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OXIDATIVE STRESS REACTIVE OXYGEN SPECIES AND ANTIOXIDANT DEFENSE SYSTEM IN PERIODONTITIS - A REVIEW

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ABSTRACT

Periodontitis is an inflammatory disease that results in the destruction of supporting periodontal tissues of the teeth, which is primarily initiated by bacterial infection and subsequently modified by anomalous (abnormal, aberrant) host response. As the oxidants and antioxidants are in dynamic equilibrium, any disturbance in one would lead to oxidative Stress. Whenever periodontitis occurs, reactive oxygen species are produced mostly by hyperactive neutrophils that could not be neutralized by antioxidant defense system, cause tissues damage. This tissues damage is mainly characterized by increased lipid peroxidation, DNA damage and protein damage leading to formation of oxidative stress markers. This article focuses on the reactive oxygen species, oxidative stress markers and antioxidant defense system in periodontitis.

KEYWORDS: Reactive oxygen species, Antioxidants, Oxidative Stress.

INTRODUCTION

Chronic periodontitis, is an inflammatory disease primarily initiated by bacterial infection and subsequently modified by anomalous host response that leads to loss of the periodontal attachment to the root surface, alveolar bone loss and ultimately result in tooth loss. Although the primary etiology of the periodontitis is bacterial infection, the majority of the periodontal tissue destruction is due to an inappropriate and extensive host response to that bacterial infection.

Recently, reactive oxygen species (ROS) have gained more attention, as they play a fundamental role in many physiologic processes. However, alongside their central role they also play role in the progression of many inflammatory diseases.[1] ROS are continuously generated by the tissues during normal cellular metabolism as metabolic by-products, but it has been well established that over‑production of ROS occurs at sites of inflammation.[2] ROS have been implicated in the tissue damage mechanisms, either directly by protein damage, lipid peroxidation, DNA damage, and oxidation of important enzymes or by stimulation of pro-inflammatory cytokine release.[3,4]

Several reactive oxygen species (ROS) are produced in physiological quantities in the human body, Thus under physiological conditions, the human body contains an array of antioxidant defense mechanisms both enzymatic and non-enzymatic antioxidants to remove harmful ROS as soon as they are formed, to prevent their deleterious effects and to repair damage caused by ROS.[5] There is a dynamic equilibrium between ROS activity and antioxidant defense capacity in normal physiology. Whenever there is shift in this equilibrium in favor of ROS, either by an increase in ROS activity or production or by a diminished antioxidant defense capacity, the oxidative stress results.[6] (Fig 1)

Oxidative stress has shown to be associated with many clinical conditions like aging,[7] diabetes,[8] many types of cancers,[9] oral pathologies,[10] and periodontits.[11-15] The aim of this review is to discuss the current status of ROS and AO systems and their role in the progression of the periodontal diseases.

REACTIVE OXYGEN SPECIES

Free radicals (FR) are the species having one or more unpaired electrons which are capable of independent existence.[16] They are highly reactive and diverse species that have potential to extract electrons from the various biomolecules vital to cell and tissue function and thereby oxidizing them. Reactive oxygen species (ROS) are a term collectively used for true free radicals and other reactive species that are not true radicals but having capability of radical formation in the intra and

extracellular environments.[17] Table 1 shows various free radicals and reactive oxygen species (ROS) and their symbols modified from Chapple et al, Battino et al.[17,18] ROS are involved in many physiologic processes and continuously generated by the cells in most tissues as an integral part of normal cellular metabolism. ROS have the potential to damage proteins, lipids and DNA, through oxidation reactions.[3]

Sources and Formation of ROS

There are several sources of reactive oxygen species in the human body. ROS can be produced by both endogenous source and exogenous stimulations. Exogenous sources of ROS include trauma, heat, smoking, radiation, infection, excessive exercise, ultrasound, ultraviolet light, ozone, and therapeutic drugs.[19] Endogenously ROS are by-products of metabolic pathways (during cellular metabolism, electrons leak from mitochondrial electron transport systems forming superoxide) or it can be generated by host defense cells (Polymorphonuclear neutrophils) and cells of the connective tissues (osteoclasts and fibroblasts) in response to infection.[17,20] Generation of ROS occur in multiple compartments within the cell like mitochondria, endoplasmic reticulum (ER) and peroxisome. The mitochondria are the largest oxygen consumption organelle, so it is considered as the main source of O2- in physiological conditions.[7] During cellular metabolism, oxygen is consumed via glycolysis to form pyruvate and generate energy within the mitochondria. Electrons produced from mitochondrial electron transport system (respiratory chains), leak at a constant rate, reducing oxygen to form superoxide radical as a byproduct of the metabolic pathway.[17,19]

However, the important source of superoxide in the periodontal tissues is thought to be a functional and purposeful, when Polymorphonuclear neutrophils (PMN) produce the superoxide via the respiratory burst as part of the host response to infection.[20] Functional generation of superoxide radical involves activation of the hexose monophosphate (NADPH-oxidase) shunt, that shunts glucose-6-phosphate from the glycolysis pathway, utilize molecular oxygen and NADPH to form the superoxide radical. This process is stimulated by a variety of antigens, mitogens, cytokines and other mediators. Superoxide (O2-) is considered as the primary ROS, from which the formation of secondary ROS occur, including hydrogen peroxide (H2O2), Hydroxyl radical (OH-), hypochlorous acid (HOCL) and singlet oxygen ($^1O_2$).[21,22,23] Fig 2 illustrates secondary ROS formation from Superoxide radical. Superoxide (O2-) radical undergoes dismutation spontaneously or by antioxidant enzyme called superoxide dismutases and get converted to less reactive H2O2.[21] This H2O2 is then removed by converting it to water and O2 by another antioxidant enzyme, catalase. Hydrogen peroxide undergoes fenton reaction, in presence of Fe2+ or Cu2+ and converted to most potent radical hydroxyl radical (•OH).[22] H2O2 also serves as substrate for myeloperoxidase enzyme, which converts it to another ROS hypochlorous acid (HOCl).[20] Further dismutation of H2O2 by superoxide dismutase enzyme converts it to singlet oxygen ($^1O_2$).[17]

ROS mediated Tissue Damage

Reactive oxygen species cause periodontal tissue destruction by various mechanisms including:[3]

1. Protein damage- Protein degradation by ROS, leading to protein fragmentation and polymerization reaction, protein folding or unfolding, protein radical formation, formation of protein-bound ROS and formation of stable end-products such as oxo-acids or aldehydes.[24,25]
2. Lipid peroxidation- Lipid peroxidation is a radical-chain process involving 3 sequences, initiation, propagation and termination. Polyunsaturated fatty acid side chain (e.g. arachidonic acid) in the lipid membrane is attack by hydroxyl or peroxynitrite radical (initiation) which abstracts a hydrogen atom and forms a carbon-centered radical (L•), which may either rearrange to form a conjugated diene, or may combine with another polyunsaturated fatty acid side-chain radical to form a covalent bond, thus causing cross-linkages and disrupt the membrane structure and function. Mostly the side chain radical reacts with oxygen and form a lipid peroxyl radical which attack another polyunsaturated fatty acid side chain generating another carbon-centered radical and a lipid hydroperoxide. Accumulation of lipid hydroperoxides disrupts the cell membrane functions.[26]
3. DNA damage- The mechanism of DNA damage by ROS mainly peroxynitrate and hydroxyl radical include; Strand breaks, conversion of guanine to 8-hydroxyguanine, Base pair mutations, Deletions, Insertions, Nicking and Sequence amplifications.[17,27]
4. Oxidation of important enzymes for example, anti-proteases such as l-antitrypsin"; and
5. Stimulation of pro-inflammatory cytokine release by monocytes and macrophages, by depleting intracellular thiol compounds and activating nuclear factor KB (NF-KB)."

Antioxidant Defense System

Antioxidants are defined as the substances which significantly delay or inhibit oxidation of the substrate, when present at low concentrations, compared to those oxidizable substrate,.[16] The Antioxidant defense system plays a crucial role in maintaining physiological oxidative state and protection of oral tissues from the deleterious effects of ROS. The specific role of antioxidant is to prevent the formation of ROS, remove harmful ROS as they form, and repair the damage caused by ROS. Various classifications of antioxidants have been proposed. They can be classified on the basis of mode of function, location of action, solubility, structure they protect, and origin (Table 2).[28,29,30]

Many antioxidants function by more than one mechanism e.g. ascorbate acts as a scavenging as well as a preventative antioxidant.[28] The efficacy of any antioxidant depends upon its location, the nature of the ROS, other interactive antioxidant, and environmental conditions (e.g., pH, oxygen tension).[17] The scavenging/chain breaking antioxidants are important in extracellular fluids, inhibiting radicals of chain initiation and propagation, as they form. The lipid soluble antioxidants more importantly act at the cell membrane and protect against peroxidation of lipid, while water-soluble antioxidants act in extracellular tissue fluids.

Oxidative Stress

Under physiological condition, the antioxidants effectively neutralize the ROS produced and prevent ROS-mediated tissue destruction, by protecting and repairing the vital tissues and molecular components. Maintenance of the balance between ROS and antioxidants is an essential aspect for periodontal health, failure of which results in shifting of this balance towards ROS, leading to oxidative stress.[30] Sies et al defined the oxidant stress as a disturbance in the pro-oxidant-antioxidant balance in favor of the former, leading to potential damage.[30] This imbalance occur, when either there are more ROS formation or reduction in antioxidants or both. The periodontal pathogen and their components or toxins stimulate the formation of ROS by polymorphonuclear neutrophils (PMNs), thus causing oxidative stress and periodontal tissue destruction.

Ros In Periodontal Diseases

Periodontal tissue destruction in periodontal diseases is mainly due to complex interactions between pathogenic micro-organism and host immune response. Upon stimulation by periodontal pathogens, host cells release various pro-inflammatory cytokines (e.g., interleukin-1a, interleukin- 1b and tumor necrosis factor-α) as part of the immune response.[31] These pro-inflammatory cytokines recruit polymorphonuclear leukocytes (PMNs) to the site of infection.[32] PMNs are believed to be the primary and predominant cells produced during the inflammatory response against periodontal pathogens. PMNs are physiologically capable of producing ROS, but following stimulation by bacterial lipopolysaccharide become functionally activated which results in increased ROS production via the metabolic pathway of the 'respiratory burst' catalysed by NADPH oxidase.[33,34] The inflammatory cells other than PMNs, like fibroblasts, vascular endothelial cells and osteoclasts also produce ROS.[22,35]

The ROS exert both direct and indirect effects on periodontal tissue destruction.[17,36] ROS directly cause cytotoxic effects such as peroxidation of lipids and phospholipids of biological cell membrane and oxidative damage to proteins and DNA.[22] ROS can also interfere with cellular growth and cell cycle progression, as well as induce apoptosis.[37,38] Direct effects of ROS also include extracellular matrix degradation by inducing the breakdown of glycosaminoglycan and matrix proteinases.[4,22,34] ROS may further degrade extracellular matrix by directly attacking the collagen and rendering it more susceptible to the enzymatic breakdown by the enzyme collagenases. ROS also cause inactivation of enzyme inhibitors such as TIMP and a1-antiproteinase.[39] Apart from direct intracellular and extracellular damage, ROS can also exert indirect effect by enhancing pro-inflammatory cytokines, chemokines, and cellular adhesion molecules.[19] ROS are believed to affect the activity of nuclear factor-κB and activating protein 1.[22] Certain ROS and the activation of NF‑kβ and AP‑1 further activate osteoclasts which ultimately lead to hard tissue destruction.[40] Periodontal tissue destruction leads to overproduction of inflammatory mediator, oxidised proteins, lipid peroxides, and other byproducts of oxidative stress. These further mediate the activation of PMNs, macrophages, and fibroblasts that again result in more ROS production. Therefore it can be stated that in the presence periodontal pathogens, ROS and tissue destruction form a circle (Fig 3).

Measuring The Oxidative Stress In Periodontitis

Oxidative stress markers are important for assessment of the periodontal disease status and progression of the diseases. The oxidative stress can be measured by any of three ways- (1) direct measurement of the ROS; (2) measurement of antioxidant; and (3) measurement of the by-products of oxidative stress induced periodontal tissue destruction. The direct measurement of ROS is a promising and valuable biomarker. However ROS have extremely short half-life so the direct measurement of these species in-vivo are much difficult and complex.[17] As it is not practical and easy task to measure different ROS because their effects are additive, total oxidant status (TOS) measurement can provide a new approach.[41,42]

Measurement of the oxidative stress through antioxidants can be done by measuring the enzymatic antioxidants like Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Catalase, and non- enzymatic antioxidants like vitamin C, vitamin E, uric acid, reduced glutathione etc.[11,12,19,43,44], Measuring the total antioxidant capacity (TAOC) of biological samples is an effective method of assessment of oxidative stress.[22,45,46] In majority of the clinical studies the oxidative stress were assessed by the measurement of the by-products of oxidative tissue destruction which provide the most direct assessment of oxidative stress.[42] The biomarkers of tissue destruction include the by-products of lipid peroxidation, oxidative damage of DNA, and protein oxidation. The markers of lipid peroxidation are Malondialdehyde (MDA), 4-hydroxynonenal (HNE), acrolein and thiobarbituric acid reactive substances (TBARS), of which MDA is most studied biomarker of the periodontal disease.[12,13,14,42] The most commonly used biomarker of DNA destruction is 8-hydroxydeoxyguanosine[47,48] and marker of protein

destruction is protein carbonyls.[25,31] So many recent periodontal researches are concerned with the determination of oxidative stress biomarkers that accurately reflect periodontal status for diagnosis and evaluation of periodontal treatment outcome.

Table 1: True radical and reactive oxygen species (ROS) and their symbols.

True radicals	ROS
Superoxide ($O_2^{\bullet -}$)	hydrogen peroxide (H_2O_2)
Hydroxyl ($OH^{\bullet}$)	hypochlorous acid (HOCl)
Perhydroxyl ($HO_2^{\bullet -}$)	singlet oxygen ($^1O_2$)
Hydroperoxyl ($HOO^{\bullet}$)	Ozone (O3)
Alkoxyl ($RO^{\bullet}$)	
Aryloxyl ($ArO^{\bullet}$)	
Arylperoxyl ($ArOO^{\bullet}$)	
Peroxyl ($ROO^{\bullet -}$)	
Acyloxyl ($RCOO^{\bullet}$)	
Acylperoxyl ($RCOOO^{\bullet}$)	
(•) indicate an unpaired electron and (− or +) indicate the molecular charge, which may be +ve or − ve or neutral (e.g. •OH).	

Table 2: Classification of Antioxidants.

Classification based on	Types	Examples
1. Mode of Action	Preventive Antioxidants	Enzymes ie superoxide dismutase (1, 2 and 3), glutathione peroxidase, catalase, DNA repair enzymes, e.g. poly(ADP-ribose) polymerase, others
		Metal ion sequestrators: superoxide dismutase, catalase, glutathione peroxidase, albumin, haptoglobin, ceruloplasmin, lactoferrin, transferrin, hemopexin, carotenoids, glutathione reductase, uric acid, polyphenolic flavenoids
	Scavenging (Chain Breaking) Antioxidants	Ascorbate (vitamin C), carotenoids, uric acid, a-tocopherol (vitamin E), bilirubin, albumin, ubiquinone, polyphenols (flavenoids), reduced glutathione and other thiols
2. Location	Intracellular	Superoxide dismutase (1 and 2), catalase, glutathione peroxidase, DNA repair enzymes, reduced glutathione, ubiquinone
	Extracellular	Superoxide dismutase 3, selenium-glutathione peroxidase, reduced glutathione, haptoglobin, ceruloplasmin, albumin, ascorbate, lactoferrin, transferrin, carotenoids, uric acid
	Membrane Associated	a-Tocopherol
3. Solubility	Water Soluble	ascorbate, haptoglobin, albumin, ceruloplasmin, uric acid, transferring, cysteine, polyphenolic flavenoids, reduced glutathione and other thiols,
	Lipid Soluble	a-Tocopherol, bilirubin, quinines, carotenoids,
4. Structure they Protect	Lipid-protective antioxidants	a-Tocopherol, ascorbate, carotenoids, reduced ubiquinone, glutathione peroxidase, reduced glutathione, bilirubin
	Protein-protective antioxidants	Sequestration of transition metals by preventative antioxidants
		Scavenging by competing substrates
		Antioxidant enzymes
	DNA protective antioxidants	Superoxide dismutase (1 and 2), glutathione peroxidase, DNA repair enzymes, reduced glutathione, cysteine
5. Origin	Exogenous antioxidants (obtained only though the diet)	Carotenoids, ascorbic acid, tocopherols (α, β, c, d), polyphenols, folic acid, cysteine
	Endogenous antioxidants (synthesized by the body)	Superoxide dismutase, Catalase, glutathione peroxidase, glutathione-S-transferase, reduced glutathione, ferritin, transferrin, ceruloplasmin, glycosylases, peroxisomes, proteases
	Synthetic	N-acetylcysteine, tetracyclines, penicillinamine

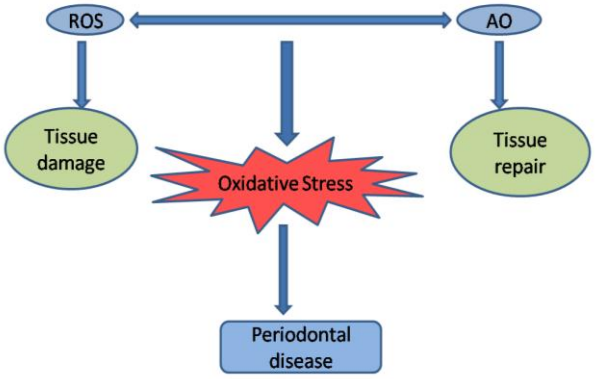

Figure 1: ROS and antioxidants balance - Oxidative stress.

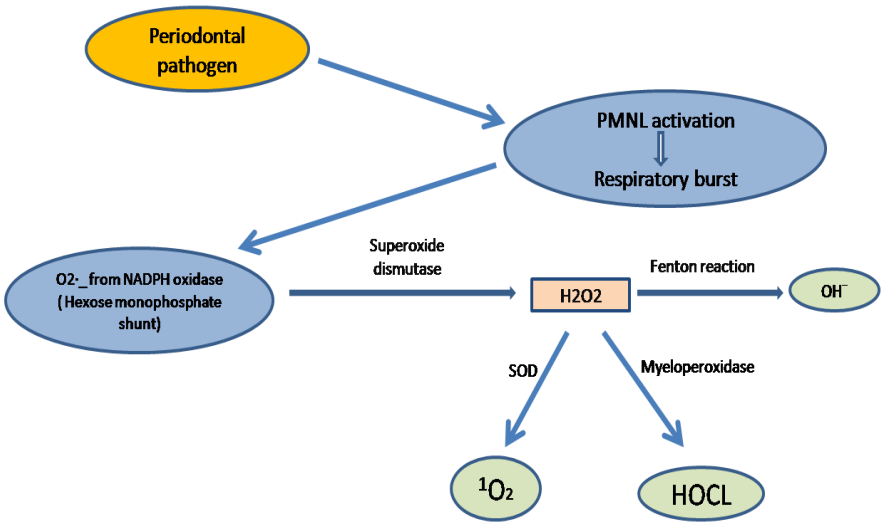

Figure 2: Formation of Reactive oxygen species.

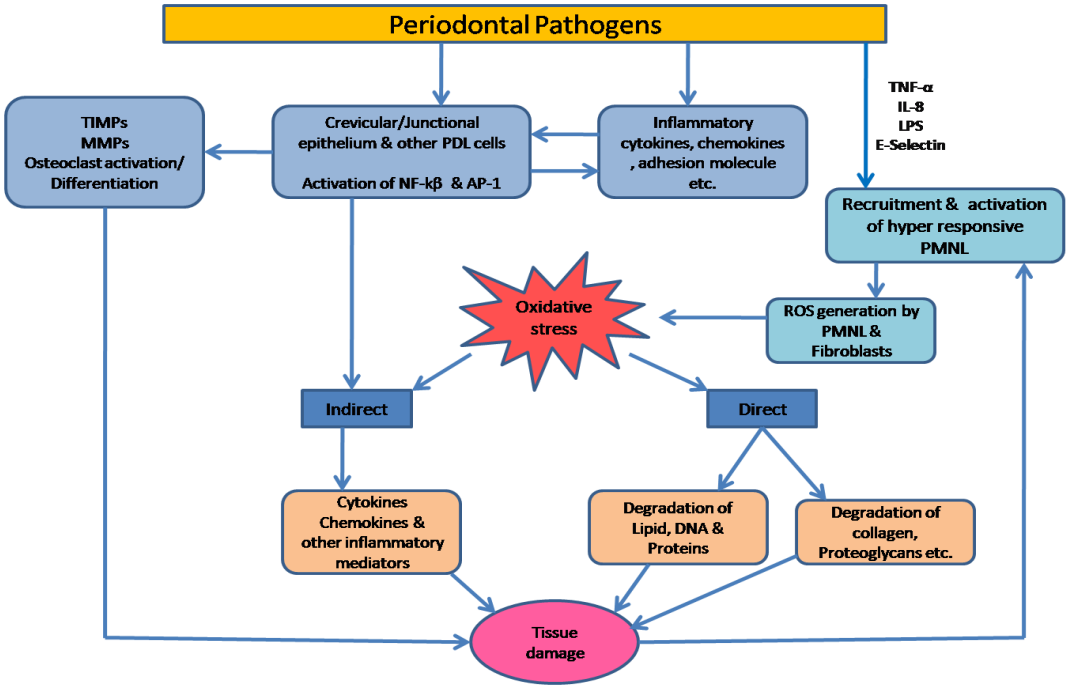

Figure 3: Diagram illustrating the role of ROS in periodontal tissue destruction.

CONCLUSION

This review highlights the association of ROS, antioxidants, and OS with the periodontal disease. Under normal physiologic conditions and at lower level ROS have beneficial antimicrobial effects, but during periodontal pathological conditions and at higher level it has detrimental effects on periodontal tissues. ROS are overproduced during periodontal disease that cannot be balanced or neutralized by antioxidants resulting in oxidative stress, which ultimately lead to periodontal tissues destruction. However ROS have extremely short half-lives, they can cause substantial tissue destruction. Furthermore, the markers of oxidative stress could be used as biomarker of periodontal disease activity and severity.

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