Relationship between Antioxidants and the Development of the Periodontal Disease

Rudrakshi C’, Prabhuji MLV, Parween S and Jyothsna S
Department of Periodontology, Krishnadevaraya College of Dental Sciences & Hospital, Hunasamaranahalli, Bangalore

Abstract
The role of oxidative stress linking the free radical damage at the cellular level causes premature aging, including periodontal disease. To overcome this, it is important to explore the role of host specifically with regard to antioxidant status along with conventional periodontal therapy. Periodontal counselling and supplementation may very well reduce inflammation and thereby enhance outcomes of conventional periodontal therapy. The purpose of this review is to summarize available research in the role of antioxidants as an adjunct to periodontal therapy.

Keywords: Antioxidants; Free radical; Inflammation; Periodontal therapy

Key Messages: Antioxidants have shown to have beneficial outcome when used as adjunct to traditional periodontal therapy. Implication of antioxidants has promising results in future.

Introduction
Periodontitis is a chronic inflammatory disease caused primarily by bacteria in dental plaque, affecting the supporting structures of the teeth. Specific periodontal pathogens such as the gram-negative anaerobic bacteria inhabiting within the sub-gingival plaque are associated with the progressive form of the disease. Although bacteria are the major etiological agents, the host immune response to these bacteria is of fundamental importance. Hence, it is evident that periodontitis is a multifactorial disease, affiliates with specific microorganisms, social and behavioral factors, genetic or epigenetic trait, all of which are modulated and controlled by the underlying immune and inflammatory responses of the host [1].

The recent focus on the progression of periodontitis is on the molecular aspects of host modulation. The discovery of the role of free radicals in chronic disease is as important as the discovery of the role of microorganism in inflammatory disease [2].

Free radicals & Reactive Oxygen Species (ROS) production is an essential component of the host response for immune system, prostaglandin biosynthesis, anti-bacterial biosynthesis and variety of insults like trauma/burns [3]. Besides, the physiological system, ROS generation are induced by several exogenous factors such as pollution, smoke, radiation, pesticides and drug consumption. Major producers of ROS are mitochondrial cytochrome P-450 reactions, peroxisomal fatty acid metabolism and NADPH oxidase activity [4]. An imbalance between the ROS production and anti-oxidant mechanism leads to oxidative stress. Oxidative stress has been associated with both onset of periodontal destruction and systemic inflammation [5,6].

Primary immune response against periodontal pathogens is elevated numbers of neutrophils seen in connective tissue, junctional epithelium (50%) and gingival crevicular fluid (90%) and it can cause loss of epithelial cell-cell attachment in junctional epithelium (>60%) leading to apical shift of junctional epithelium [7].

Neutrophil have several mechanisms for controlling bacterial invasion which includes both intracellular and extracellular oxidative and non-oxidative killing mechanisms [8]. When neutrophils and macrophages get stimulated by a phagocytic stimulus, produces a 'respiratory burst', which is characterized by an increase in oxygen consumption, activation of the Hexose-Monophosphate (HMP) shunt and generation of Free Radicals (FR), Reactive Oxygen Species (ROS) and their metabolic products. At sites of chronic inflammation (periodontium in case of periodontitis), there is considerable over production of FR and reactive species.

Research with scientific evidence confirmed that inflammation in the oral tissues especially that associated with periodontitis can be a factor in chronic illness such as cardio and cerebro vascular diseases [9,10], diabetes mellitus, rheumatoid arthritis, advance pregnancy outcomes and a growing list of other conditions such as cancer, psoriasis, chronic kidney disease and anemia. Several hypotheses proposed to describe the interlinking between periodontal diseases and systemic diseases. One of the explanations is imbalance between systemic oxidants and antioxidants [11-13]. "An antioxidant is any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate" [14].

Hence the concept of inhibition of building up oxidative stress within cells through anti-oxidative therapy is implicated in inflammatory disorders and periodontitis along with gold standard therapy for periodontitis is through removal of subgingival biofilm through scaling and root planing has shown to be advantageous. The present article reviews on the comprehensive appraisal of the newer aspects of anti-oxidative therapy and its applications in Periodontology (Figure 1).
Reactive Oxygen Species (ROS)

Every day, numerous free radicals and ROS are produced in each cell by various exogenous and endogenous sources and these ROS are counteracted or balanced by anti-oxidants present within each cell, thereby preventing oxidative stress build up. Reactive oxygen species and their sources are enumerated in table 1.

Recent literature postulate that, non-radicals which have the capability of radical transformation in the extra- and intracellular environment (singlet oxygen, hydrogen peroxide and hypochlorous acid) are grouped under reactive oxygen species category [15,16]. Tissue damage by ROS [5,15,17] (Table 2).

<table>
<thead>
<tr>
<th>Free radicals Sources</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen derived free radical</td>
<td>Non-Oxygen derived free radical</td>
</tr>
<tr>
<td>Superoxide (O2-)</td>
<td>Singlet oxygen (‘O2)</td>
</tr>
<tr>
<td>Hydroxyl (OH-)</td>
<td>Ozone (O3)</td>
</tr>
<tr>
<td>Hydroperoxyl (HOO)</td>
<td>Hypochlorous acid (HOCl)</td>
</tr>
<tr>
<td>Alkoxyl (RO)</td>
<td>Hydrogen peroxide (H2O2)</td>
</tr>
<tr>
<td>Arylperoxyl (ArOO)</td>
<td></td>
</tr>
<tr>
<td>Peroxy ROO</td>
<td></td>
</tr>
<tr>
<td>Acylperoxyl (RCOOO)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1: Reactive oxygen species and their sources.**

<table>
<thead>
<tr>
<th>ROS (Mainly Hydroxyl and peroxynitrate anion)</th>
<th>Target molecule</th>
<th>Example</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>Gingival hyaluronic acid and Proteoglycans</td>
<td>• Folding/unfolding, Fragmentation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Protease degradation of the modified protein</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Formation of protein radicals and protein bond ROS</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Polymerization of proteins</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Formation of stable end products (acetaldehydes)</td>
<td></td>
</tr>
<tr>
<td>Lipid</td>
<td>Cell membrane (activation of cyclooxygenases and lipo-oxigenases pathways)</td>
<td>• Peroxidation and formation of products these are bioactive molecules, Conjugated dienes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Lipid Proxides, Aldehydes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Volatile-hydrocarbons, Prostaglandin-E2 (PG-E2) production</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>eg: Malondialdehyde, Acrolein, Isoprostanes and Neuroprostanes</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>Activating nuclear factor kB (NFkB)</td>
<td>• Strand breaks, Base pair mutations, Deletions, Insertions, conversion of guanine to 8-hydroxyguanine, Nicking and Sequence amplifications.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stimulation of pro-inflammatory cytokine release by monocytes and macrophages</td>
<td></td>
</tr>
<tr>
<td>Enzymes</td>
<td>Antiproteases such as α-1antitrypsin</td>
<td>Oxidation</td>
<td></td>
</tr>
<tr>
<td>Superoxide and hydrogen peroxide</td>
<td>Cells</td>
<td>Osteoclast activation</td>
<td>Bone resorption</td>
</tr>
</tbody>
</table>

**Table 2: Tissue damage by ROS.**
Halliwell established 4 criteria for causal relationship between ROS and disease [18]:

1. ROS or the oxidative damage caused must be present at the site of injury.
2. The time course of ROS formation or the oxidative damage caused should occur before or at the same time as tissue injury.
3. Direct application of ROS over a relevant time course to tissues at concentrations found in vivo should reproduce damage similar to that observed in the diseased tissue.
4. Removing or inhibiting ROS formation should decrease tissue damage to an extent related to their antioxidant action in vivo.

Growing evidence exist in causal relationship between oxidative stress and periodontitis [19] (Table 3).

Evidence suggesting causal relationship between ROS and periodontitis

It is more than a decade the physiological and pathological roles of ROS in the development of periodontitis have been studied.

Chapple and Matthews postulated that periodontal tissue destruction results in increased production of ROS and increased ratio between elastase and lactoferrin by peripheral neutrophils [5].

Agnihotri have been reported that increased levels of ROS in smokers was responsible for excessive periodontal tissue destruction [21].

Patil et al., have reported that the severity of tissue destruction due to excessive ROS is more when periodontal disease is associated with type 2 DM, indicating that oxidative stress is common factor involved in tissue destruction [22].

Di Meo et al., suggested that low levels of ROS are beneficial, but excessive generation or antioxidant deficiency results in periodontal tissue destruction [23].

Liu et al., He et al., have shown the potential link between ROS and autophagy in periodontitis [16,24].

Antioxidants (AO)

The concept of inhibition of building up oxidative stress within cells through anti-oxidative therapy is implicated in inflammatory disorders and periodontitis. Antioxidants are agents which scavenge free radicals or otherwise reactive oxygen species, thereby preventing damage caused by them. Antioxidant therapy as adjunct to conventional treatments such as scaling and root planing has shown to be advantageous. Antioxidant can be classified in the following way (Table 4).
Antioxidant enriched diet and periodontal diseases [25]

Recent evidence has demonstrated a significant role for oxidative stress in promoting bone resorption via activation of certain transcription factors (Fox Os, which decreases want signalling), Modulated by insulin resistance and increasing age [26]. Antioxidant micronutrients combat such pro-inflammatory cascades through modulation of oxidative stress by directly scavenging Reactive Oxygen Species (ROS) and also by down-regulation of some redox-sensitive pro-inflammatory gene transcription factors such as nuclear factor-kappa B and activator protein-1 while up regulating anti-inflammatory gene transcription factors such as nuclear factor [5]. Following are some of the examples of antioxidant diets.

Green Tea

Green tea is a non-fermented product of tea (Camellia sinensis) leaves that is consumed as a beverage worldwide. Green tea extract is documented to have antibacterial, antioxidant, anti-inflammatory and anticarcinogenic properties. It is a rich source of flavonoids, mainly catechins. The four major catechins include

- Epigallocatechin-3-gallate (59%)
- Epigallocatechin (19%)
- Epicatechin-3-gallate (13.6%)
- Epicatechin

Local drug delivery of green tea extract has also shown promising results in treating periodontal diseases [27]. Dentifrice and mouthwashes can be contemplated as vehicles for self-application of chemo-therapeutics. Recently, the addition of green tea catechins to dentifrice has shown reduction of periodontal inflammation in the rat model, by decreasing gingival oxidative stress and expression of pro-inflammatory cytokines [28].

Hrishi et al., conducted a study to evaluate the effect of adjunctive use of green tea dentifrice in periodontitis patients and concluded that green tea dentifrice may serve as a beneficial adjunct to non-surgical periodontal therapy as it showed greater reduction of gingival inflammation and improved periodontal parameters on comparison with fluoride-triclosan dentifrice [29].

Grape Seed Extracts (GSE)

Grape seed extract is a naturally occurring polyphenolic compound obtained from seeds of Vitis vinifera. It possesses a wide range of biological activities such as immunomodulator agent, antioxidant, anticancerous, and anti-inflammatory effects [30,31]. The immuno-modulator effect is particularly due to its proanthocyanidin content. GSE may beneficial for the treatment of inflammation associated with bone destruction as it may strongly inhibit osteoclast differentiation, reduce osteoclast activity, and stimulate bone formation through its positive action on osteoblast differentiation [32]. The anti-inflammatory effect is modulated by calibrating the delicate balance between pro-inflammatory and anti-inflammatory cytokines through regulating their release and gene expression delicate balance between pro-inflammatory and anti-inflammatory cytokines through regulating their release and gene expression [33].

Ozden FO et al., investigated the effects of GSE application on periodontium before and after ligature induced experimental periodontitis [34], using histological and immunohistochemical analyses. Histopathological findings showed improvements in the inhibition of periodontal inflammation and destruction following GSE intake.

Propolis Extract

Propolis, sometimes called bee glue, is a natural resinous substance collected by honey bees (Apis mellifera L.) from plant buds and bark exudates. Bio-flavonoids are the key contributors to propolis’ properties. Propolis is found to have strong inhibitory effects on at least 21 species of bacteria, 9 species of fungi, 3 species of protozoa, and a wide range of viruses.

A number of studies have presented evidence that propolis has strong hepatoprotective, antitumor, antioxidant, antimicrobial and anti-inflammatory properties. Coutinho A in the year 2012 in their clinical study demonstrated the benefits provided by propolis extract and indicated that it should be considered for use as an adjuvant to scaling and root planing [45-48].
Salivary Antioxidant Status

Saliva is rich in antioxidants, mainly uric acid accounting for more than 70% of the total antioxidant activity of resting and stimulated saliva from both healthy and periodontally compromised subjects. Other antioxidants present in lesser concentration than uric acid are albumin, ascorbate and glutathione [49]. Albumin concentration is comparatively low, about 10 nM, apparently in the same range of that of ascorbic acid [49,50]. Accounting for the 5-10% of antioxidant activity in saliva are the traces of other antioxidants such as transferrin, lactoferrin and caeruloplasmin. They are capable of binding metal ions are found in both saliva and GCF [51].

Antioxidant status in gingival crevicular fluid

An excess of reactive oxygen species and depletion of antioxidant levels in gingival crevicular fluid are responsible for the chronic local activation of periodontal inflammation and tissue destruction [5,52]. Gingival crevicular fluid probably contains a locally derived antioxidant from local three sources such as plaque bacteria, neutrophils and crevicular epithelium [53].

Significantly more intense levels of superoxide dismutases in gingival crevicular fluid comprise the most important antioxidant enzyme defense system against reactive oxygen species.

The reduced and oxidised glutathione concentrations in the gingival crevicular fluid are significantly lower in patients with chronic periodontitis, supporting that the glutathione levels in gingival crevicular fluid can play an important role in the pathogenesis of periodontitis [54].

Total antioxidant capacity

It is the measure of the antioxidant capacity of all antioxidants in a biological sample rather than the antioxidant capacity of a single compound. Total Antioxidant Capacity (TAC) of saliva has been measured by only 3 methods using 3 different biochemical techniques [55];

- Spectrophotometric assay
- Enhanced chemiluminescence assay
- Cyclic voltammetry assay

Salivary TAC decreases or does not changes in periodontal disease.

Mechanism of Action [5,15]

Superoxide Dismutase (SOD)

It is an anti-oxidative enzyme, which has been localized within human periodontal ligament and represent an vital defense mechanism for gingival fibroblasts against superoxide ions by catalyzing and accelerating the reaction (approximately 10,000 times) from superoxide ion to oxygen and less reactive H₂O₂ (Figure 3).

In humans SOD exists in 3 forms (Figures 4 and 5)
- Cytosolic Cu/Zn-SOD
- Mitochondrial Mn-SOD (major role)
- Extracellular SOD (EC-SOD)

Catalase (CAT)

It is heme bound iron anti-oxidative enzyme, majorly located in peroxisomes. It protects the cells from hydrogen peroxide by converting it into water (Figure 6).

Reduced Glutathione (GSH)

GSH is an essential antioxidant enzyme, which AIDs as A- Anti-oxidant, I-Immunobooster, D- Detoxifier. It regulates IL-2 dependent T lymphocyte proliferation (Figure 7).
Glutathione Peroxidase

It is a selenium containing peroxidase which reduces variety of hydroperoxides, $\text{H}_2\text{O}_2$, and lipids, thereby protecting the cell particularly against low levels of oxidative stress. It has 5 types of isoenzymes, these are GPX1, GPX2, GPX3, GPX4, and GPX5.

Uric acid

Moore et al., in 1994 proposed that uric acid is responsible for 70% antioxidants capacity of saliva and it is one of the major antioxidant present in saliva. Diab-Ladki in 2003 [56] found decreased concentrations of uric acid, ascorbic acid and albumin in saliva of periodontitis patients.

Ascorbic acid (vitamin C)

It is a water soluble vitamin, function as antioxidant by scavenging water-soluble peroxyl radicals, scavenging superoxide and perhydroxyl radicals, prevention of damage mediated by hydroxyl radicals on uric acid, scavenger of hypochlorous acid, decreases heme breakdown and subsequent Fe$^{3+}$ release thereby preventing Fenton reactions, scavenger of singlet oxygen and hydroxyl radicals, re-forms α-tocopherol from its radical and also protects against ROS-release from cigarette smoke.

Conclusion

Following conclusion can be drawn with respect to the role of reactive oxygen species and antioxidants in periodontitis

1. Oxidative stress and reactive oxygen species appear to play a significant role in the patho-physiology of periodontal diseases.
2. Adjunctive use of antioxidants with traditional therapies should be considered to improve the periodontal treatment outcome.
3. This review presents with evidence within the biomedical literature, provides exciting new opportunities for future development of host modulation therapies in periodontontology.

Future Research Directions

The results of the review may have relevance for the development of treatment protocols. The impact of the combination of periodontal therapy with antioxidants in terms of antioxidant/oxidative stress parameters requires further investigation with longer follow.

References


