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OXIDATIVE STRESS AND PERIODONTAL DISEASE. REVIEW

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ABSTRACT

ROS (reactive oxygen species) have become increasingly worthy of attention because of their central role in the progression of many inflammatory conditions. They are described as oxygen free radicals and any other non-radical oxygen derivative involved in the production of oxygen radicals. ROS are involved in normal cellular metabolism and are continuously generated by cells in most tissues. Another category of substances called antioxidants exist in the cell that can effectively delay or completely inhibit ROS-induced oxidation. Under physiological conditions, ROS production is drastically increased largely due to the immune system cells and the phagocytosis process through the metabolic pathway. Consequently, high levels or increased activities can not be balanced by the antioxidant defense system, which leads to oxidative stress and tissue damage. ROS can directly damage the tissue by their action against DNA, proteins and oxidation of important enzymes. At the same time, they can act as signaling pathways or as mediators of inflammation.

In recent years, numerous clinical and experimentional studies have demonstrated a strong association between oxidative stress and periodontitis. A better understanding of this association can lead us to a detailed knowledge of the pathogenic mechanisms of this disease, and so can guide us towards a more structured therapy.

Key words: Oxidative stress, Reactive oxigen species, Periodontal disease

INTRODUCTION

Oxidative stress is a mechanism involved in many inflammatory conditions that cause lipid, nucleic and protein damage (oxidative distress). On the other hand, it is also a physiological process that allows the immune system to handle microorganisms and intracellular signaling (oxidative eustres). The physiological functions of free radicals have been neglected for years so much more is known about their pathological role. However, oxidative stress is usually defined as an imbalance between free radical production and antioxidant mechanisms. Free radicals are not simply a negative product of oxygen metabolism; they are involved in immune hepatic metabolic responses but also in intracellular signaling pathways. Hypotheses have been made that under physiological conditions even large concentrations of a single species of reactive oxygen or reactive nitrogen do not lead to oxidative lesions, considering the reparative mechanisms of the cell.

Periodontitis is an inflammatory disorder that affects the teeth supporting structures that ultimately lead to the loss of alveolar bone and teeth. The main causal

factor is the microorganisms that colonize the subgingival dental plaque that induces an inflammatory host response. However, inflammation affects healthy surrounding tissue that ultimately leads to the destruction of said tissues and finally to periodontitis. Although proteolytic liposomesaccharides and enzymes are essential in periodontitis, exaggerated inflammatory response, genetic predisposition, smoking, poor oral hygiene and malnutrition are also important in the pathogenesis of periodontitis (Kinane et al., 2017) [1].

Progression of periodontal disease is dependent on the delicate balance between host immune response, susceptibility and the bacterial challenge imposed by dental plaque.

The role of oxidative stress in periodontitis has been postulated decades ago, however, the suggested implication was not clear. Some studies have shown that leukocytes from patients with periodontitis are depleted and have low oxidation activity while other studies have shown a higher production of free radicals by leukocytes in periodontal patients. The contradictory findings may be related to the dynamics of the mechanisms during the pathogenesis of the disease, but could also be explained by different forms of periodontitis. More recently, studies have pointed to oxidative stress as being part of the pathogenesis of periodontal diseases (Solomon et al)[2]. Oxidative stress is a condition caused by a pathological increase in the production of reactive oxygen species (ROS), which are important signalling molecules in the regulation of several cellular processes. Consequences of this kind of oxidative stress include adaptation, damage or cell death through a variety of mechanisms, such as DNA, lipid and protein damage. Regarding the prevention of ROS formation, enzymatic and non-enzymatic antioxidant mechanisms have been studied and reported in the literature. Enzymatic mechanisms are responsible for direct ROS neutralization and these mechanisms are constituted by primary enzymes involved in human organism protection in an attempt to maintain the ROS levels in a normal range. Examples of these enzymes are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).

The reactions of superoxide, nitric oxide, and other primary reactive species are reversible and ideal for intracellular signaling thus, measuring oxidative stress using a single marker can lead to erroneous interpretations. This means that the administration of antioxidants can effectively treat oxidative stress-related conditions such as diabetes.

Intracellular interaction between ROS and antioxidants is crucial and is regulated by the transcription process at the genetic level. An upward shift in intracellular oxidants can cause damage to vital cellular structures and biomolecules, destroying cell membranes, and ultimately leading to cell death through necrosis or apoptosis. An increased extracellular oxidant level can cause damage to mineral and non-mineral matrices and to their constituents.

ROS cause tissue damage through various mechanisms, including protein destruction, protein fragmentation, and polymerization actions. Another pathway is through lipid peroxidation that leads to a wide range of toxic metabolites that destroy cell membrane integrity and lead to cellular destruction. The effect on DNA leads to mutations.

Overproduction of ROS and periodontitis

Neutrophils are the most abundant leukocytes that belong to the first line of defense against bacterial infections. After initiating the host response by pathogen

biofilm, neutrophils become the most common inflammatory cells collected in periodontal tissue and gingival sulcus and are believed to be the primary source of ROS in periodontitis. Following stimulation by pathogens, PMNs produce O2 through the metabolic pathway called NADPH-oxidase-catalyzed respiratory burst during phagocytosis. This product can be released in the extracellular space and then converted into various radical and non-radical derivatives such as hydrogen peroxide, hypochloric acid and hydroxyl radical.

Numerous studies have focused on neutrophils located in the peripheral circulation in patients with periodontitis and have demonstrated that their ROS production activity is greater than that of neutrophils present in healthy individuals. Consistent results have shown that PMNs in individuals with chronic periodontitis or aggressive periodontitis significantly generate more ROS in stimulation with purified S. aureus compared to peripheral PMNs of healthy individuals.

A study by Fredriksson et al confirmed that increased production of ROS by neutrophils occurs by stimulating the Fc R and not by the CR3 complement receptor. PMN hyperreactivity of both patients (with periodontitis) was also demonstrated in stimulation with a neopsonized periodontal pathogen Fusobacterium nucleatum. It has been demonstrated that, even despite any stimulation, PMN of the periodontal affected individuals releases more ROS than in the case of healthy individuals.

A study by Almerich-Silla et al in 2015 [3] has shown that periodontal therapy can reduce the production of reactive oxygen species via the Fc R pathway but have no effect on ROS already located extracellular. The same study noted that unstimulated SOR production was higher in periodontitis than in the healthy control group, thus concluding that both mechanisms contribute to PMN hyperreactivity in periodontitis.

A recent study has shown that PMNs in the peripheral circulation of individuals affected by chronic periodontitis produce more extracellular superoxide with or without the stimulation of *F. nucteatum and P.gingivalis*. This overproduction was reduced following non-surgical therapy, indicating that PMN hyperactivity is related to both constitutional and reactive mechanisms. Additionally, the level of superoxide released by pre-therapy PMNs was significantly correlated with CRP levels in the blood. This correlation can be partially explained by the fact that CRP increases the number of toll-like receptors induced by the release of PMN superoxide, thus increasing oxidative stress (Hirschfeld J et al., 2017) [4].

ROS metabolic products in periodontitis

ROS are very active and their life span is extremely short, also they can cause direct tissue damage resulting in a variety of metabolites of lipid peroxidation, DNA damage and damage to proteins, which are usually used to assess tissue destruction.

Lipid peroxidation products are the most investigated derivatives of ROS in periodontitis.

Lipid peroxidation by free radicals causes changes in structural integrity and cell membrane function. Several lipid peroxidation products, such as malondialdehyde (MDA), 4-hydroxyl nonenal (HNE), and isoprostane were used to evaluate systemic and local oxidative damage associated with periodontitis.

MDA is a well-established lipid peroxidation product that evaluated oxidative stress and is also investigating lipid peroxidase produced in periodontitis. Periodontitis has been shown to be associated with higher levels of MDA in blood plasma

and systemic red blood cells, as well as gingival crevicular fluid (GCF) and gingival tissue locally. The association between the rise in MDA and the deterioration of periodontal status was also present in adult saliva (Ahmadi-Motamayel et al., 2017) [5].

A study by Baltacioglu et al. [6] compared the level of MDA in saliva between individuals with chronic periodontitis (CP), aggressive periodontitis (AgP) and healthy controls, and found that AgP and CP groups had significantly higher levels of MDA than the control group but there were no differences between AgP and AgP.

It has also been demonstrated that higher local MDA levels in patients with periodontitis may be diminished after periodontal therapy. There are also some studies investigating the MDA level in the serum of parodontopathic patients; however, unlike local MDA levels, their results are controversial. MDA levels were measured in GCF, saliva, and serum of CP patients, showed that periodontitis had no effect on systemic MDA levels, although MDA local levels were increased in patients with periodontitis (Wei et al., 2010) [7]. This finding suggests that the influence of periodontitis on systemic oxidative stress may be limited. However, a meta-analysis by Liu et al., which evaluated studies on systemic MDA in periodontitis showed that patients with periodontitis had a higher incidence of circulating MDA than healthy controlls (Liu et al., 2014) [8].

Meanwhile, studies involving patients with diabetes mellitus, hyperlipidemia and acute coronary syndrome have indicated that periodontitis could also contribute to a higher level of MDA in systemic circulation among people with these systemic diseases (Fentoglu et al. 2015, Nguyen et al., 2017). [9,10]

HNE is another major product of finite aldehydes associated with lipid peroxidation, but data on this periodontal biomarker are limited so far. A study by Hendek et al. [11] investigated the impact of periodontitis, smoking and periodontal treatment on HNE levels in GCF, saliva and serum, and found significant different levels of HNE in smokers with periodontitis and non-periodontal healthy nonsmokers (Hendek et al., 2015) [11]. In contrast to this study, Onder et al. in 2017[12] showed that HNE levels are increased by periodontitis only in serum, but not in saliva.

Protein Carbonyl (PC) groups are relatively stable end products resulting from the oxidation of proteins generated by multiple forms of ROS. It is the most used biomarker for evaluating oxidative damage to proteins with higher stability compared to lipid peroxidation products. The association between periodontal status and PC groups was investigated in GCF, serum and saliva, the serum and salivary level being increased and consecutively linked with a worsened periodontal status (Celec 2017) [13].

ROS may react with DNA and cause lesions to purine and pyrimidine. Oxidative stress biomarker 8-hydroxy-deoxyguanosine (8-OHdG) is most often used to quantify such damage although it may not accurately reflect the entire DNA damage resulting from the oxidative stress. Numerous studies have shown a higher level of 8-OHdG in GCF in saliva of patients with periodontitis compared to healthy patients, as well as their significant association with clinical parameters (Dinç G et al., 2018) [14].

The antioxidant system is extremely complex and therefore total antioxidant capacity (TAOC) has been developed as a cost-effective tool for assessing the activity of the entire antioxidant system.Some studies suggested that periodontitis is associated with a compromised TAOC and have also indicated that periodontitis could influence

TAOC in the general circulation (Tripathi V et al., 2018) [15].

TAOC in plasma and saliva has been shown to correlate with periodontal parameters. Therefore, additional controlled trials on the effect of periodontal therapy on the local and systemic TAOC system are required. A recent study has shown no relationship between TAOC and bacterial load in periodontitis suggesting that changes from TAOC could be related to host immune response rather than bacterial load (Tartaglia et al.,2017) [16].

TAOC associated with periodontitis may be affected by systemic conditions such as gender, smoking, pregnancy and systemic illness. Some studies suggest that men have higher serum TAOC than women (Chapple et al., 2007) [17]. There is a study indicating that salivary TAOC among smokers is significantly lower than that of non-smokers (Gadham et al., 2017) [18]. Vincent et al. in 2018 [19] have shown that both periodontitis and diabetes mellitus could contribute to lower TAOC in saliva and also the decrease in TAOC in saliva was positively correlated with periodontal disease among patients with diabetes.

It was confirmed that periodontitis is associated with hyperactivity of peripheral blood neutrophils, which are the predominant source of ROS. Numerous studies have suggested that periodontitis could contribute both locally and systemically to oxidative stress. Lipid peroxidation products, proteolysis and DNA damage can be used as biomarkers of oxidative stress associated with periodontitis. Local, and systemic activities of antioxidants can also be influenced by periodontitis. Studies that measure oxidative stress parameters confirmed the increase in local and systemic oxidation and stress was associated with inflammation resulting from periodontitis, but their sensitivity to be used as biomarkers for oxidative stress associated with periodontitis should still be verified.

Antioxidants

Under normal, physiological conditions, there is a balance between ROS and antioxidants. Oxidative stress occurs only when the antioxidant defense system can not neutralize the increase in ROS production. Antioxidants can be classified into two categories based on their mode of action.

The first category includes preventive antioxidants including enzyme antioxidants such as superoxide dismutase, catalase, glutathione peroxidase (GPx), glutathione reductase and DNA repair enzymes, but also albumin.

The second category includes antioxidants such as ascorbic acid (vitamin C), carotenoids (including retinol-vitamin A), uric acid, α-tocopherol (vitamin E), reduced glutathione and polyphenols (flavonoids). The activity of such antioxidants were measured in human gingival tissue and oxidative stress has been shown to be reduced, but there was also observed a clinical inprovement of periodontal indexes such a probing depth and gingival bleeding (Ambati et al., 2017)[20].

The activity of these enzymes in the periodontal ligament was considerably lower than the activity at the level of the edema and falls with age. In vitro studies on gingival biopsies demonstrated a progressive reduction in catalase activity and SOD. It has been hypothesized that a decreased activity of catalase and SOD leads to tissue destruction by the production of oxidants.

Chapple et al., suggested that GCF and total antioxidant plasma capacity is significantly reduced in patients with periodontal disease compared to healthy periodontal tissue.

Different antioxidants have been applied as supplements to conventional

periodontal treatment and optimistic results have been obtained, which offers new possibilities in the field of periodontal therapy.

It is worthwhile to note that antioxidant treatment may be effective only in a subset of patients during a specific periodontitis stage. The consumption of some antioxidant agents may be useful in gingivitis or in periodontitis patients but further studies are necessary to assess the precise benefit but also the limitations of such a therapy as the literature is still quite controversial in that field and the development of randomised controlled clinical trials is strongly recommended.

The role of oxidative stress in periodontitis is not clear despite decades of research. Numerous studies have been published that indicate the potential benefit of oxidative stress markers for screening, diagnosis or disease monitoring, but none are commonly used clinically. Similarly, animal experiments, as well as most patient intervention studies, indicate that antioxidant treatment should be effective in periodontitis therapy, but no such treatment has been approved. The absence of conclusive proof could be due either to lack of solid evidence for clinical utility or due to obstacles in the application of results, including the low or no commercial interest of major stakeholders. From the perspective of research, an important issue is the lack of specificity - both in diagnosis and treatment.

Current data do not support the use of a single oxidative stress marker. It is likely that a set of markers covering both oxidative damage and antioxidant status will be needed. The low specificity of oxidative stress markers requires caution in interpreting the results, even if multiple markers are used. The inter-individual and intra-individual variability of the markers analyzed is very high. This prevents their use at the level of individual diagnosis.

CONCLUSSION

It can be speculated that oxidative stress is an important factor in periodontitis and the study of antioxidant defensive mechanisms will help to understand tissue damage and will help us to design new therapeutic strategies. Antioxidant enzymes can be considered as an important biomarker for periodontal disease. Antioxidant supplements can also help to reduce periodontal condition without extra effort.

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