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Review

Oxidative Stress and Oral Mucosal Diseases: An Overview

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Abstract. *Background: Reactive oxygen species (ROS) and free radicals are physiologically produced during cellular metabolism. When their balance is disrupted in favor of ROS, a condition called oxidative stress occurs. Oxidative stress represents a widespread phenomenon involved in several pathological conditions. The aim of the present review was to report current knowledge on oxidative stress related to oral mucosal diseases. Materials and Methods: Articles from 2000 to 2018 were selected for relevance, validity and quality, from results obtained in PubMed, MEDLINE and Google Scholar using the following search terms: oxidative stress and oral lichen, oral pemphigus, aphthous stomatitis, oral leukoplakia, oral cancer, oral squamous cell carcinoma and oral carcinoma. All articles were independently screened for eligibility by the authors. Results: This narrative review integrates extensive information from all relevant published studies focusing on oxidative stress in oral mucosal diseases. We outline the pathogenetic function of oxidative stress in the most frequent inflammatory, potentially malignant and malignant diseases of the oral mucosa and provide detailed findings from human research. Conclusion: Although variability in findings between individual studies exists, it justifies the conclusion that oxidative stress is a significant process in the oral mucosal diseases pathogenesis.*

This article is freely accessible online.

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Several biological processes lead to the production of endogenous free radicals, which are highly reactive molecules due to the presence of a spilled electron (1). This chemical peculiarity gives them high instability and, consequently, the urgency to react to achieve a higher level of stability. The radical, during its short existence, is pushed to capture an electron from other molecules, resulting in a chain reaction that irreversible alters the chemical structure of the cellular components it comes into contact with. The cellular components most exposed to the harmful action of free radicals are lipid structures, low-density lipoproteins, proteins and nucleic acids (2). Interaction with these structures is responsible for the oxidative damage to the body, favoring mutagenesis and carcinogenesis.

Physiologically, free radicals are produced during immune reactions, tissue repair, ATP synthesis (3, 4); on the other hand, they can also be generated after long and repeated exposure to ionizing radiation (*e.g.* U.V. rays), to chemical (some drugs, pesticides, pollutants, *etc.*), cigarette smoking, and prolonged stress (5, 6).

Antioxidant system or 'defense'. The human body counteracts oxidative stress by activating antioxidant defense systems. Antioxidants act as free radical scavengers and neutralize excess of reactive oxygen species (ROS). The antioxidant system comprises low molecular weight enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx). SODs are a family of metalloenzymes found in all aerobic organisms and are the first enzymes to be implicated in antioxidant defense. They

catalyze the dismutation of superoxide to hydrogen peroxide.

During oxidative stress, cells respond to ROS with SOD. SOD defends cells from injury caused by superoxide (O_2^-) and hydroxyl radical, liberating H_2O_2 in the process. While SOD lowers the steady state level of O_2^-, catalase and peroxidases have the same behavior for H_2O_2. GPx also represents the first defense against oxidative stress, which in turn requires glutathione in the reduced form as a co-factor. It detoxifies peroxides by acting as an electron donor in the reduction reaction, producing glutathione disulfide as an end product (7).

Similarly, saliva has non-enzymatic antioxidants molecules such as uric acid (UA), albumin and ascorbic acid. UA is the main antioxidant found in saliva, responsible for most of the total antioxidant capacity (8). UA neutralizes radicals being transformed in the process to allantoin (9). UA has been found to be an important salivary biomarker, with clinical importance in monitoring oxidative stress (10); UA is the principal antioxidant molecule in saliva.

Free radicals in the pathogenesis of diseases. When a stressed organism becomes unable to neutralize an excessive level of endogenous free radicals, these highly reactive substances irreversible damage cell structures and induce mutations implicated in the pathogenesis of several diseases. Oxidative stress plays a pivotal role in the acceleration of the aging process and in the development of chronic and degenerative diseases (11-13). Very often the evolution of the pathway triggered by the action of free radicals remains hidden and only becomes apparent when the clinical picture is already severe.

An aberrant production of free radicals is involved in the onset of several pathologies such as cancer, arthritis cataract, *retinitis pigmentosa*, autoimmune disorders, cardiovascular (hypertension, atherosclerosis, stroke and heart attack) and neurodegenerative diseases (14-18). Low-density lipoproteins, mainly metabolized by plasma cholesterol, are oxidized in contact with free radicals, altering their physico-structural properties and biological activity, and transformed into causal factors of the atherosclerotic process (19).

Superoxide anion is also capable of destroying nitric oxide (NO), an endogenous molecule that regulates the volume of vessels, causing hypertension (20).

An excess of free radicals can also induce uncontrollable autoimmune diseases. Some researchers believe that the etiology of central nervous system pathologies, including Alzheimer's dementia and Parkinson's disease, may be found in the activity of radical species on brain neurons (21, 22).

Data from the literature report a direct link between free radical levels, oxidative stress and inflammatory states. This association is also found in the most common inflammatory diseases and potential neoplastic lesions of the oral cavity (23, 24). Inflammatory diseases involving the oral cavity affect approximately 50% of the adult population. They consist in reduction of the gingival tissue and, in the most severe cases, retraction of the underlying bone tissue, as recorded in 10-15% of the world population (25, 26). The initial occurrence of these inflammatory pathologies is represented in most cases by massive bacterial colonization of the gums (27). These infections trigger the activation of host defense systems by increasing the production of ROS to disturb pathogenic microorganisms. ROS does not distinguish between pathogenic bacteria, which also have antioxidant defenses, and host structures and can, thus, damage the tissue of the organism that has produced them as a 'defense weapon' (28, 29). ROS action is exacerbated in patients with periodontal disease who have a weakened antioxidant defense system (25).

The purpose of this review was to report current knowledge on the production of ROS and oxidative stress in the most common oral mucosal diseases.

Materials and Methods

Articles from 2000 to 2018 were selected for relevance, validity and quality, from results obtained in PubMed, MEDLINE and Google Scholar using the following search terms: oxidative stress and oral lichen, oral pemphigus, aphthous stomatitis, leukoplakia, oral cancer, oral squamous cell carcinoma and oral carcinoma.

Only English language, experimental studies were considered, while reviews and single-case reports were not included. Any study published before 2000 was excluded from this review.

All articles were independently screened for eligibility by the Authors,

Results

A total of 326 citations were identified, of which 30 were potentially relevant, but only 22 publications finally fulfilled our eligibility criteria.

Oral lichen planus (OLP). A common chronic inflammatory disease of the oral mucosa whose etiologic basis is not yet fully understood, OLP is manifested as an exaggerated response to the body's immune system (30). The epidemiological data are also somewhat uncertain, attesting a rate of incidence ranging from 1.27-2.0% in the general population (31). OLP is considered a potential malignant oral condition, although the rate of malignant transformation is low (32, 33).

Clinically, OLP presents with a wide range of features that vary from white papules, striae and plaques, to red, erosive and ulcerated lesions. The most common is the reticular form, which is characterized by white keratotic dots and lines, called Wickham's striae, surrounded by an erythematous area, reflecting subepithelial inflammation. Most lesions are bilateral and located on the buccal mucosa, on the tongue, in the vestibule and on the gingivae (34).

The histological diagnostic criteria for OLP include the presence of a well-defined band-like zone of cellular infiltration in the *lamina propria* and indications of necrolysis in the basal cell layer (35). Specific and non-specific immune responses are described that outline this pathology as the immune response of T-lymphocytes to the antigen offered by the keratinocytes of the basal epithelium layer, resulting in apoptosis (36, 37).

The role of oxidative stress in the molecular etiology of OLP has been highlighted by recent studies (38, 39). As evidence of oxidative stress, patients with OLP have high levels of lipid peroxidation products such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (40, 41). Although their role in OLP is not clear, these oxidative stress markers trigger a sequence of biological responses (including apoptosis initiation) by influencing the levels of B-cell lymphoma 2 and BCL2-associated X proteins, the recruitment of pro-inflammatory T-lymphocytes and nuclear localization and activity of factor kappa B (42, 43). There are few data about the state of antioxidant defenses in patients with OLP; a recent study effectively demonstrated a reduction of antioxidant molecules in the saliva of such patients in relation to the same elements measured in healthy individuals (44).

Recurrent aphthous stomatitis (RAS). The most common ulcerative disorder of the oral mucosa, RAS is characterized by painful single or multiple round shallow ulcers with well-demarcated erythematous margin and yellowish-greyish pseudomembranous central area (45). RAS has a distinctive burning sensation that persists from 2 to 48 hours before an ulcer appears. The ulcers last from 7 to 14 days and reappear at intervals of a few months to a few days.

RAS occurs in apparently healthy individuals, with high prevalence among young adults (46), and must be differentiated from other causes of recurrent ulceration, such as Behçet disease, gluten-sensitive enteropathy, inflammatory bowel diseases, hematinic deficiencies and some syndromes (47-49).

Lesions of this type are most commonly found in non-keratinized mucosa, particularly on the buccal and labial mucosa and tongue. Participation of the heavily keratinized mucosa of the palate and gum is less frequent (50). About 20% of the world population is affected by RAS, but the rate varies from 5-50% in relation to ethnic and socioeconomic conditions (51).

The etiology of RAS lesions is not entirely clear, but several local (trauma) (52), systemic, genetic (53), immunological (54, 55), nutritional (56, 57), allergic (58, 59) and microbial (60, 61) factors have been proposed as causative agents.

Furthermore, the administration of certain immunosuppressive drugs such as calcineurin and mammalian target of rapamycin protein kinase inhibitors have been associated with severe aphthous-like stomatitis (62, 63).

All these issues can perturb the oxidant-antioxidant equilibrium of the organism thus triggering the formation of free radicals (64). The immune system may, therefore, be compromised by an oxidative stress situation. Triggered by an increase in free radicals, it may lead to cell damage. In order to protect themselves against oxidative stress, cells have antioxidant systems consisting of enzymes such as superoxide dismutase, catalase and GPx. Non-enzymatic antioxidants are the vitamins A, E, C, melatonin, UA and reduced glutathione (GSH) (65).

In a recent study, Ziaudeen and Ravindran evaluated the role of oxidative stress in the pathogenesis of RAS by measuring the levels of salivary oxidants and antioxidants: They found an increase in mean salivary SOD and a reduction of the activity of GPx and UA in the study group compared to the controls. They also found that infiltration of immune cells into the lesion led to an increase in free radical concentration. Since several molecules of SOD are required to bring about dismutation of superoxide radicals, this could explain the higher levels found (64). The dismutation reaction leads to an over production of H_2O_2 which is detoxified by GPx, while GSH is consumed in the process. The MDA level was also increased (64). Other authors also indicated that the enzymatic antioxidant defense system is impaired in patients with RAS with active lesion and it seems to play a crucial role in the pathogenesis of the disease (66). This in accordance with what was previously reported by Tugrul *et al*., who described that the total oxidative status and oxidative stress index values were significantly higher in a group with RAS compared to the control group, while total antioxidant status values were significantly lower. In the RAS group, DNA damage was observed to be significantly higher than in the control group. An important correlation was found between DNA damage and the oxidative stress index and total oxidative status values in patients with RAS (67). However, it should be remembered that not all research groups have found data in agreement with those reported above (68, 69).

Oral pemphigus vulgaris (OPV). Pemphigus vulgaris is an autoimmune disease involving both the skin and mucosal areas, in which acantholysis (the loss of cell adhesion) causes intraepithelial blister. Oral lesions often herald the disease and are initially vesiculobullous, but rupture readily to leave ulcers (70). Diagnosis is based on clinical manifestations and confirmed with histological and direct immunofluorescent analysis (71).

The onset and the course of OPV depend on a variable interaction between genetic predisposition and inducing factors. Drug intake, viral infection, stress, contact allergens, diet, hormonal disorders, malignancy are possible precipitating factors, which may trigger pemphigus initiation in susceptible individuals or be exacerbated in affected patients (72).

The pathogenesis of this disease is not yet completely defined (73). Pemphigus is typically characterized by impairment of the desmosomes by IgG-antibodies, against the extracellular domains of desmogleins with intraepithelial immune deposits. It is known that these autoantibodies play an imperative role in the pathogenesis and development of *pemphigus vulgaris* (74). The involvement of oxidative stress and variations of antioxidant elements in the pathogenesis of this disease is poorly analyzed. From our bibliographic research, a single study of 2015 established that it is possible to detect the highest activity of antioxidant enzymes (SOD, catalase and GPx) and a reduced total antioxidant capacity in patients with OPV compared to healthy controls (75). An explanation for this observation is likely to be due to an effect of antioxidant enzymes counteracting the decline in non-enzymatic antioxidant levels.

Oral leukoplakia. Oral leukoplakia is the most common potentially malignant disorder of the oral mucosa (76). At present, oral leukoplakia is defined as "a white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer" (77). The diagnosis is typically of exclusion and is based on a combination of the patient history, clinical considerations and histopathology (78). The estimated pooled prevalence for oral leukoplakia is 1.49% to 2.60% with a high incidence among older individuals and in India, due to the widespread use of areca nut (79).

Clinically, oral leukoplakias are divided into homogeneous and nonhomogeneous lesions. The former type is usually thin, flat and uniform well-demarcated white plaque; the latter is characterized by the presence of speckled, nodular or verrucous areas (78). The histopathology of oral leukoplakia shows atrophy or hyperplasia that may or may not include epithelial dysplasia (77).

Many cases of oral cancer are preceded by a variety of potentially malignant oral disorders, of which leukoplakia appears to be most common. Moreover, the high survival rate and low morbidity associated with leukoplakia makes their detection of utmost importance (80). Clinicians face a major challenge in the early identification of such innocuous lesions. Early detection methods and biomarkers have become progressively important for various ongoing research studies.

Changes in saliva composition, caused by pathological processes as oral potentially malignant lesions, have suggested the use of saliva for measurement of markers of oxidative stress. Vlkovà *et al.* compared salivary markers of lipoperoxidation and carbonyl stress in patients with oral leukoplakia and healthy controls by biochemical analysis. Salivary markers of stress, such as thiobarbituric acid-reacting substances and advanced glycation end-products, were found at significantly higher levels in patients than in controls. Salivary SOD and total antioxidant capacity were lower in patients than in controls (81).

The continuum of the disease process from normal mucosa to leukoplakia and then to oral cancer opens an important chance for research implications of ROS in premalignant lesions. Srivastava *et al.* found a statistically non-significant increasing trend of product of lipid peroxidation in clinicopathological stages of leukoplakia except in stages I and II and a significantly decrease in levels of GSH, GPx, catalase, and SOD in patients with leukoplakia compared to healthy control groups (82). This study also indicated a significant decline of all antioxidant enzymes along progressive stages of leukoplakia when compared with negative controls.

Oral cancer. Oral cancer, well-defined as oral squamous cell carcinoma (OSCC), is one of the most common types of cancer in the world, with delayed clinical detection, poor prognosis, without specific biomarkers, and with expensive therapeutic alternatives (83). OSCC represents more than 90% of malignancies of the oral cavity. The global estimated incidence is approximately 300,000 cases per year, with a wide geographical variation due to differences in habits, environmental factors and socioeconomic status (84). Tobacco and alcohol are considered the main etiological factors in oral carcinogenesis; moreover, human papillomavirus infection, exposure to radiation and chemicals, and family history of cancer are considered other risk factors for oral cancer (85).

The most common sites for the presentation of oral cancer are the tongue, floor of the mouth and lower lip (86). OSCC commonly appears as a bleeding ulcer or a sore that does not heal and persists for over 3 weeks. A high percentage of OSCC develops from oral potentially malignant disorders, including leukoplakia and erythroplakia, which are the most common ones (77).

Several studies have been conducted on the importance and relevance of oxidative stress in OSCC. Rathan Shetty *et al.* investigated the implications of low serum antioxidant capacity in oral cancer development (87). Although they did not find any statistically significant difference between pre- and post-surgical serum antioxidant capacity, patients with oral cancer had lower serum antioxidant levels than normal. They concluded that serum antioxidant capacity is a predisposing factor in oral carcinogenesis rather than the pathological effect of cancer and it remains unchanged even if the cancer is treated. Srivastava *et al.* reported that ROS and their deleterious consequences such as lipid peroxidation, have been implicated in the pathogenesis of oral cancer (88). They showed significantly increased lipid peroxidation with a decrease in antioxidants in the venous blood of patients with OSCC at various clinical stages when compared with healthy controls. The same authors confirmed their previous results reporting increased tissue levels of free

radicals and reduced concentrations of SOD, GSH, GPx and catalase in stage II, III and IV OSCC (89).

Another interesting study, carried out by Metgud *et al*., reported the diagnostic efficacy of saliva in evaluating levels of MDA and GSH in smoking patients with OSCC (90). Tobacco represents an exogenous source of ROS that subsequently leads to oxidative stress. Free radicals, ROS and their metabolic products, such as MDA, have a pathognomonic role in multistep cancerogenesis and progression. Metgud *et al*. showed a significant increase of MDA and reduction of GSH progressively from healthy controls to pre-cancerous and to patients with OSCC, in line with a state of oxidative stress related to OSCC. This finding supports the hypothesis that ROS metabolism is markedly altered in cancer cells, leading to enhanced production of ROS compared to non-neoplastic cells, and the suppression of the antioxidant system that mediate body's defense mechanisms (90). For OSCC, as for most other cancer types, the prognosis depends largely on lifestyle factors, medical comorbidity, grading and tumor staging. Lifestyle choice is strictly related to mechanisms regulating oxidative stress and antioxidant defense system. Avoiding oxidant sources (cigarettes, alcohol, stress) and taking a diet rich in antioxidants must be considered important to prevent this disease.

Conclusion

In this review, the association between the most frequent inflammatory, potentially malignant and malignant diseases of the oral mucosa and oxidative stress is well established. Although the molecular mechanisms underlying the etiology of these pathologies still need to be explained and understood in depth (30, 50, 74), evidence that oxidative stress plays an important role regarding the pathogenesis of oral diseases has been presented. The increase in free radicals in oral tissues and saliva in patients and the reduction in activity of antioxidant defense systems support this thesis. Based on these considerations, further studies in this direction will help in clearly understanding whether the onset of oxidative stress causes or is a predisposing factor in these diseases. A reliable and unambiguous diagnostic marker among the different radical molecules or elements of the antioxidant barrier might be found in the future and used as a therapeutic target in clinical practice.

Declarations

Ethics approval: not applicable.

Consent for publication: The manuscript does not contain any individual person's data in any form (including individual details, images or videos).

Data availability statements: The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The Authors declare that they have no competing interests

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