ROLE OF ANTIOXIDANTS AS AN ADJUNCT IN TREATMENT OF PERIODONTAL DISEASE

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Abstract:
Periodontal disease is a chronic inflammatory disease of the supporting structures of tooth and is related to the oxidative state of the tissue. In its most common form, chronic periodontitis (CP) is initiated by the microorganisms which are a part of the dental biofilm, causing a local host immune response which results in host mediated tissue destruction and unresolved inflammation. This condition occurs usually in individuals with a tendency towards high rate of accumulation of plaque and calculus deposits in the oral cavity. In the prolonged presence of deposits, the tissue is further exposed to bacterial endotoxins, which leads to the inflammatory responses in the host. These endotoxins cause heightened oxidative stress and discharge of reactive oxygen species. There exists a natural dynamic state of equilibrium between reactive oxygen species (ROS) and antioxidants (AOs). Any disruption in one would lead to modification in the value of other. The values of antioxidant enzymes like Superoxidase dismutase (SOD) and Glutathione (GSH) are thus affected during periodontitis. Instituting periodontal therapy, reduces the levels of these ROS considerably, thus limiting the tissue damage and leading to resolution of the inflammation.

Key words: Antioxidants; Periodontal disease; Periodontitis; Oxidative stress

I.Introduction:

Periodontitis is a chronic inflammatory condition affecting the periodontium because of endotoxins released predominantly by gram negative bacteria of dental plaque origin [1]. Though the etiology is dental plaque, the destruction of the tissue can be greatly corelated to the exaggerated host response towards the pathological micro-organisms and their products, thus resulting in disease progression [2]. Inflammation and host response are integral part of periodontitis and Reactive oxygen species (ROS) have a major role in progression of inflammation, ROS plays a vital role in maintaining periodontal health [3,4].

Oxidative stress as defined by Sies in 1985, "A disturbance in the pro-oxidant – antioxidant balance in favour of the former leading to potential damage" [5]. It is basically an imbalance amongst the Reactive oxygen species (ROS) and host's ability to repair the subsequent damage. Oxygen is a must for survival of life, but excessive of the same can be possibly toxic. Normally, oxygen binds to the mitochondrial electrons to form ATP. The electrons which are leaked out of the mitochondria, get exposed to the oxygen, thus leading to formation of free radicles, which further leads to toxicity among the tissue. The host tissue tries to adapt itself under the constant bombardment of these free radicles. Antioxidants act as a defence system against these ROS. A continuous state of equilibrium is maintained by the body between the ROS and the AO. Whenever this balance is disturbed, the ratio of ROS to AO increases, resulting in 'Oxidative Stress'[6]. Neutrophils have a major part in initiating host response towards periodontal pathogens. These neutrophils produce free radicals for destruction of the pathogens. The excess amount of free radicles are responsible for most of the collateral tissue damage in periodontitis. [7]

Antioxidants (AO) can be defined as 'the substances, when present at a low concentration, compared to those of an oxidizable substrate, will considerably delay or impede oxidation of the specific substrate' [8]. AO can effectively delay or inhibit ROS-induced oxidation and under normal physiologic conditions, can under physiological conditions, ROS are efficiently neutralized by AOs, thus, reducing tissue damage. But, during inflammatory conditions, the ROS production is increased dramatically, as a result of inflammatory cell damage, e.g. Neutrophils and macrophages during the process of phagocytosis through the metabolic pathway of the "respiratory burst." Consequently, this increase in the ROS is not effectively handled by the AO in the tissue, thus leading to tissue damage. [9,10]

Multiple studies have associated oxidative stress in the pathogenesis of chronic inflammatory diseases including periodontitis. The disparity between the ROS-AO has been associated as one of the radical or pathogenic factors for periodontitis. [9,10,11,12]

This review article aims at understanding role of AO in limiting the tissue damage related to ROS.

II.Role of Oxidative stress on periodontal tissues:

Free radicles are of two forms namely 'True Radicals' and 'Reactive Oxygen Species' (ROS) as given by Battino in 1999[Table 1][13]. Free radicals are highly reactive and diverse species including not only oxygen species but also nitrogen and sulphur species, while the hydrogen radical (H^+) is same as the hydrogen atom which is the simplest free radical containing only one proton and one electron.[14].

ROS is a collective term which includes oxygen derived free radicals (ODFR), such as the superoxide radical (O_2^-), hydroxyl radical (OH^-) and nitric oxide radical (NO) species and non-radical derivatives
of oxygen, constituting of hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). The presence of one or more unpaired electrons in the outer orbitals of ODFR makes such species, especially the OH species,
extremely reactive in nature.

True radicals	ROS
Superoxide	Hydrogen peroxide
Hydroxyl	Hypochlorous acid
Perhydroxyl	Singlet oxygen
Hydroperoxyl	Ozone
Alkoxyl	
Aryloxyl	
Arylperoxyl	
Peroxyl	
Acyloxyl	
Acylperoxyl	

Table1: Reactive Oxygen Species

It has been rationalised that more than 1 billion reactive species are released by every cell in the body each day. As these ROS may be responsible for causing both direct and indirect tissue damage.[13]

III.Halliwell's Postulates:

Halliwell in 2000 [15] stated that, for the ROS to be considered as the facilitators of tissue damage, they should be able to fulfil the following four criteria:

1) ROS or the oxidative damage caused must be present at 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.

IV.ROS in Periodontal Destruction:

ROS are involved in tissue damage through a variety of different mechanisms, including DNA damage, lipid peroxidation, protein damage, oxidation of enzymes, and stimulation of pro-inflammatory cytokine. ROS also play an active role in degradation of bone proteoglycans and collagen.(Fig 1) [16]

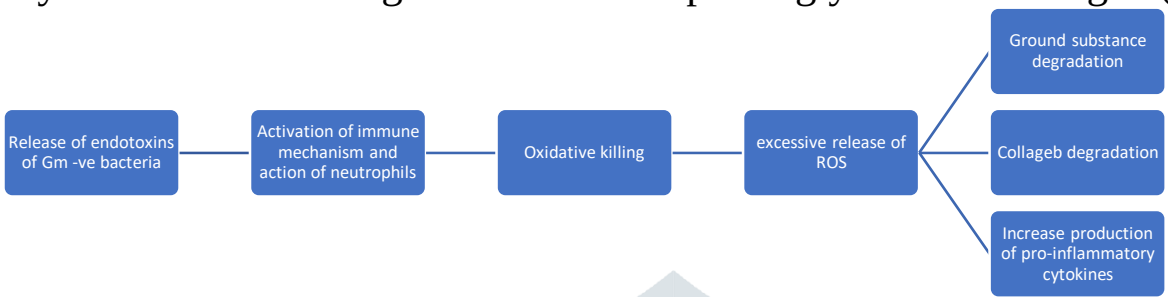

Fig. 1 Action of ROS on Periodontium

The pro-inflammatory cytokines (e.g.TNFα. IL-1), chemokines (e.g. IL-8) and cellular adhesion molecules are expressed due to excess production of ROS are responsible for the activation of factors like NF-kβ and AP-1, resulting in further damage to the periodontal structures, thus corroborating to indirect action of ROS. It is also considered that ROS may lead to increase apoptosis, specifically due to NO• causing damage to cell DNA. [17]

The damage caused can be further divided under metabolites of lipid peroxidation such as Malondialdehyde (MDA), 4-hydroxy-nonenal(HNE) and Isoprostane. These metabolites are also considered as markers used to assess the damage caused by the ROS.[19]

V.Role of Antioxidants:

Antioxidants, as the name suggests, helps the host tissue to prevent the damage caused by ROS.[18] Studies have linked the damage caused by free radicals at the molecular levels to periodontal disease. Free radicals are highly unstable and extremely reactive molecules or atoms with deficit electron. Antioxidants are the nutrients most found in fruits and vegetables which can neutralize these free radicals by contributing an electron without themselves becoming unstable.[20]

If the diet lacks certain nutrients, it becomes further difficult for the oral mucosa to resist infection and assault by the microorganisms on the periodontium. Though poor nutrition may not result directly in periodontitis, it has been hypothesised that there is significant increase in the rate of disease progression due to the compromised host response owing to inadequate nutritional status. [19]

VI.Classification of antioxidants

The antioxidants can be classified depending on several criteria like according to their nature, mode of action, location, origin, or solubility [21,22,23] (Fig 2).

Depending on nature

a) **Enzymatic antioxidants**: Enzymes that collaborate to catalyze the transformation of various types of ROS into hydrogen peroxide. E.g. Super oxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GSH)

b) **Non enzymatic antioxidants**: Molecules that prevent oxidation of other compounds by donating their own electrons. E.g. GHS, Vitamin E, Ascorbic acid, Uric acid, polyphenols ,bilirubin and albumin.

Depending on their mode of action

a) **Preventive antioxidants:** e.g. Enzymes Superoxide dismutase (SOD), catalase , glutathione peroxidase (GPx), glutathione reductase , DNA repair enzymes.

b) **Scavenging (chain breaking antioxidants):** Ascorbate (vitamin C), carotenoids (including retinol – vitamin A), uric acid, α-tocopherol (vitamin E), polyphenols (flavenoids)

Depending on the origin

Endogenous: SOD, GPx, Catalse, Tranferin, Ferritin, Proteases.

Exogenous: Ascorbic acid, Folic acid, Polyphenols, Cysteine.

Depending on location of action

Extracellular: SOD, 3 selenin GPx, Tranferin, Ferritin, Haptoglobin

Intracellular: SOD, Catalse, GPx DNA repair enzyme

Membrane associated: Tocopherol

Depending on solubility

Water soluble: Haptoglobin, Albumin, Uric acid, Ascorbate, Cysteine, Transferin

Lipid soluble: Tocoperol, Carotenoids, quinones

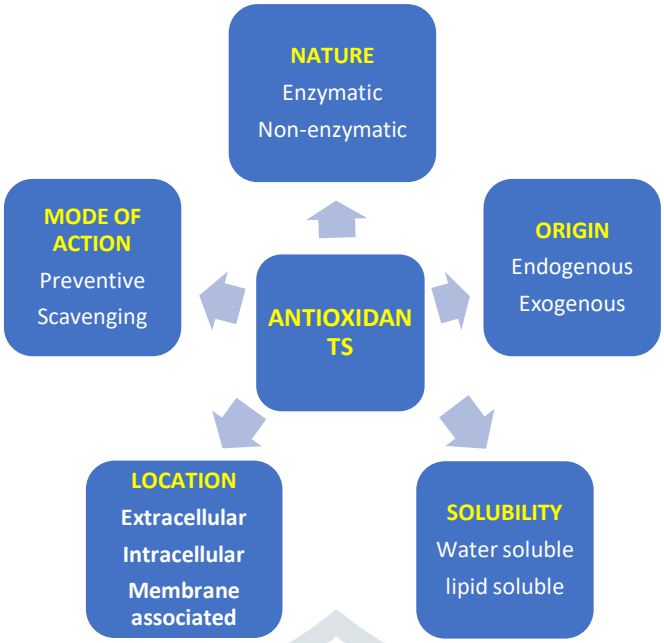

Fig. Classification of Antioxidants

VII.Role of Antioxidants:

Current research has shown promising results in ability of AO to reduce the inflammatory process in periodontal disease. A study by Chappel et al [24] compared the effect of an antioxidant and phytonutrient rich fruit and berry juice powder against placebo in sixty volunteers with chronic periodontitis, it was seen that there was improvements in probing depth and reduction in bleeding on probing in patients taking the antioxidant and phytonutrient supplements.

Similarly, Chandra et al (2005)[25] evaluated another systemic antioxidant composition in patients with gingivitis. The antioxidant capsules contained primarily lycopene in addition to vitamin A, vitamin C, β-tocopherol acetate, and other antioxidants. The patients were randomly subjected to AO capsules and placebo. Results showed that lycopene group showed the greatest percentage reduction in GI, thus concluding that there may be an additive effect using routine oral prophylaxis along with antioxidant treatment.

VIII.Salivary antioxidant status:

Saliva is abundant with antioxidants, comprising primarily of uric acid, which forms almost upto 70% of the total antioxidant activity of both resting as well as and stimulated saliva. This is equally true for healthy and periodontally compromised subjects. Saliva also consists of other lesser antioxidants such as, ascorbate and glutathione [26]. Furthermore, rest of the 5-10% of antioxidant activity in saliva is due to presence of other traces antioxidants such as transferrin, lactoferrin and caeruloplasmin. They function by binding to the metallic ions in both saliva as well as GCF. [27]

IX.Antioxidant status in gingival crevicular fluid

GCF as a source for ROS is again important as excess these free radicles are responsible for the local activation of periodontal inflammatory mediators followed by chronic tissue destruction. [9,28] GCF also is a source of locally derived antioxidants form neutrophils and crevicular epithelium. [29]

Superoxide dismutase (SOD) present abundantly in GCF comprises the defence system against the ROS. Concentration of Glutathione is significantly lower in GCF in patients with chronic periodontal conditions. [30]

X.Total antioxidant capacity

It is the total measure of the 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 [31]

- Spectrophotometric assay
- Enhanced chemiluminescence assay
- Cyclic voltammetry assay

Salivary TAC does not change in periodontal disease.

XI.Antioxidants in management of Periodontitis:

Antioxidants change the progress of oral problems such as periodontitis, gingivitis by compromising antioxidant capacity of crevicular fluid and plasma [32]. One of the conditioning factors for gingivitis is ascorbic acid deficiency. So, antioxidant support is preferred against struggling periodontal diseases. [33,34] Plant oils and green, leafy vegetables can break free radical chain reactions thus may contribute in reducing periodontal inflammation. Flavanoids acquired from antioxidants can possess anti-inflammatory properties that reduce inflammatory molecule expressions in immune system warriors such as monocytes within the gingival connective tissues.[35] In addition, the cranberry fraction could prevent biofilm formation by Phorphyromonas Gingivalis that is a major pathogen of chronic periodontitis. [36] Another study authors stated that Vitamin E has a potential to reduce oxidative damage in experimental periodontitis but does not prevent alveolar bone loss and could cause anxiety. [37]

Decades of research have proven that precise combinations of the antioxidants phloretin, tetracurcuminoid and ferulic acid, including formulations applied topically, can neutralize cell-damaging free radicals, particularly those caused by UV rays, nicotine, alcohol, and hydrogen peroxide. [38] A research has further confirmed that certain antioxidants, including phloretin, silymarin, and hesperetin, significantly inhibit the inflammatory response associated

with *Actinobacillus actinomycetemcomitans*, one of the pathogens that cause periodontal disease.[39] Clinical research also demonstrates the efficacy of topical antioxidants on improving the condition of gingival tissues.[40]

XII.Conclusion:

Diet rich in antioxidants can not only decrease the risk of developing gingival disease but influence its severity as well and this has implications beyond periodontal disease since periodontal pathogens have been shown to play a role in heart disease, lung disease and diabetes in addition to destroying periodontal tissue and bone. Nutritional counselling and supplementation may very well reduce inflammation and thereby enhance outcomes of conventional periodontal therapy. There is increasing evidence from various studies, that an increased intake of antioxidants is associated with a diminished risk for several diseases.

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