional relationship of diabetes and periodontitis, the adverse pregnancy outcomes related to periodontitis, and other systemic effects of periodontitis like on the cardiovascular system, respiratory system, rheumatoid arthritis have been suggested. The exact mechanism of which has not been elucidated, so it may be considered that oxidative stress is one of the missing links between these relationships.

Keeping in mind the above facts, it may be speculated that oxidative stress is an important factor in periodontitis and studying the antioxidant defense mechanisms will help in better understanding of underlying tissue damage mechanisms and novel therapeutic interventions/strategies. The antioxidant enzymes may be considered a useful biomarker for periodontal diseases. Also, antioxidant supplementation may be of help to reduce the burden of periodontal destruction without having much extra effort.

Key messages: Oxidative stress is an important factor in periodontitis and studying the antioxidant defense mechanisms will help in better understanding of underlying tissue damage mechanisms and novel therapeutic interventions/strategies. The antioxidant enzymes may be considered a useful biomarker for periodontal diseases.

Conflicts of interest

The authors have none to declare.

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