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Role of Oxidative Stress and Glutathione as Antioxidant in Periodontitis: Review Article

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Abstract: *Reactive oxygen species production plays an important role in the pathogenesis of periodontal disease. In periodontitis, polymorphonuclear leucocytes appear to be functionally activated and exhibit increased production of reactive oxygen species. Reactive oxygen species may lead to damage the periodontium. The use of antioxidants in the treatment of periodontitis prevent tissue damage caused by reactive oxygen species. Glutathione is the most important intracellular antioxidant for ROS detoxification and a good marker for investigating the antioxidant defense mechanism in periodontitis caused by oxidative stress.The tissue damage associated with oxidative stress, and enhancing wound healing with glutathione cannot be underestimated, so need to be evaluated further through randomized controlled trials.*

Key Words: *Glutathione, Reactive oxygen species, Periodontitis, Antioxidants, oxidative stress*

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I. Introduction

Periodontitisis a complex disease in which disease expression involves intricate interactions of the biofilm with the host immune-inflammatory response and subsequent alterations in bone and connective tissue homeostasis [32], ending with periodontal ligament, alveolar bone and root cementum destruction which leads finally to tooth loss. [18]

The primary clinical features of periodontitis include clinical attachment loss (CAL), alveolar bone loss (BL), periodontal pocketing, and gingival redness and edema. In addition, enlargement or recession of the gingiva; bleeding of the gingiva following application of pressure; and increased mobility, drifting, and/or tooth exfoliation may occur.[23]

Periodontal disease results from the complex interplay between the subgingival biofilm and the host immune-inflammatory events that develop in the gingival and periodontal tissues in response to the challenge presented by the bacteria. Subgingival plaque biofilm is composed of up to 150 bacterial species present at individual sites, however only specific groups of bacteria are related to periodontal breakdown. [44]These include the red complex of Porphyromonasgingivalis, Tannererella forsythia, and Treponema denticola as well as Aggregatibacter actinomycetemcomitans. [43]

The subgingival biofilm initiates and perpetuates the inflammatory responses in the gingival and periodontal tissues. Lipopolysaccharides are considered one of a range of microbe associated molecular patterns (MAMPs) recognized by pattern recognition receptors (PRPs) such as Toll like receptors (TLRs) on host cells. The sensing of TLRs by MAMPs results in the establishment of innate immune responses. The TLRs are also expressed on immune cells thus contributing to the development of adaptive immune responses.[30]

The initial immune response in periodontitis occurs following colonization of the gingival sulcus by periodontopathic bacteria. The presence of the bacteria induces the production of cytokines and chemokines by the gingival epithelium. This results in the expression of adhesion molecules, increased permeability of gingival capillaries and chemotaxis of polymorhponuclear neutrophils through the junctional epithelium and into the gingival crevice. [29]

Periodontitis is a chronic inflammatory condition initiated in response to plaque biofilm, characterized by exaggerated inflammation and loss of periodontal and bone support, with the excessive production of reactive oxygen species (ROS) and proteolytic enzymes.[45]Periodontitis patients reported having reduced total anti-oxidant (AO) capacity in whole saliva,[10]and lower concentrations of reduced glutathione (GSH) in serum and gingival crevicular fluid (GCF).[11,49]

II. Role of oxidative stress in pathogenesis of periodontal diseases:

Oxidative stress is characterized by an increased level of reactive oxygen species (ROS) that disrupts the intarcellular reduction-oxidation (redox) balance. [52] ROS is a term often used to define oxygen radicals

(superoxide [O_2^-], hydroxyl [$^{\cdot}OH$]), and certain non-radicals that are either oxidizing agents and/or are easily converted into radicals such as hypochlorus acid (HOCL), singlet oxygen ($1O_2$) and hydrogen peroxide (H_2O_2). [13]

The largest amounts of ROS are produced in mitochondria by the transfer of unpaired electrons from the respiratory chain to molecular oxygen (O_2). This results in (O_2^-) formation that is converted to H_2O_2 by manganese-dependent superoxide dismutase (SOD) in the mitochondrial matrix and by Cu- and Zn-dependent SOD in the cytosol. H_2O_2 is detoxified by either catalase or glutathione peroxidase activity. Oxidative stress may result from mitochondrial electron leakage during normal metabolism. [53]

In this situation increased nutritional intake of refined sugars or saturated fats can overload the Krebs cycleproducing excess $O2^{-}$ radical and downstream ROS which overwhelm the mitochondrial SOD.

PMNLs play a vital role in host defense and constitute the first line of defense against microbial invasion and infection in the body. In the oral cavity, following plaque accumulation and the development of clinical inflammation, 90% of leukocytes that enter the gingival crevicular fluid (GCF) and 50% of those that

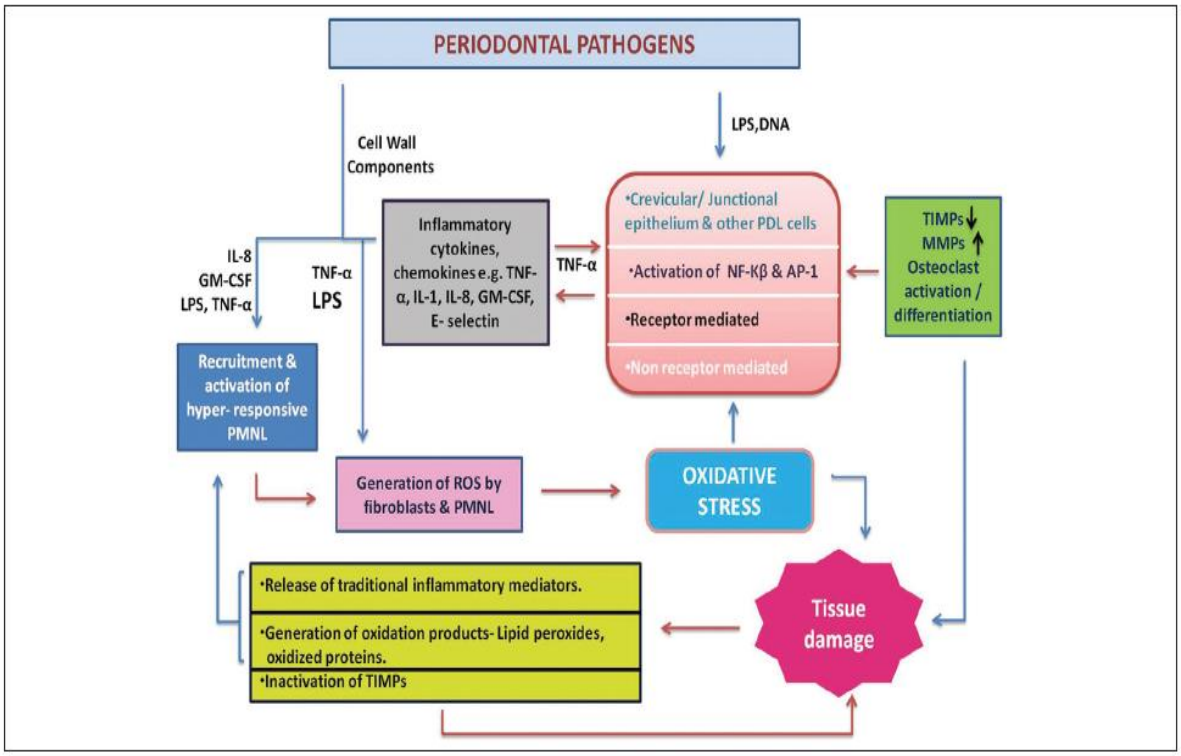

Fig. (1) Diagram illustrating a role of reactive oxygen(ROS) species in generating chronic inflammation and tissue damage in response to periodontal pathogens. MMP – Matrix metalloproteinase; TIMP – Tissue inhibitor of matrix metalloproteinase; NF-κβ– Nuclear factor kappa beta; AP-1 – Activating protein-1; PDL – Periodontal ligament; TNF – Tumor necrosis factor; IL – Interleukin; GM-CSF – Granulocyte–macrophage colony-stimulating factor; LPS – Lipopolysaccharide; (Dahiya, et al, 2013)[14]

infiltrate junctional epithelium are PMNLs. [10]These neutrophils help in controlling the microbial invasion by several intracellular and extracellular oxidative and non-oxidative killing mechanisms. [17]This oxidative killing mechanism of neutrophils leads to the formation of ROS/Free Radicals.The specific cytokines and chemokines produced by this initial response lead to a perivascular Tcell/macrophage dominated inflammatory infiltrate in the connective tissues. If this cell-mediated immune response does not control the bacterial challenge, progression to a B cell /plasma cell lesion occurs. [29]

One of the endogenous source for radical oxygen formation is the functional generation by phagocytic cells during an inflammatory response following stimulation by opsonised particles, bacterial DNA or peptides. This leads to activation of respiratory burst that utilizes molecular oxygen and NADP as electron donor. [12,50]The superoxide generated by this enzyme serves as the starting material for the production of a vast assortment of ROS. The different ROS species are used by phagocytes to kill periodontal pathogens but also cause collateral damage to nearby tissues. [36, 37,50]

In periodontitis, PMNLs appear to be functionally activated and exhibit increased production of reactive oxygen species (ROS*)*.[25]The degree to which ROS influence the progression of periodontal diseases is

as yet unclear, but their role cannot be considered in isolation, given the range of antioxidant species that protects against excess ROS activity and maintains a delicate equilibrium within host tissues.[13]

Effect of reactive oxygen species in systemic health:

In addition oxidative stress may be induced by exogenous sources as heat, trauma, ultraviolet light, ozone, smoking, radiation and drugs. [12,15] ROS underpins the pathogenesis of diseases as cardiovascular diseases [54], type 2 diabetes mellitus [55] and neurological disorders. [48] ROS cause tissuedamage by a variety of mechanisms as lipid peroxidation, DNA damage,protein damage and stimulation of proinflammatory cytokine release bymonocytes and macrophages. Importantly, ROS activatestress sensitive gene transcription factors as NF-κB and AP-1 that triggeractivation of proinflammatory cytokine production. [13]

Effect of reactive oxygen species in periodontitis:

In periodontitis, polymorphonuclear leucocytes appear to be functionally activated and exhibit increased production of reactive oxygen species.[11] Periodontium are subject to neutrophilic inflammation in response to microbial insult, within an environment high in activating cytokines produced by the lining epithelium and underlying fibroblasts and inflammatory cells. Normally, intracellular concentrations of GSH are high (0.5–10mM) but extracellular fluid values are low (0.5–5mM in human plasma). The high concentrations of GSH found in GCF are likely to influence the regulation of proinflammatory cytokines, involved in the processes that lead to host tissue damage.[13]

ROS production is also involved in mineral tissue homeostasis and contributes mostly to bone loss by inhibiting osteoblast differentiation and enhancing osteoclastogenesis. ROS induced bone resorption occurs through the modulation of kinases and transcription factor activation in both osteoclasts and osteoblasts.[22]

The majority of tissue destruction in periodontitis is considered to be the result of an aberrant inflammatory/immune response to microbial plaque adjacent to the gingival margin and to involve prolonged release of neutrophil enzymes and ROS. Most published work in the periodontal literature has focused on markers of ROS reactions with lipids. Till date, only thiobarbituric acid reactive substances has been investigated in periodontitis. All the published studies have suggested that patients with periodontitis have higher levels of lipid peroxidation than periodontally healthy controls. [14]

Antioxidants:

Antioxidants (AOs) may be regarded as "those substances which when present at low concentrations, compared to those of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate".[10]Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species. A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables and tea, etc. [34]

According to Kumar[33]antioxidantscan be divided into natural antioxidants and synthetic antioxidants. Naturally occurring antioxidants of high or low molecular weight can differ in their composition, in their physical and chemical properties, in their mechanism and in their site of action. They can be divided into enzymes and low molecular weight antioxidants. Enzymes such as superoxide dismutase (SOD) and glutathione peroxidase attenuate the generation of reactive oxygen species by removing potential oxidants or by transferring ROS/RNS (reactive nitrogen species) into relatively stable compounds.

The Low molecular weight antioxidants include lipidsolubleantioxidants (α tocopherol, carotenoids, quinones, bilirubin and some polyphenols) and water soluble antioxidants (ascorbic acid, uric acid and polyphenols). These delay or inhibit cellular damage mainly through free radical scavenging property.[33]Antioxidants can be classified by mode of action into preventive and scavenging (chain breaking) antioxidants.[12] The preventive antioxidants function by enzymatic removal of superoxide and hydrogen peroxide or by sequestration of divalent metal ions preventing subsequent hydroxyl radical formation. The scavenging or chain breaking antioxidants are low molecular weight species that denote electrons before becoming oxidized requiring subsequent reduction or replacement to maintain the body antioxidant capacity. The lipid soluble antioxidants (α-tochopherol and the carotenoids) act at the cell membrane level and protect against lipid peroxidation, whereas the water soluble scavengers are more important within the extracellular tissue fluids.[12]

TABLE (1) ANTIOXIDANTS CLASSIFIED BY THEIR MODE OF ACTION[12]

Mode of action	Example
Preventative antioxidants	Enzymes:superoxide dismutase enzymes (1, 2 and 3), catalase, glutathione peroxidase, DNA repair enzymes, e.g. poly (ADPribose) polymerase, others. Metal ion sequestrators: albumin, lactoferrin, transferrin, haptoglobin, ceruloplasmin, hemopexin, carotenoids, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, uric acid, polyphenolicflavenoids.

Scavenging(chain breaking)antioxidants	Ascorbate (vitamin C), carotenoids (including retinol– vitamin A), uric acid, α-tocopherol (vitamin E), polyphenols (flavenoids), bilirubin, albumin, ubiquinone (reduced form), reduced glutathione and other thiols (free or protein bound)

Antioxidants and periodontal disease

Few studies have considered the effect of the imbalance between oxidants and antioxidants in patients with periodontitis, which predisposes such individuals to the damaging effects of ROS in the periodontium.[8]Huang et al. [25]found that within periodontitis subjects, glutathione peroxidase levels correlated negatively with pocket depth and attachment loss and increased post-therapy. On the other hand,Wei et al. (2004) found that lactoferrin levels in GCF have been shown to be increased in periodontitis as indeed have glutathione peroxidase levels. The total antioxidant capacity in plasma is reduced in periodontitis patients.[9,31]In addition,Panjamurthy et al.[36]demonstrated lower plasma levels of vitamin C, vitamin E and reduced glutathione (GSH) in periodontitis patients compared with healthy controls.

Several studies in animal have demonstrated the role of some biological substances with antioxidant capacities in the modulation of host inflammatory response by reducing inflammatory biomarkers and bone loss.[56]The role of flavonoids and cocoa enriched diet in relation to periodontal diseases that investigated on gingival oxidative stress in a rat-periodontitis model[46]and the results also showed the degree of alveolar bone loss and the densities of polymorphonuclear leukocytes and TRAP positive osteoclasts were significantly lower than in the periodontitis group with no specific diet. There are many important intracellular antioxidants for ROS detoxification and a good marker for investigating the antioxidant defense mechanism in chronic inflammatory diseases caused by oxidative stress.[24,41]

III. Glutathione:

Glutathione is consider master antioxidant present in every one of our cells.[39]It is a low-molecular weight, major non-protein cellular thiol, and as potent AO that prevents ROS mediated damage to essential components of the cells and acts as a cofactor for enzymes in the destruction of ROS. It serves as a reservoir for cysteine, participates in detoxification reactions for xenobiotics and metabolism of numerous cellular compounds (e.g., NO), and is required for synthesis of some prostaglandins (PGs).[16]It also has a significant role in cell metabolism, cell apoptosis, cell proliferation, immune response, cytokine production , gene expression and protein synthesis.[49]

Glutathione is a cellular thiol present in human cells at concentration 0.5-10 mmol/L. 85% to 90% of cellular glutathione is present in the cytosol, and rest 10-15% is distributed in many intracellular organelles. Concentration of mitochondrial glutathione is similar to that of the cytosol (10-14 mM) by the volume of the mitochondrial matrix.[35]The greatest concentration of glutathione is found in the liver, the organ involved in elimination of toxins.[13, 14, 51]

Glutathione is synthesized intracellularly, by two enzymes namely glutamate-cysteine ligase (GCL) or γ-glutamate-cysteinyl-synthetase (GCS), which is the rate-limiting step and glutathione synthetase, and is regenerated by six enzyme-catalyzed reactions known as γ-glutamyl cycle[13]of the three building blocks of glutathione, viz., cysteine, glutamic acid and glycine, the sulphydryl or thiol (SH) group in cysteine serves as a proton donor, and acts as a limiting factor in glutathione synthesis. In the absence of reduced cysteine, the addition of an acetyl group to cysteine (N-acetyl-cysteine [NAC]) provides the ability for a molecule to cross the cell membrane and promotes intracellular glutathione synthesis.[14]

Glutathione is a low molecular weight thiol (up to 5–10 mM) made from the combination of three amino acids, that is, cysteine, glutamate, and glycine.It present in the two forms which are oxidized glutathione (GSSG) (10%) and reduced GSH (90%) forms in the body.[4,11, 24] The reduced GSH maintain the survival of the cell. The deficiency of GSH in the cell lead to risk of oxidative damage. It plays an important role in the body as anti-oxidant function, detoxification function and immune function.[4] The level of GSH and GSS decrease in the gingival crevicular fluid in patients with periodontitis than in the control subjects.[11]

IV. Glutathione in periodontitis

Glutathione is one of the most important AO (radical scavenger), which controls inflammatory process.[24]Reduced glutathione has important role in the regulation of pro-inflammatory cytokines is of great importance in periodontal disease. Some studies revealed increase in the concentrations of the cytosolic cysteine, and thus glutathione of monocytes and macrophages using a synthetic form of cysteine called NAC, blocks H_2O_2 mediated activation of NF-kB, and thus pro-inflammatory cytokines (interleukin [IL]-1 β, IL-6 and tumor necrosis factors [TNF]-α) by this route. TNF-α, IL-1 β and IL-6 are associated with activation of bone resorbing processes, and IL-8 is reported to polymorphonuclear leukocyte activity.[10]

Disturbances in neutrophils with periodontitis patients, which are associated with deregulation of anti-inflammatory transcription factor Nrf2 pathway.The activation of the redox sensitive protein acid sphingomyelinase lead to promote lipid raft formation, and therefore assembly of the NADPH oxidase enzyme was also mediated by a disturbance in redox balance. Both mechanisms, result in persistent hyperactivity in neutrophils of periodontitis patients.[21]

In patients with Periodontitis, the studies showed decreased levels of GSH and lower antioxidative activity in GCF and direct correlation with symptoms of this disease in compared to high concentrations of GSH in patients with healthy gums.[9,11] The lower concentrations of glutathione (reduced, oxidised, and total) in the GCF could be the result of a variety of factors leading to decreased synthesis and enhanced local degradation which have important implications for the pathogenesis and treatment of periodontal disease.[11]GSH and GSSG concentrations in the GCF of periodontally disease patients remain lower than that of healthy subjects.[42]Albuali found that GSH concentrations in the blood in obese individuals were lower than those of normal weight control individuals.[1]

The studies showed the total anti-oxidant such as GSH concentrations in the saliva was higher in the patients with severe periodontitis in compared to the healthy or gingivitis control before periodontal treatment.[27,28,47]Some studies have demonstrated that reduced in the total antioxidant capacity in periodontitis patientsin the blood and the GCF.[9] Other studies showed the GSH levels increased in the gingival crevicular, fluid, plasma and saliva when compared to healthy subjects after therapy.[6]Scaling and root planing (nonsurgical therapy) restores GSH concentration in GCF post 1-month and 3 months along with redox balance (GSH: GSSG ratio), but at 6 months the balance is not maintained. [40]Other studiesdemonstrated that successful, non-surgical therapy does not fully restore GSH concentrations in GCF but restore the redox balance (GSH:GSSG ratio).[11]

There are novel therapeuticapproaches can lead toelevate theGSH buffering capacity within the tissues such as the use of N-acetyl-cysteine (NAC) drug which promotes intracellular glutathione synthesis.[13,57]Alkadasi et al. showed a significant reduction in the level of periodontal pocket in the three months followingadjunctive use NAC as a systemic antioxidant with non-surgical periodontal treatment[3], or with surgical periodontal treatment.[4]The use of micronutritional supplements to boost antioxidant concentration in the tissues is recommended as adjunctive to nonsurgical periodontal therapy topreserve elevatingof GSH at the inflamed sites, to maintain redox balance for longer duration.[40] The local alterations in the redox balance and a systemic increase in ROS following obesity may induce gingival oxidative damage, and this may lead to progression of periodontal inflammation. No literature data are available that show the effect of obesity on glutathione values in patients with periodontitis.[1]

V. Conclusions

Oxidative stress is one of the most important factors that lead to destruction of the periodontium so we need antioxidant therapy specially glutathione which consider a master antioxidant to help in removal ROS and maintain the periodontium.The effect of oxidative stress on the pathogenesis of periodontitis and the adjunctive use of glutathione in management of periodontitis, cannot be underestimated; however, it needs to be evaluated further through multi-centered randomized controlled trials.

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