INFLAMMATION AND ITS CONSEQUENCES

Inflammation and oxidative stress have become major health issues in current years, the subject of many researches. Inflammation is body’s natural reaction to invasion by an infectious agent, toxin or physical, chemical or traumatic damage. There are some inflammatory situations in dental diseases; their names are pulpitis, gingivitis, periodontitis etc. Inflammatory process is often associated with free radical damage and oxidative stress. The sources of free radicals are both external (chemicals, drugs, tobacco, electronic pollution, stresses of all kinds) and internal (mitochondrial respiration). If body is unable to stop the amplification free radical chain reaction, oxidative stress results. Oxidative stress damages cellular proteins, membranes and genes and leads to systemic inflammation.

Oxidative stress and inflammation biomarkers, as well as anti-oxidants and anti-inflammatory factors, need to be measured concurrently. This is due to the fact that...
metabolic processes overlap and the lack of balance between these stressors and protectors may guide to development of oral diseases. The formation of reactive oxygen species (ROS), tumor necrosis factor (TNF) could potentially affect oral healing or the response to bacteria induced periodontitis, by direct effects on osteoblastic or fibroblastic cells, such as reduced expression of collagen, or indirectly through promoting inflammation and apoptosis of these matrix-producing cells (Figure 1). Thus, by enhancing the production of ROS, TNF, toxins may impair the healing response or progression of dental disease.¹

There are many biomarkers of inflammation and oxidative stress. This review presents the role of oxidative stress and inflammation in the tissue destruction and explains the biochemical markers during these processes.

OXIDATIVE STRESS AND ANTIOXIDANT SYSTEM

In recent years, investigations have linked oxidative stress with the pathophysiology of dental tissues. Oxidative stress characterized by an increased level of reactive oxygen species (ROS) that disrupts the intracellular reduction-oxidation balance. Actuality, depending on their concentration, ROS can either have beneficial or deleterious effects on mineralized tissues. ROS are mainly represented by the superoxide radical (O₂⁻), hydroxy radical (OH) and nitric oxide radical (NO) species, and non-radical derivatives of oxygen, such as hydrogen peroxide (H₂O₂) and hypochlorous acid (HOCI).² The presence of unpaired electrons in the outer orbitals of oxygen derived free radicals makes such species, especially the OH species, extremely reactive in nature.³,⁴ ROS are produced in cells at various sites including plasma membrane, mitochondria, endoplasmic reticulum and cytoplasm.

Polymorphonuclear leukocytes (PMN) are widely believed to be the initial and predominant defense cell produced during the host response against bacterial pathogens in a variety of pathological conditions including inflammatory dental diseases. Several enzymatic complexes are responsible for ROS production, including NADPH oxidase, cytochrome P450, xanthine oxidase, monoamine oxidase, cyclo-oxygenase and lipoxygenase. Following stimulation by bacterial antigen, PMN produce O₂⁻ via the metabolic pathway of the “respiratory burst” catalyzed by NADPH oxidase, during phagocytosis. O₂⁻ is released into the phagosomal space and also into the extracellular environment. In addition, NO is produced by macrophages via nitric oxide synthase during acute inflammation by vascular endothelium. The non-radical species, HOCI, is also a potent antimicrobial agent and is generated by azurophilic enzyme myeloperoxidase (MPO), during phagocytic degranulation. The production of HOCI has been shown to have a positive correlation with periodontal diseases.⁵ Data regarding gingival crevicular fluid myeloperoxidase and elastase-like activity present periodontal disease and health status. MPO activity in gingival tissue displayed a significant increase in patients with periodontal disease when compared with the control group.⁶⁻⁹ Tissues may be exposed to a variety of free radicals during inflammatory reactions. Especially where PMN and macrophages are in abundance and respond with significant quantities of oxygen derived free radicals, which may cause significant cellular and tissue damage. The effects of free radicals on cells and tissues are many and varied. These include disruption to cellular proteins, nucleic acids and plasma lipids as well as causing de-polymerization of matrix components such as collagen, hyaluronan and proteoglycans. In addition to free radicals, cytokines also contribute to connective tissue degradation. Those harmful effects are controlled by enzymatic and non-enzymatic antioxidant system.
The agents that scavenge reactive species or prevent their formation should decrease the tissue damage to an extent related to their antioxidant action in vivo. This means that their ability to decrease formation of or damage by reactive species should correlate with their ability to prevent tissue injury.\textsuperscript{9} Important antioxidants include: 1) the chain breaking or scavenging ones like vitamin E (\(\alpha\)-tocopherol), vitamin C (ascorbic acid), vitamin A (\(\beta\)-carotene), urate, 2) those substances containing thiol groups-preventative antioxidants-that function largely proteins by nature (albumin, transferrin, ceruloplasmin, ascorbic acid and glutathione) and 3) enzyme antioxidants that are enzyme systems that function by catalyzing the oxidation of other molecules like catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione S-transferase (GST), glutathione reductase (GR) (Figure 2).\textsuperscript{10,11,12} These agents acting as scavengers help to prevent cell and tissue damage that could lead to cellular damage and disease.

The significance of antioxidants is under research in clinical dentistry. During tissue inflammation, molecules such as H\(_2\)O\(_2\) and O\(_2\)\(_2\) are released from host cells; these oxidizing agents are able to destroy bacterial components as well as normal components of the surrounding cells and matrices. So as to evade excessive tissue destruction, host cells such as neutrophils release several enzymes that can degrade these oxidizing agents. There are several studies on antioxidant enzymes related with dental diseases such as gingivitis and reversible-irreversible pulpitis. While the activities of SOD, CAT were increased in reversible pulpitis, there was a decrease in activities of these enzymes in irreversible pulpitis and gingivitis when the experimental and control groups were compared.\textsuperscript{6,13-16} One probable rationalization for these responses is that the patients with oral diseases were in divergent stages of the disease. By the way, it is well known that the antioxidant responses found in different pathologies rely on the severity or expansion suffered by the patients, and long term chronic conditions may have jeopardized the antioxidant defenses.\textsuperscript{17} The analyses of the enzymes such as GST, GSH-Px, GR revealed a significant increase in gingival tissue of individuals with periodontal disease.\textsuperscript{18-20} All of these enzymes are related with reduced glutathione. The ubiquitous tri-peptide glutathione acts directly as a generic ROS scavenger or as a cofactor of GSH-Px and GST. The enzyme GR has an important antioxidant function related to GSH-Px and GST, GR intervention continuously regenerates GSH from GSSG in the presence of NADPH, therefore preventing cellular loss of GSH.\textsuperscript{20} GSH/GSSG ratio is important for evaluation oxidative stress degree.\textsuperscript{21,22} That’s why to assess both of GSH and GSSG level and GSH related enzymes activities is significant for situation of cells. Oxidative damage markers can measure easily in oral tissues such as pulpa, dentin, gingiva and oral fluids such as gingival crevicular fluid, peri-implant sulcular fluid, saliva.

**TISSUE DEGRADATION: “CYTOKINES-MMP CONNECTION”**

Endogenous and exogenous factors activate collagen degradation. The endogenous factors include a local variation in membrane thickness and a reduction in collagen content. The exogenous factors include the effects of bacterial metabolism and host inflammatory response. Infection-induced activation of Matrix Metalloproteinases (MMPs) has recently been shown to be related with excessive collagen turnover and membrane weakening, leading to tissue destruction. MMPs are involved in physiological process such as tissue development, remodeling and wound healing. They also play important roles in the regulation of cellular communication, molecular shedding and immune functions by processing bioactive molecules including cell surface receptors, cytokines, hormones, defensins, adhesion molecules and growth factors.\textsuperscript{23} Cytokine activation of cells can lead to increased processing of MMPs from inactive zymogens to the active enzymes. Cytokines and their receptors can also be substrates for MMP action. Many of the membrane-bound cytokines, receptors, and adhesion molecules can be released from the cell surface by the action of a subset of metalloproteinases called convertases (Figure 3).\textsuperscript{24,25}

The toxins, enzymes and metabolites of potent pathogenic bacteria present in dental plaque are considered to be the initiating factors of the host immune response which is primarily responsible for the tissue destruction. It is the host cells which produce and release pro-inflammatory mediators (cytokines and chemokines) as well as ROS, prostaglandins and cysteine proteases and matrix metalloproteinases (MMPs). Lipopolysaccharides (LPS) derived from bacterial membrane have the capacity to activate host epithelial cells to express and release pro-inflammatory cytokines IL-1\(\beta\), IL-6 and TNF-\(\alpha\). It is also important to point out that macrophages comprise the main source of TNF-\(\alpha\) in periodontal tissues.\textsuperscript{26}
Endogenous and exogenous factors activate collagen degradation. The endogenous factors include a local variation in membrane thickness and a reduction in collagen content. The exogenous factors include the effects of bacterial metabolism and host inflammatory response. Infection-induced activation of MMPs has recently been shown to be coupled with excessive collagen turnover and membrane weakening, leading to tissue damage. Collagenolytic process is mediated by MMPs, each of which degrades a type-specific substrate. According to these substrates, 28 MMPs are identified in vertebrates, of which 24 are found in humans. MMPs are classified into six different subfamilies: 1) collagenases, 2) gelatinases, 3) stromelysins, 4) matrilysins, 5) membrane type MMPs, and 6) others. Collagenases are the most efficient MMPs to cleave not only collagens I, II, and III, but other extracellular matrix (ECM) molecules and proteins as well. The main substrate of gelatinase subfamily is gelatine, but both collagenases and gelatinases are capable of digesting a number of other ECM molecules. MMP-2 (Gelatinase A) efficiently digests collagens I, II, and III in the same manner as the collagenase subfamily does. Stromelysins digest a number of ECM molecules and participate in pro-MMP activation. MMPs present in oral fluids (gingival crevicular fluid-GCF-, peri-implant sulcular fluid-PISF-, mouth-rinses and saliva) can be utilized to develop non-invasive, chair/bed-side, point-of-care diagnostics for periodontitis and dental peri-implantitis. MMPs have been suggested to play an important role in the destruction of dentin organic matrix following demineralization by bacterial acids and, therefore,
in the control or progression of carious decay. They may be activated by an acidic pH brought about by lactate release from cariogenic bacteria. Just the once activated, they are able to digest de-mineralized dentin matrix after pH neutralization by salivary buffers.\textsuperscript{27,28} There are several regulatory mechanisms that can influence the ultimate impact of an MMP on ECM degradation. MMP activity is tightly regulated not only via control of transcription and translation but also at the post-translational level (activation of zymogen forms of MMPs) as well as at the tissue level (by specific regulators known as the tissue inhibitors of metalloproteinase (TIMPs)). In humans there are four members of the TIMP family.\textsuperscript{29} The major function of TIMPs is the inhibition of MMPs, they can also take part in MMP transportation and stabilization.\textsuperscript{30,31} It has been recommended that periodontal destruction is an outcome of the imbalance between MMPs and their inhibitors.\textsuperscript{32} Increase in MMP and decrease in TIMP levels start collagen degradation. MMP-8, also called neutrophil collagenase-2, is one of the major collagenases that have a major part in the destruction of connective tissue and alveolar bone in periodontitis. MMP-8 can be activated by other MMPs (namely MMP-2, MMP-13, and MMP-14), ROS and inhibited by TIMP-1 and TIMP-2.\textsuperscript{32} Abundant information is available on the role of MMPs in health and disease, but information on TIMPs is limited, especially with respect to their role in oral diseases.

Like in other inflamed tissues MMPs are present in inflamed dental pulp tissue and periapical lesions.\textsuperscript{24,33,34} The level of MMP-8 in periapical exudates decreases during successful root canal treatment, while in cases with persistent inflammation the levels remain high, indicating that MMP-8 dip-stick analysis from periapical exudate could be used to monitor inflammatory activity and the achievement of treatment in teeth with periapical lesions.\textsuperscript{34}

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\caption{Cytokine activation of cells can lead to increased processing of MMPs from inactive zymogens to the active enzymes. Cytokines and their receptors can also be substrates for MMP action. Many of the membrane-bound cytokines, and adhesion molecules can be released from the cell surface by the action.}
\end{figure}
The presence and role of different MMPs in dentin has recently been under increasing interest. Dentin MMPs have been suggested to be involved in caries progression, degradation of hybrid layer under composite fillings and in pulpal and periapical inflammation. The physiological roles of dentin-embedded MMPs remain unknown, but they have been speculated to be involved, e.g. in peritubular dentin formation, mineralized dentin maturation and liberation of growth factors from dentin during wear and caries. The role of MMPs in oral diseases still requires basic research to find the right combination of MMP inhibitors or possible activators. There are series of studies of Professor Sorsa T, Professor Tjaderhane L and collaborators on MMPs and inhibitors in oral diseases. In addition to destructive enzymes like MMPs, it is possible to evaluate tissue breakdown products such as osteonectin, laminin, tip I collagen peptides for tissue injury. The excessive amount of collagen degradation products, such as pyridinoline cross linked carboxyterminal telopeptide of type I collagen (ICTP) in GCF and/or in saliva is regarded to predict alveolar bone loss in periodontitis.

**CELL DEATH**

Proteolytic damage by any of the proteases can trigger cell death, although activation of a protease is not necessarily identical with apoptosis. However, during proteolysis of several biologically active intra- and/or extra-cellular molecules, MMPs can up or down-regulate apoptosis in a context-dependent manner, in which the relative concentrations, tissue specificity, and spatiotemporal balance between MMPs and TIMPs will also influence the proteolytic cascade, determining the commitment to programmed cell death (PCD).

Apoptosis constitutes an endogenous physiologic mechanism of cell death, triggered through a diversity of internal or external. Extracellular signals may include free radicals, viral infections and bacterial toxins, cytokines, growth factors, extracellular matrix that inhibit cell death or endorse cell survival. There are two major pathways, intrinsic and extrinsic, throughout which apoptotic signals are transmitted, resulting in activation of caspases. Many organelles and molecules are involved in these pathways, including mitochondria, endoplasmic reticulum, cytochrome c, death receptors and their corresponding ligands, and adaptor molecules. Besides these, different genes and their products play a vital role in the control and execution of apoptosis. Apoptosis plays omnipresent role in the body. Amongst other things, apoptosis is a key process in oral development, the evolution of oral disease, wound healing and in certain pharmacological effects. The understanding of the ability of clinical materials to selectively induce or inhibit apoptosis is leading to new treatment modalities. An understanding of the mechanisms of apoptosis may lead to adjunctive treatments for pulpal and periodricular diseases through yet unknown pathways. Since the extended presence of chronic inflammatory cells producing osteolytic factors may be a key stimulator of chronic bone destruction, reduced lymphocyte apoptosis could be an important factor in the pathogenesis of periodontal disease. Several studies have investigated the role of apoptosis in periodontal disease. Bacterial products have been shown to induce apoptosis as well as prolong the lifespan of inflammatory cells.

**CONCLUSION**

The accelerated expression of antioxidant and proteolytic enzymes along with cytokines were associated with the degree of oral tissue inflammation. The elevated levels of these molecules and enzymes eventually reflect the extent of the dental inflammations, thus suggesting that the host derived molecules can participate in the progression of periodontal and peri-implant inflammatory diseases. Antioxidant enzymes such as GR, GSH-Px, GST, SOD, CAT, acting as scavengers help to prevent cell and tissue damage that could lead to cellular damage and disease. It is important to evaluate infection-induced activation of MMPs and MMPs/TIMP ratio. The balance between MMPs and TIMPs will also influence the proteolytic cascade, that’s why determining the pro-apoptotic, apoptotic and anti-apoptotic proteins levels. To determine the degree of inflammation there is a need to assess cytokines, especially TNF, IL-1β. These biomarkers may prove to be diagnostically useful tools and also targets of medication in the future.

**REFERENCES**


