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Role of free radicals and antioxidant defences in oral cavity-related pathologies

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Free radicals play a key role in the development of several pathological conditions. Therefore, several methods have been developed to measure oxidative stress from bodily fluids including blood, urine and, more recently, saliva. Free radical and antioxidant defences within the oral cavity may play a key role in odontostomatological pathologies. This review provides an update of the literature concerning the association of oxidative stress with pathological conditions associated with the oral cavity. It focuses on the diagnostic and therapeutic importance of the tests based on saliva specimens in a preventive perspective.
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Keywords: antioxidant; dentistry; free radical; oral cavity; oxidative stress; periodontal; saliva

Introduction

Oxidative stress has been described as a process derived from the inability of the body's endogenous antioxidant defences to scavenge free radical species and has been related to many pathologies such as ageing, cardiovascular disease, neurodegenerative disorders, cancer, complex regional pain syndrome and many others (1). This evidence suggests that free radicals play a key role in the development of several pathological conditions. Free radicals are highly reactive chemical species, characterized by very short half-life. They are made up of a single atom or several atoms that form a molecule with a free electron. This electron is responsible for the high reactivity of free radicals, which can bind other free radicals or subtract an electron from the surrounding molecules (2). Living cells are constantly exposed to oxidants deriving from a variety of sources that can be exogenous, i.e. pollution, ozone, radiations, chemical substances and pathogenic micro-organisms, and endogenous, such as the mitochondrial electron transport chain, inflammatory cells and enzymes (3). When free radical-derived oxidative damage to nucleic acids, proteins and lipids of the cellular and extracellular matrix is observed, it produces damages of clinical importance and severity. One of the most crucial concerns is the mutation-induced carcinogenesis, the damage to cellular membrane lipoproteins and lipid-mediated oxidative damage leading to tegument ageing evolution. Oxidative damage represents the main threat for the genome integrity in the greater part of living organisms. Reactive oxygen species (ROS) can be generated as a product of the normal aerobic cell metabolism which, although is limited to the mitochondria, can lead to the formation of oxidized bases, apurinic–apyrimidinic sites (AP) and DNA breaks. 7,8-Dihydro-8-ossiguanina (8-oxoG) and 2-hydroxy-adenine (2-OH-A) represent the two main pre-mutagen bases (4, 5). The elevated mutagenic characteristics of these oxidized bases are the consequence of their ability to create unusual pairings. In particular, the 8-oxoG can pair with the cytosine and adenine nucleotides with the same efficiency, causing the transversions GC → TA e A → CG. The 2-OH-A can pair in an unconventional way leading to the transversion GC → TA.

The 8-oxoG is a particularly frequent lesion, and its danger to the cell is evident from the presence of several control systems, which are able to remove the oxidized base. The presence of ROS inside a cell can cause not only DNA direct oxidation, but also purine and/or pyrimidine oxidation of the nucleotide pool (dNTPs). The oxidized dNTPs can be incorporated in the DNA by the polymerases involved in the replication during the synthesis phase.

Low-density lipoproteins (LDL) that contain hundreds of phospholipids, cholesteryl esters and triglycerides are particularly targeted by the attack of free radicals and therefore subject to lipid peroxidation. The LDL oxidative modifications alter their biological properties increasing the atherogenic power. Oxidized

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LDL stimulate the endothelial cells to produce inflammatory markers leading to potential innate and adaptive immune responses; they are also involved in the formation of foam cells that inhibit macrophage motility and NO-induced vasodilatation (6). Among lipids, fatty acids are the most commonly subject to oxidation because the double bonds, which compose their chemical structure, are easily attacked by free radicals, leading to lipidic oxidation. When free radicals interact with unsaturated fatty acids, which are found in cell membranes or inside lipoproteins, the lipid peroxidation mechanism is automatically triggered off. They are converted into the primary product, commonly called lipid peroxide, and consequently a chain reaction is triggered off, leading to the amplification of the lipoperoxide number with the formation of dienes, lipid hydroperoxide, cyclic endoperoxide and malondialdehyde (MDA) (7).

The difficulty in measuring oxidative stress with histochemical, spectrophotometric or proteomic and genomic assays and its aetiopathogenic link with pathological conditions are among the reasons that have hold back research and development in this field so far. Therefore recently, new practical and reliable instruments have been set up. They only require a blood drop or a sample of urine or saliva and are able to provide a generic value of the patient's oxidative stress quantifying his amount of free radicals. Furthermore, several other tests, which are not found in the literature, are available on the market. An example is given by the BioxTest Oraxx (Bioxvita Diagnostics, Getvital® Healthcare GmbH, Klosterweg, BiliesKastel, Germany), a commercially available colorimetric test able to measure the increase in free radicals through the analysis of urine specimens. This test is based on the ability of pararosaniline, a triphenylmethanol derivative, to bind the aldehyde compounds normally found in the urines, leaving a coloration that can vary between pink and violet, but its reliability has not been proven yet. Recently, a new non-invasive instrument (FRAS 4 EVOLVO – Free radical system; H&D, Parma, Italy; Fig. 1) has been introduced in the clinical setting. It analyses a sample of saliva giving the grade of oxidative stress in the oral cavity, and it can be useful for the study of particular pathological conditions affecting this district. Everything has been achieved thanks to an instrument that allows the measurement of the antioxidant concentration starting from a simple saliva specimen. The patient receives a cotton swab that has to be rolled for a minute in his mouth to stimulate salivation. The swab is then weighed inside a glass and subsequently squeezed out to allow the collection of saliva. In a cuvette containing R1 reagent, 40 μl of the reactive reagent R2 is added, and after shaking the cuvette for 10 s, the blank reading is obtained. After that, 10 μl of the previously collected saliva is withdrawn and introduced into the cuvette containing the two reagents, and after agitation, it is inserted in the instrument to be read. At the end of the reading, the instrument prints the result directly, and after that, it is confronted with the reference values. With this simple procedure, it is possible to give a comparative diagnosis of the current pathological condition in which the role of free radicals has been identified. It involves the pathogenesis with pre- and post-medical or surgical therapy references and protects the oral cavity health and, in a more general sense, the patient.

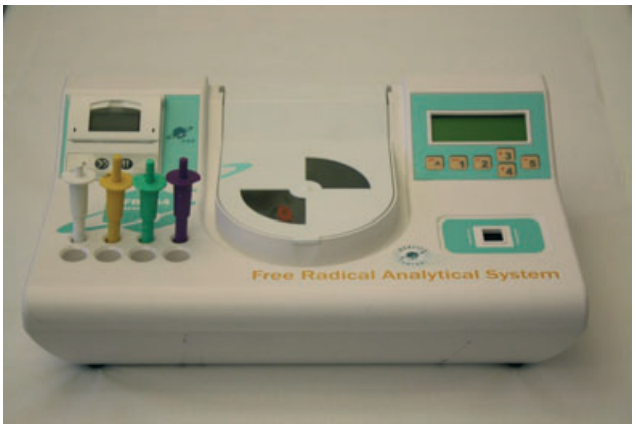

Figure 1 FRAS 4 EVOLVO, H&D, Parma, Italy – Free Radical Analytical System used for antioxidant identification in saliva specimens.

This article provides an update of the literature concerning the association of oxidative stress with pathological conditions associated with the oral cavity. It includes the pathologies associated with otolaryngology and the ones related to the oro–cervico–facial district, focusing on the diagnostic and therapeutic importance of the tests based on saliva specimens in a preventive perspective.

Searching criteria

A PubMed search was performed by using the following key words (separately or combined): 'antioxidant', 'oxidative stress', 'free radical', 'dentistry', 'periodontal', 'saliva' and 'oral cavity'. Selected papers, including reviews, were chosen on the basis of their content (quality and novelty). The main focus was on free radical species and antioxidants in relation to the oral cavity.

Salivary physiology in the redox balance

A consistent number of recent studies have showed how oxidative stress can cause pathologies affecting the periodontium, although the periodontitis triggering event is due to the presence of bacteria forming a biofilm in the subgingival plaque. However, the disease progression would be due to the immune system adverse response to these organisms that, through the polymorphonucleated leucocytes, causes an acute inflammation releasing ROS (8). The resulting disequilibrium between the ROS generated in this process and the existing antioxidants leads to a situation of oxidative stress causing pathological conditions at the level of the periodontium, but also other diseases, such as rheumatoid arthritis (9), acute respiratory distress syndrome

(10), AIDS (11), Alzheimer's disease (12) and Parkinson's disease (13).

Several studies show how free radical levels increase in saliva and in crevicular gingival fluid (CGF) resulting in a failure to keep their protective function within the oral cavity. Saliva is the biological liquid of the oral cavity that is generated for the most part, i.e. 65%, by the submandibular glands, for the 20% by parotid, for the 7–8% by the sublingual glands and in a low percentage by minor salivary glands localized in various mucosal sites of the oral cavity. Saliva production is around 1–1.5 l daily (14). Water is the most abundant component of saliva (99%), followed by a variety of electrolytes including sodium, potassium, calcium, magnesium, bicarbonate and phosphate. In saliva, there are several inorganic compounds, that is, urea, ammonia, uric acid, glucose, cholesterol, fatty acids, mono-, di- and triglycerides, glycolipids, amino acids, hormones, steroids and proteins, and they all exert a key role in the maintenance of the oral cavity protection or health. They include mucins, amylases, agglutinins, glycoproteins, lysozyme, peroxidase, lactoferrin and secretory IgAs (15). All these substances play an important role in the function of saliva:

1 Bicarbonates, phosphates and urea modulate pH;
2 Proteins and mucins act as disinfectant and play an important preventive role against micro-organisms;
3 Calcium, phosphate and proteins are all involved in the modulation of tooth mineral integrity;
4 Immunoglobulins and enzymes contribute to the bactericidal action, in particular lysozyme, which is able to lyse the Gram-positive bacterial wall catalysing the beta 1, four bond hydrolysis between *N*-acetylmuramic acid (NAM) and the *N*-acetylglucosamine (NAG), which are the main components of peptidoglycan.

Changes in the composition of these compounds may be indicative of the patient's health. Therefore, saliva can be used as a diagnostic tool, and its components may be used as pathological markers (see Fig. 2).

Antioxidants, free radicals and odontostomatology-related diseases

Antioxidant defensive systems also play an important role in the oral cavity. Among them, salivary peroxidase (SPO) and myeloperoxidase (MPO) represent the two most important ones (16). SPO catalyses thiocyanate ion (SCN^-) peroxidation to generate oxidation products, such as O2SCN^-, O3SCN^-, (SCN)2, HOSCN and $OSCN^-$ that inhibit the growth and the metabolism of many micro-organisms. MPO is able to catalyse CL^- with a consequent formation of hypochlorite (OCL^-), a ROS that induces the cleavage of peptide bonds with a consequent formation of a low molecular weight potential bactericide compound, that is, chloramine. According to several studies, the myeloperoxidase could accumulate while sleeping, when the salivary flux is low, i.e. between meals, removing the original products from the polymorphonuclear neutrophils.

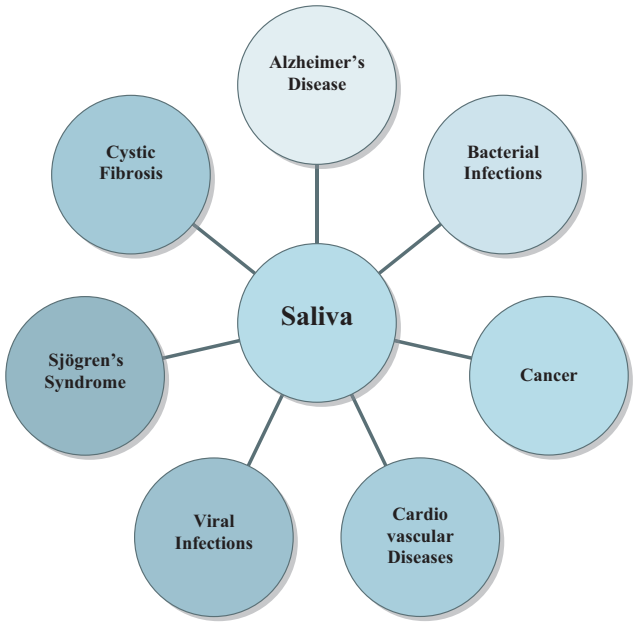

Figure 2 Pathologies in which saliva can be used for medical diagnosis.

Crevicular gingival fluid, an exudate of the periodontal tissue, which pours in the gingival sulcus passing through the sulcular epithelium, is similar to the composition of saliva and, in part, its possible expression. Passing through the deepest tissues, it catches the subproducts of the local metabolism. It is largely demonstrated that variations in the type and quantity of several components of CGF, as its total antioxidant capacity (TAOC), is an indicator of the periodontium health status. This fact is supported by Chapple et al. (8), who measured the CGF TAOC in 17 patients with slight chronic periodontitis and 18 patients with serious chronic periodontitis, comparing them to 32 controls. This study showed that at baseline, the total antioxidant levels were lower in patients affected by the disease if compared to healthy controls (680 ± 371 μMteq in ill patients compared to 1129 ± 722 μMteq observed in healthy subjects), pointing out that this parameter is compromised during the periodontium inflammation. TAOC levels can increase until they reach the ones found in controls as a consequence of non-surgical treatment at the level of the periodontium. Another research group (17) also analysed free radical production in the CGF level, stating that free radical production is involved in the pathophysiological progression of periodontal diseases because it might contribute to oxidative stress generation. The authors investigated the role of glutathione peroxidase (GPx), an antioxidant enzyme of lactoferrin and myeloperoxidase, i.e. two glycoproteins that reflect the amount of oxidative stress and the role of interleukin 1 beta (IL-1β), a pro-inflammatory cytokine that plays a key role in the regulation of the inflammatory and immunitary reaction in the periodontium-associated diseases in humans. They collected CGF from 27 patients, aged 24–62 years. Nineteen patients were affected by periodontitis, while

eight subjects were healthy and were used as controls. ELISA test was used for the purposes of this study. A significant increase in GPx, lactoferrin, myeloperoxidase and IL-1β was observed in subjects affected by the disease, if compared to healthy controls. Moreover, they were positively correlated to plaque and gingival indices. The presence of these markers of oxidative stress is strictly correlated to the severity of the disease, and in particular, the disequilibrium in the myeloperoxidase/IL-1β levels and GPx/lactoferrin could be the result of the tissue damage previously generated by ROS and fuelled by the pathogens that were present in the oral cavity. Superoxide anion has also been studied in relation to the disease affecting the oral cavity. A study performed by Krol (18) in patients affected by periodontitis aimed at determining the production of superoxide anion in blood, measuring the reduction of cytochrome *c*, identifying the effects of oxidative stress in patients with periodontal disease and analysing the lipid peroxidation and the degradation of DNA bases in peripheral and gingival blood. Moreover, this study wanted to prove whether there was a correlation between superoxide anion production, lipid peroxidation, 8-hydroxy-deoxyguanosine (8-OHdG) concentration and clinical parameters. The total antioxidant status in peripheral and gingival serum was also studied and correlated with the periodontal clinical status; several periodontitis risk factors, related to oxidative stress, were evaluated too. Fifty-six patients, affected by untreated periodontitis, and 25 healthy volunteers were enrolled in this study. The results evidenced a negative correlation between cytochrome *c* reduction and the index of periodontal disease, a positive correlation between oxidised anti-LDL autoantibodies in gingival blood and the levels of 8-OHdG in venous blood. Moreover, a negative correlation between the 8-OHdG concentration in venous blood and the total antioxidant status in patients' gingival blood and a positive correlation in healthy controls were observed. Increased levels of 8-OhdG in gingival blood were also observed, while significantly lower levels of total antioxidant status in venous blood, from patients affected by periodontitis if compared to healthy controls, were reported. There was also a negative correlation between gingival blood 8-OhdG concentration and the total antioxidant status in venous blood serum from patients affected by periodontitis, if compared to healthy controls. This study points out that oxidative stress in patients affected by periodontitis is expressed by elevated concentrations of ROS and associated with a suppression of gingival blood antioxidant activity and may contribute to a rapid formation of lesions in periodontal tissues. In 2002, for the first time, Takane et al. (19) evaluated the levels of 8-OHdG in saliva of patients affected by periodontitis, before and after periodontal treatment, to demonstrate that there is a strong connection between the presence of this molecule in their saliva and periodontal health status. Seventy-eight patients with periodontitis and 17 healthy controls participated in this study and 8-OhdG saliva levels were detected by immunoenzymatic essay. The mean value of 8-OhdG levels in subjects affected by periodontitis increased significantly if compared to the value of healthy subjects (4.28 ± 0.10 ng/ml and 1.56 ± 0.10 ng/ml, respectively), and a decrease in this parameter was observed after a therapy, concluding that 8-OHdG levels in saliva are proportional to the periodontium health status. Another study (20) also evaluated pro-inflammatory and oxidative stress markers in gingival tissues from patients affected by chronic periodontitis. Eighteen subjects were divided into two groups: an experimental one (age: 52.9 ± 5.0) and a control one (age: 51.1 ± 9.6). For both groups, catalase, GPx, glutathione S-transferase, glutathione reductase (GR), total glutathione, redox glutathione, glutathione oxidase, thiobarbituric acid-reactive substances and myeloperoxidase were evaluated. This study showed that, in subjects affected by periodontitis, there was a significant increase in gingival tissues concerning myeloperoxidase, GPx, glutathione S-transferase, oxidized glutathione and thiobarbituric acid-reactive substances if compared to the control group, showing a correlation between oxidative stress and periodontitis. Therefore, research on antioxidant total power in saliva and serum of patients affected by pathologies of the periodontium is a useful approach to determine a connection between disease and oxidative stress, highlighting that the disequilibrium between ROS and antioxidants, in favour of ROS, leads to the production of free radicals and a defective antioxidant activity of saliva. This evidence was also supported by a study performed by Diab-Ladki et al. (21) who compared the antioxidant activity of saliva from 17 patients affected by periodontal disease, requiring a dental treatment, to the one from 20 healthy controls. The results showed that healthy subjects' saliva was more efficient (40–50%) in the removal of *in vitro*-generated free radicals. In patients affected by periodontitis, saliva antioxidant activity decreased significantly, although this fact did not interfere with the levels of the three main traced antioxidants, namely uric acid, albumin and ascorbic acid (AA). Another study (3) focused on the evaluation of TOC in serum samples from 30 subjects with clinically diagnosed periodontitis and 30 healthy controls using spectrophotometric quantification. A mean antioxidant value of 28.5 μg/dl ± 4.6 was observed in patients affected by periodontitis, and this value almost doubled in control subjects (50.6 μg/dl ± 3.1), showing that the antioxidant ability decreases with periodontitis and suggesting a negative correlation with the periodontal parameters.

During the past few years, in the odontostomatological and oropharyngeal fields, several studies have addressed the issue of peculiar oral diseases related to a direct damage caused by an excess in free radical production. The literature shows how MDA is one of the most studied products of polyunsaturated fatty acid peroxidation, which increases in an oxidative stress–dependant manner. In a recent study (22), MDA levels and total oxidative stress (TOS) in serum, saliva and GCF have been measured from 36 patients (19 men and 17 women, aged between 32 and 55, with mean age 40.66 ± 5.31) affected by chronic periodontitis and

from 28 healthy controls (13 men and 15 women, aged between 29 and 51 with mean age of 38.5 ± 6.10). Data analysis showed that MDA values in saliva and in CGF and TOS values in serum, saliva and CGF were significantly higher in the group of patients affected by the disease if compared to the control group, suggesting that lipid peroxidation plays an important role in the periodontal pathology. However, no significant differences in serum MDA levels between the two groups were observed. It is noteworthy that MDA and TOS concentrations appeared to be lower in saliva and higher in gingival crevicular fluid.

Mitochondrial DNA also represents an excellent biomarker of oxidative stress and its possible deletions, in gingival tissues of patients affected by periodontitis, were studied by Canakçi et al. (23). In this study, blood and gingival tissue samples were collected from 30 patients with chronic periodontitis and 30 healthy controls. Possible mitochondrial DNA deletions, corresponding to 5 and 7.4 kbp, were analysed through PCR. No deletions were observed in the two groups of blood samples and in the mitochondrial DNA, corresponding to 7.4 kbp. In the mitochondrial DNA, deletions, corresponding to 5 kbp, were found in 24 ill subjects [on a total of 30 patients (80%)], while no deletions were found in healthy subjects, showing that the ROS overproduction can cause oxidative damage at this level. The studies concerning antioxidants, free radicals and odontostomatology-related disease are summarized in Table 1.

Oxidative stress and oral cavity tumours

On a worldwide scale, the tumours of the oral cavity, together with the ones associated with the larynx and pharynx, represent nearly 10% of all malignant

Table 1 Antioxidants, free radicals and odontostomatology-related diseases

Author	*Patients*	*Methods*	*Results*
Takane et al. (19)	78 patients with untreated periodontitis and 17 healthy controls	Saliva samples were collected to evaluate 8-OHdG production and eventual changes after periodontal treatment	8-OHdG levels in subjects affected by periodontitis were significantly increased if compared to healthy subjects. A decrease in 8-OHdG levels was observed after treatment
Diab-Ladki et al. (21)	17 patients affected by periodontal disease requiring a dental treatment and 20 healthy controls	Saliva TAOC in removing free radicals like xanthine–xanthine oxidase system was determined Uric acid, albumin and ascorbic acid were measured in saliva specimens	In healthy subjects, saliva was more efficient in the removal of free radicals. In patients affected by periodontitis, saliva antioxidant activity decreased significantly although it did not interfere with the production of uric acid, albumin and ascorbic acid
Wei et al. (17)	19 patients affected by periodontitis and 8 healthy controls	CGF levels of glutathione peroxidase, lactoferrin, myeloperoxidase and IL-1β were analysed by ELISA test	A significant increase in glutathione peroxidase, lactoferrin, myeloperoxidase and IL-1beta was observed in subjects affected by periodontitis if compared to healthy controls
Krol (18)	56 patients affected by periodontitis and 25 healthy controls	The production of superoxide anion was determined in peripheral and gingival blood TAOC status in peripheral and gingival serum was also studied and correlated with periodontal clinical status	Increased levels of 8-OHdG in gingival blood were observed in patients affected by periodontitis. On the other hand, significant lower levels of TAOC status in venous blood were observed in these patients
Canakci et al. (23)	30 patients with chronic periodontitis and 30 healthy controls	Mitochondrial DNA was analyzed in blood and gingival tissue samples to investigate its deletions	In mitochondrial DNA, deletions, corresponding to 5 kbp were found in 24 ill subjects (80%), while no deletions were found in healthy subjects
Chapple et al. (8)	17 patients with slight chronic periodontitis, 18 patients with serious chronic periodontitis and 32 controls	TAOC of CGF and plasma before and after a non-surgical therapy was evaluated	At baseline, CGF TAOC was lower in patients affected by disease Periodontal therapy did not alter plasma TAOC, but CGF TAOC increased in patients with periodontitis
Borges et al. (20)	18 subjects divided into 2 groups: experimental group including patients with chronic periodontitis and control group	Catalase, glutathione peroxidase, glutathione S-transferase, glutathione reductase, total glutathione and reduced glutathione, oxidized glutathione, thiobarbituric acid-reactive substances and myeloperoxidase activity were evaluated in gingival tissues	In subjects with periodontitis, there was a significant increase in gingival tissues concerning myeloperoxidase, glutathione peroxidase, glutathione S-transferase, oxidized glutathione and thiobarbituric acid-reactive substances
Akalin et al. (22)	36 patients affected by chronic periodontitis and 28 healthy controls	MDA levels and TOS in serum, saliva and CGF were measured	MDA values in saliva and in CGF and TOS values in serum, saliva and CGF were higher in patients affected by periodontitis No significant differences in serum MDA levels were observed in both groups
Pendyala et al. (3)	30 subjects with clinically diagnosed periodontitis and 30 healthy controls	Serum TAOC was evaluated using a spectrophotometric quantification	In the control group, antioxidant values doubled if compared with patients affected by periodontitis

TAOC, total antioxidant capacity.

neoplasia in men and 4% in women. In Italy, every year, 4500 cases of oral cavity tumours are diagnosed and 3000 deaths are registered. Several studies have investigated the presence of free radicals in this area to determine a possible correlation between free radicals and neoplasia incidence. As far as tongue carcinoma is concerned (5% of incidence among all malignant tumours worldwide and 0.43% incidence in the world's population), a potential correlation with a high index of oxidative stress and a weak antioxidant ability has been reported (24). This type of neoplasia, very frequent in India, has been selected in 60 patients affected by squamous cell carcinoma (stadium III–IV) compared with a group of 60 healthy controls to evaluate serum lipid peroxide, glutathione reductase (GSH), conjugated dienes, vitamin C and E, GPx and superoxide dismutase (SOD). Elevated levels of lipids, peroxides and dienes were observed in serum of patients affected by this type of carcinoma, while low levels of antioxidants were detected. According to this study, we could suggest to use therapeutic and prophylactic supplementation with vitamins and nutraceuticals, while the measurement of free radicals and the spontaneous antioxidant individual profile might represent a predictive value of risk. Fiaschi et al. (25) compared the intratumoral and haematic levels of GSH, AA and antioxidant enzymes in the tumour tissue affected by squamous cell carcinoma of the mouth to better understand the genotoxic and mutagen role of free radicals. Therefore, a study was developed to compare 18 patients affected by squamous cell carcinoma and 20 healthy controls comparing the levels of GSH, AA, SOD, GPx and GR. The patients, affected by mouth cancer, had increased levels of GR, GPx, GSH and AA and decreased levels of SOD in the tumour tissue and in the surrounding healthy mucosa. On the other hand, the same values of GSH, GPx, GR, AA and SOD had significantly decreased in blood from patients with cancer if compared to healthy subjects. This interesting observation let us assume that the antioxidant defences are confined in the oral district to tackle the neoplastic degeneration, although, in this way, an antioxidant reserve is built in the tumour cells and it could paradoxically protect them from necrosis and apoptosis because of oxidative stress.

The hypothesis of a programmed and therapeutic use of antioxidant compounds to reduce the frequency of oral tumour malignancy has still to be confirmed. Another study (26) evaluated plasma levels of thiobarbituric acid-reactive compounds (TBARS), vitamin E, GSH, SOD activity, catalase (CAT) and GPx. This study involved 48 patients, affected by different stages of oral cancer, who were divided into groups of 16 patients at the II, III and IV stages and compared to 16 healthy controls of the same age and sex. In this study, an increased lipid peroxidation and a decrease in antioxidant enzymes were reported. Ma et al. (27) studied the formation of DNA damage in the oral epithelium of 19 patients affected by oral leukoplakia using double-labelled immunofluorescent immunohistochemistry. They reported that in the leucoplastic tissue, as well as in the dysplastic epithelium and with surrounding phlogistic reaction, the accumulation of 8-nitroguanine, which represents DNA damage caused by nitric oxide, and of 8-oxo-7,8-dihydro-2′-deoxyguanosine, which is another marker of oxidative stress, induced DNA damage.

None of these immunoreactions resulted positive in normal mucosa, while the nitric oxide synthetase, an enzyme that promotes the damage, was present at the same time in leucoplastic tissue. Moreover, the histochemical examination showed the presence of nuclear proliferating antigen and p53 gene.

According to the authors, the increased nitrosylation linked to the phlogistic state results in any case jointly responsible for the disproliferative event, and as such, it is subject to adjustment in a prophylactic manner. In neoplastic diseases affecting other districts, the salivary profile also plays a key role allowing the achievement of a better quality of life. Therefore, Avivi et al. (28) realized a study, on the damage induced by oral mucositis, in 25 patients affected by myeloma and treated with melphalan at high doses to undergo autologous bone marrow transplantation. Among the tests used to establish the oral mucositis level, after 3 and 7 days from the transplantation, they measured secretory IgA and TAOC, while the damage to the mucosa was measured by albumin and carbonyl assay. The iatrogenic mucositis, measured at day 3 and 7 post-transplantation, showed significant reductions in the secretory IgA, and even more significant reductions in antioxidant levels, TAOC and uric acid levels. The sum of these investigations is without any doubt a useful measure of iatrogenic damage, and it suggests the need to start an antioxidant therapy as a protective measure. The human studies related to oxidative stress and oral cavity tumours are summarized in Table 2.

Oxidative stress and various pathologies of the oral cavity

The pathology of the oral cavity also affects the Waldeyer ring corresponding to the adenotonsil tissue. On this subject, two studies described the relationship between hypertrophy and pathology of the lymphatic tissue and free radicals. Doğruer et al. (29) studied 29 children affected by adenotonsillar hypertrophy compared to 51 age-matched controls. All the patients showed rhonchus and obstructive sleep apnoea and underwent adenotonsillectomy under general anaesthesia. Venous blood, withdrawn at baseline and 4 weeks post-surgery, was used to measure the levels of MDA, SOD, GSHPx and CAT. The results showed that the levels of enzymes and MDA were very high pre-operatively, but they normalized post-operatively. The levels of catalase did not show any variations pre- and post-operatively. This fact shows that there is an imbalance between oxidation and antioxidation in young patients affected by adenotonsillar hypertrophy and surgery is able to restore the systemic balance. Kiroglu et al. (30) designed a study in 20 children affected by chronic adenotonsillitis and 19 children with adenotonsillar hypertrophy who underwent

Table 2 Oxidative stress and oral cavity tumours

Author	*Patients*	*Methods*	*Results*
Fiaschi et al. (25)	18 patients affected by squamous cell carcinoma of the mouth and 20 healthy controls	Antioxidant levels were estimated in tumour tissue and blood samples	Patients affected by squamous cell carcinoma of the mouth showed an increase in GR, GPx, GSH and AA values and a decrease in SOD activity in tumour tissue and surrounding healthy mucosa. The same values were significantly lower in the blood from patients with cancer if compared to healthy subjects
Manoharan et al. (26)	48 patients, affected by different stages of oral cancer and 16 healthy controls	Plasma levels of TBARS, vitamin E and GSH, and activity of SOD, CAT and GPx were assayed	Elevated lipid peroxidation and decline in non-enzymatic and enzymatic antioxidant status were noticed in patients with oral cancer if compared to healthy subjects. The TBARS levels had gradually increased, whereas antioxidants were gradually reduced from stage II to stage IV of patients with oral cancer
Ma et al. (27)	Biopsy specimens of 19 patients affected by leukoplakia and normal oral mucosa (NM) obtained from 4 specimens	The formation of 8-nitroguanine and 8-oxo-7,8-dihydro-2′-deoxyguanosine was investigated with immunohistochemistry methods	Mutagenic 8-nitroguanine and 8-oxo-7,8-dihydro-2′-deoxyguanosine production was observed in oral epithelium of patients with leukoplakia. Little or no immunoreactivity was observed in normal oral mucosa
Avivi et al. (28)	25 patients affected by myeloma treated with melphalan followed by autologous SCT were enrolled to evaluate alterations in the normal salivary composition	Salivary samples were collected post-transplantation to analyse secretory IgA and antioxidant capacity The degree of mucosal damage was assessed by measuring the salivary carbonyl and albumin levels	After transplantation, there was a significant reduction in secretory IgA, TAOC and uric acid levels. The increase in salivary Alb and carbonyl levels indicates mucosal and oxidative damage
Sharma et al. (24)	60 patients affected by advanced squamous cell carcinoma of the tongue and 60 healthy controls	Single blood samples were taken to assess lipid peroxides, conjugated dienes, GSH, vitamin C and E, GPx and SOD alterations	Elevated levels of lipids, peroxides and dienes and low levels of antioxidants were detected in serum of patients with cancer
Rai et al. (43)	Patients with oral leukoplakia, oral submucous fibrosis or lichen planus, and healthy individuals	Salivary and serum oxidative markers, such as MDA, 8-OHdG and vitamins C and E were measured just prior to the intake of curcumin, a pigment with antioxidant, anti-inflammatory and pro-apoptotic activity	After a week from curcumin intake, serum and saliva vitamin C and E levels increased, while MDA and 8-OHdG decreased in patients affected by leukoplakia

GR, glutathione reductase; GPx, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase; TAOC, total antioxidant capacity.

adenotonsillectomy. MDA pre-operative levels and serum and erythrocyte catalase of both compounds at tonsillar and adenoidal levels were studied. All the checked parameters were significantly higher than expected, showing that the two pathologies share the same pathological mechanism and confirming the study previously described. Yilmaz et al. (31) also examined 38 children with chronic tonsillitis and adenoid hypertrophy, who were undergoing adenotonsillectomy, and compared them to 23 healthy controls. Levels of circulating retinol, β-carotene, alpha tocopherol, lycopene, AA, SOD, GSHx and peroxidation product and MDA, before and 1 month post-operatively, were measured. The same assay was used on the previously collected adenoid-tonsillar tissue. In the group of patients affected by pathology, an increased level of antioxidants and a reduction in the oxidative damage, comparing pre- and post-operative values, were observed, although they never reached the healthy subjects' balanced equilibrium. According to the authors, the oxidative stress imbalance represents a peculiar feature of the oro–lymphatic perioral pathology in paediatric population, and it does not get back to normal even after the elimination of pathological tissue.

Zhang et al. (32) conducted a study to detect and quantify DNA damage in human oral mucosa cells from 25 smokers and 25 healthy controls. This study used a monoclonal antibody specific from MDA-DNA adducts and showed a significant difference in the raising of the DNA damage. This method appears to be useful to detect MDA-DNA adducts, which could represent an important biomarker of DNA damage. A study performed by Yamamoto et al. (33) showed how in rats, fed on a high-cholesterol diet, the topical application of phlogogen compounds (lipopolysaccharides and proteases) in the gingival sulcus augmented the effect of diet on steatosis, inflammation and oxidative damage in their liver. This phenomenon was measured quantifying the levels of hexanol-lysine, a value that is a symptom of steatosis and inflammation and the concentration of 8-hydroxydeoxyguanosine in the hepatic parenchyma. These values were significantly elevated and associated

with the hepatic damage morphologically observed if compared to rats put on a normal diet, and also displaying oxidative damage in the oral cavity and hepatic parenchyma with less serious morph-functional consequences. The steatosic phlogistic damage, consequently strengthened by an atherogenic diet at a high level of lipid oxidation, can lead to a real systemic damage if this experimental observation will be confirmed in clinical studies involving human subjects. As to tooth motility and reabsorption of deciduous teeth, relevant from the orthodontic point of view, there are experimental studies that show a correlation between osteoplasty or readsorption and manganese-dependent SOD activity and lipoperoxides (34). The orthodontic motility of teeth (35) would be a function of the local activity of nitric oxide promoted by the administration of L-arginine in rodent studies. According to Poplawski et al. (36), compounds, such as the urethane dimethacrylate, used for orthodontic purposes, would determine the cause of oxidative damage as they induce DNA damage to the leucocytes in the oral mucosa that can be counteracted by vitamin C and chitosan. In conclusion, toothpastes, according to Moore et al. (37), must be taken into consideration as a potential cause of lesions to the soft tissues, as shown by experimental studies on keratinocytes exposed to commonly available detergents. In particular, increasing concentrations were evaluated in pluronic acid, showing that a partial oxidative damage exists *in vivo* and it is neutralized by salivary flux. These investigations demonstrate the opportunity to regularly monitor the physiological level of salivary antioxidants for homoeostasis especially in the presence of exogenous insults, even if they are introduced for hygienic sanitary purposes. The human studies related to oxidative stress and various pathologies affecting the oral cavity are summarized in Table 3.

Role of antioxidants in the oral cavity

Antioxidants, according to the definition given in 1989 by Halliwell and Gutteridge, are 'substances which slow down the rate at which something decays because of oxidization'. This definition includes both non-enzymatic and enzymatic compounds. The endogenous enzymatic antioxidants are represented by SOD, catalases and glutathione system. The SODs are a class of ubiquitous enzymes that efficaciously catalyse the rupture of the superoxide anion in oxygen and hydrogen peroxide. In mammals, three different enzyme isoforms were identified. SOD1 was found in cytoplasm, in nuclear compartments and in lysosomes, and it contains Ra and Zn in its catalytic centre. SOD2, which exists as a homotetramer, presents Mn as cofactor, and it has been localized at mitochondrial level and has been observed to play a role in the promotion of cellular differentiation and tumorigenesis and the protection of pulmonary toxicity induced by hyperoxia. Finally SOD3, which is also a homotetramer, was found in extracellular fluids like human plasma, lymphatic fluid and cerebrospinal fluid (38). The catalase is an enzyme that belongs to the class of oxidoreductase, and it catalyses the following reaction:

$$2\ H_2O_2 \rightleftharpoons O_2 + 2\ H_2O$$

It is a tetramer that is constituted by four polypeptidic chains highly compacted which cause an unusual stability of this enzyme. Inside the tetramer, there are 4 Fe groups that allow the reaction of the enzyme with hydrogen peroxide: it is therefore degraded to two molecules of water and one of oxygen.

The glutathione system consists of GPx and glutathione S-transferase. This system is found in animals, plants and micro-organisms. The glutathione is a tripeptide that is soluble in water and synthesized starting from glutamate amino acids, cysteine and glycine. Glutathione is one of the most important antioxidants that are present at cellular level. It plays a key role in the detoxification of a variety of electrophilic compounds and peroxides through reactions catalysed by GST and GST (39).

It has been observed that cells lacking in this enzyme suffer from oxidative stress with a consequent degeneration at mitochondrial level. The tripeptide can exist intracellularly in GSSG and GSH. The GPx is an enzyme that, as SOD and catalase, has the function to protect the cell from the damage provoked by ROS. It detoxificates peroxides acting as an electron donor in the reduction reaction producing GSSG as a final product. The expression of this enzyme is induced by oxidative stress, and one of its aberrant expression has been associated with a variety of pathologies such as hepatitis, HIV, skin, kidney, breast and intestine cancer. The GST is a member of the family of enzymes that are coevoluted with glutathione system. It catalyses the conjugation of the glutathione to exogenous and endogenous electrophilic compounds including arene oxides, unsaturated carbonyls and organic acids (40). Many compounds that induce GST expression are often substrates of the enzyme, suggesting that the induction represents a mechanism of adaptive response. Instead, among the non-enzymatic antioxidants, we can find several compounds like vitamin C, vitamin E, vitamin A, carotenoids and polyphenols. L-AA or vitamin C is a hydrosoluble vitamin that plays numerous functions, including the antioxidant function, in the human organism. It is a compound that is very hydrosoluble, highly acidic and appears in the form of odourless and tasteless crystals with a pH nearly 2.5. Vitamin C has a strong reducing action due to the presence of an enediol group; in particular, it is able to donate an electron forming semidehydroascorbic acid, which donates a second electron generating dehydroascorbic acid. The redox potential of these reactions is as follows: semiascorbic/AA 0.28 V and dehydroascorbic acid/semihydroascorbic acid −0.17 V. This fact makes vitamin C an effective electron donor. Finally, dehydroascorbic acid is reduced by dehydroascorbate reductase regenerating AA.

Vitamin E is made up of a group of eight natural isoforms like alpha, beta, gamma and delta tocopherol

Table 3 Oxidative stress and various pathologies affecting the oral cavity

Author	*Patients*	*Methods*	*Results*
Zhang et al. (32)	25 smokers (range 20–30 cigarettes/day) and 25 race-, sex- and age-matched non-smokers	Oral mucosa cells were collected to evaluate DNA damage measuring MDA	DNA damage had increased in smokers if compared with non-smokers
Dogruer et al. (29)	29 children affected by adenotonsillar hypertrophy who underwent adenotonsillectomy and 51 age-matched controls	MDA, SOD, GSHPx and CAT levels and activity were analysed in venous blood specimen	MDA levels and SOD-GSHPx activity were significantly higher in the pre-tonsillectomy period than in the post-tonsillectomy period, while CAT activity was not different pre- and post-operation
Yilmaz et al. (31)	38 children with chronic tonsillitis and adenoid hypertrophy who underwent adenotonsillectomy and 23 healthy controls	Circulating retinol, beta-carotene, alpha-tocopherol, lycopene, ascorbic acid, SOD, GSH and MDA levels were measured before and 1 month after operation	An increased level of antioxidants and a reduction in oxidative damage, comparing pre- and post-operative values, were observed following adenotonsillectomy
Kiroglu et al. (30)	20 children affected by chronic adenotonsillitis and 19 children with adenotonsillar hypertrophy who underwent adenotonsillectomy	Pre-operative blood levels of erythrocyte and serum MDA and CAT, and adenoidal and tonsillar tissue levels of MDA and CAT were studied	All parameters were significantly higher than expected, showing that the two pathologies share the same pathological mechanism
Ergun et al. (46)	21 patients affected by oral lichen planus and 20 healthy controls	TAOC and MDA were measured in serum and saliva	Salivary MDA was significantly higher, while serum TAOC was significantly lower in patients affected by oral lichen planus if compared to healthy controls. A significant correlation between serum and saliva TAOC values was observed in patients with oral lichen planus and control subjects. A significant correlation between serum and saliva MDA values in control group was reported. A significant inverse correlation between salivary MDA and TAOC values was observed in the control group
Agha-Hosseini et al. (47)	30 patients affected by oral lichen planus and 30 healthy controls	Salivary MDA and TAOC levels were assayed by thiobarbituric acid and ferric-reducing antioxidant potential (FRAP) respectively in both groups	Higher levels of salivary MDA were observed in patients with oral lichen planus
Upadhyay et al. (48)	22 patients affected by oral lichen planus, 10 patients with oral lichenoid reaction and 15 healthy controls	Protein thiol oxidation, MDA and TAOC were estimated in both the groups and compared with that of normal subjects	Serum MDA levels were significantly higher if compared to healthy controls. Serum thiol levels were significantly lower in patients with oral lichen planus and oral lichenoid reaction if compared to controls. Therefore, these studies point out that oxidative stress may play a key role in pathologies affecting the oral mucosa such as oral lichen planus and oral lichenoid reaction. Both salivary and serum MDA have increased in patients with oral lichen planus confirming its importance as an oxidative stress biomarker

MDA, malondialdehyde; SOD, superoxide dismutase; TAOC, total antioxidant capacity.

and alpha, beta, gamma and delta tocotrienols, which are known to display antitumoral properties because of their antioxidant features. Among these isoforms, alpha tocopherol is a more potent form of vitamin E, and it has a high biological and nutritional value. Tocopherols cannot be synthesized in humans and animals; they must be taken with the diet through vegetable oils such as soy, maize and sesame oil; they can also be found in cotton seeds and in hazelnuts. The tocotrienols are mostly present in palm oil (41).

Carotenoids are a class of organic pigments that can be found in plants or photosynthetic organisms like algae and some bacterial species. We know more than 600 varieties of carotenoids, and they are normally divided into two classes: carotenes, which are hydrocarbons lacking in oxygen, and xanthophylls, which contain oxygen. They are accessorial pigments that during the photosynthetic process allow absorbtion of wavelengths different from chlorophyll, protecting the latter from photo-oxidation. They protect against the effects of unpaired oxygen and form ROS generated in the presence of sunlight.

The typical colour of carotenoids, which goes from pale yellow to orange until bright red, is a direct

consequence of the molecular structure of these compounds. In fact, the polymeric chains that compound them are characterized by the presence of double bonds that interact among them, allowing the electrons of the atoms involved to move more freely. As soon as the double bonds in the chain increase, there is also an increase in the freedom of electron movement. This fact allows the light spectrum, absorbed from these molecules, to decrease and, as a consequence, the reflected light wavelength increases appearing of a colour verging on red (42).

Polyphenols are a family of around 5000 organic molecules that are largely present in the vegetable kingdom. As meant by their name, they are characterized by the presence of several phenolic groups organized in more or less complex structures, generally characterized by a high molecular weight. These compounds are the product of plant secondary metabolism. Polyphenols are natural antioxidants present in plants, and they can prevent oxidation of lipoproteins and exert positive biomedical effects at cardiovascular level and in diseases related to ageing and tumoral growth.

The problem associated with the polyphenol intake with diet is their low bioavailability. In particular, they are found in bodily fluids in a non-native form, but as metabolites (sulphates, methylates and glucoronates) after an extensive metabolization in the intestine and liver.

A recent study, performed in Belgium (43) in 2010, has examined the potential antioxidant, anti-inflammatory and pro-apoptotic effects of curcumin, a yellow pigment extracted from its plant, in subjects affected by tumours of the oral cavity like leukoplakia and submucous fibrosis, and by oral lichen planus. Twenty-five patients with oral leukoplakia (13 men, 12 women), 25 patients with oral submucous fibrosis (11 men, 14 women), 25 patients with oral lichen planus (12 men and 13 women) and 25 healthy subjects, aged between 17 and 50 years, were selected. Consequently, oxidative stress markers, present in saliva and serum, were measured. MDA was measured using TBARS according to the methods described by Buege and Aust (44), 8-OhdG according to the method described by Toyokuni (45) and vitamin C and E with liquid chromatography. The measures were executed before curcumin intake after a week from curcumin treatment after clinical cure for pre-cancerous lesions and after 209 days. The values of vitamin C and E, measured in serum and saliva, increased, while MDA and 8-OHdG decreased after a week from curcumin intake if compared to the values before treatment in all the ill patients. The values were particularly significant after the cure of the disease, and in particular, the lesion entity improved as for the lesion size. Therefore, these results suggest that curcumin significantly increases the antioxidant local and systemic status and vitamin C and E levels, while lipid peroxidation and DNA damage decrease in patients with pre-cancerous lesions, showing that the anti-pre-cancerous effects of curcumin are mediated by pro-oxidative and antioxidative pathways. A study by Ergun et al. (46), involving 21 patients affected by oral lichen planus (11 men, 10 women), showed significantly higher salivary MDA ($z = 2.24$, $P = 0.03$), while serum TAOC was significantly lower ($z = 2.48$, $P = 0.01$) if compared to healthy controls ($n = 20$; 11 men, nine women). This study also showed a significant correlation between serum and saliva TAOC values in patients with oral lichen planus ($r = 0.714$ and $P = 0.0001$) and control subjects ($r = 0.69$ and $P = 0.001$) and between serum and saliva MDA values in control group ($r = 0.464$ and $P = 0.04$). A significant inverse correlation ($r = -0.598$ and $P = 0.005$) between salivary MDA and TAOC values was observed in control group. The higher levels of salivary MDA in patients affected by oral lichen planus were also confirmed by another study involving 30 patients affected by the same disease (15 men and 15 women (47). Another study (48), involving 22 patients affected by oral lichen planus and 10 patients with oral lichenoid reaction, also showed that serum MDA levels were significantly higher in patients affected by these pathologies if compared to healthy controls ($n = 15$; $P < 0.001$). Serum thiol levels were also significantly lower in patients affected by oral lichen planus and oral lichenoid reaction if compared to controls ($P < 0.0047$). These studies point out that oxidative stress may play a key role in pathologies affecting the oral mucosa such as oral lichen planus and oral lichenoid reaction. Both salivary and serum MDA have increased in patients affected by oral lichen planus confirming their importance as oxidative stress biomarkers. The finding that serum thiol levels have decreased in these patients is also of interest because plasma thiols are considered key oxidative targets (49), further strengthening the importance of oxidative stress in these pathologies.

Conclusions

This literary excursus is meant to draw the clinical-scientific readership's attention to the role that the dental surgeon, the otolaryngologist and generally the physician can play in an early biochemical and clinical diagnosis intended to characterize the diseases that are the object of a specialized study.

In summary, this review points out the key role played by oxidative stress in pathologies concerning the oral cavity. For instance, salivary 8-OHdG levels significantly increase in periodontitis, while they decrease after treatment, strongly underlying the key role played by this oxidized nucleoside of DNA in this condition (19). This finding is supported by another study showing increased levels of 8-OHdG in periodontitis, although a different specimen was analysed, that is, gingival blood (18). Mitochondrial DNA deletions have also been detected in subjects affected by chronic periodontitis (23), a condition that is also characterized by a decrease at baseline TAOC values which, on the other hand, increase after therapy (8). Furthermore, in subjects affected by periodontitis, a significant increase in myeloperoxidase, GPx, glutathione S-transferase, oxidized glutathione and thiobarbituric acid-reactive substances

has been reported in gingival tissue (20). Saliva, CGF and serum MDA levels, and saliva and CGF TOS values were also higher in patients affected by this condition (22). Another interesting feature of this condition is the significant increase in GPx, lactoferrin and myeloperoxidase, as reported by Wei et al. (17). In the setting of periodontal disease, saliva antioxidant activity decreases significantly, if compared to healthy peers (21). Another study showed how serum TAOC levels double in patients affected by periodontitis, if compared to controls (3).

As to cancer affecting the oral cavity, in patients with squamous cell carcinoma, GR, GPx, GSH and AA values had increased, while a decrease in SOD activity was observed in tumour tissue and surrounding healthy mucosa (25). An increase in oxidative stress in this condition was also confirmed by another research group who reported elevated levels of lipids, peroxides and dienes and low levels of antioxidants in serum from patients with squamous cell carcinoma (24). In patients affected by oral cancer, lipid peroxidation increased, and a decrease in non-enzymatic and enzymatic antioxidant status was reported, further supporting a key role of oxidative stress in this condition (26). Patients affected by myeloma present an increase in salivary Alb and carbonyl levels, which are an indicator of mucosal and oxidative damage. Following transplantation, a significant reduction in salivary secretory IgA, TAOC and uric acid levels was observed (28). Two studies also investigated the role of oxidative stress in leukoplakia. Mutagenic 8-nitroguanine and 8-oxo-7,8-dihydro-2′-deoxyguanosine production was observed in oral epithelium of patients affected by this pathology (27). Curcumin intake promoted serum and saliva vitamin C and E increase, while MDA and 8-OHdG decreased in patients with oral leukoplakia, oral submucous fibrosis or lichen planus, suggesting that an antioxidant treatment may be useful to face these conditions or as a supportive therapy (43).

It is also interesting that adenotonsillectomy results in a decrease in MDA levels and SOD-GSHPx activity in patients affected by chronic tonsillitis and adenoid hypertrophy (31). Adenotonsillitis and adenotonsillar hypertrophy are pathologies sharing an increase in blood levels of erythrocyte and serum MDA and CAT, and adenoidal and tonsillar tissue levels of MDA and CAT that decrease following adenotonsillectomy (30).

Oxidative stress has also been investigated in the context of oral lichen planus. For instance, salivary MDA was significantly higher, while serum TAOC values were significantly lower in patients affected by this condition. Furthermore, the same study showed a significant correlation between serum and saliva TAOC values in patients with oral lichen planus and control subjects. A significant correlation between serum and saliva MDA values in control group was reported (46). Another study also reported higher levels of salivary MDA in patients affected by oral lichen planus (47). This evidence was also confirmed by Upadhyay et al. (48), who also reported the same finding in patients affected by oral lichenoid reaction. Furthermore, the same study showed that serum thiol levels were significantly lower in patients with oral lichen planus and oral lichenoid reaction if compared to controls. Building upon these foundations, these findings strongly underline how oxidative stress may be involved in both oral lichen planus and oral lichenoid reaction.

The syndromes or the diseases, induced or aggravated by oxidative stress, can be evaluated at the right time, using corrective, instrumental, pharmaceutical or nutraceutical treatments able to support the primary treatment, namely antibiotics, anti-inflammatories, steroids or being themselves the primary corrective substratum for a particular pathology.

In our experience, the values of free radicals and antioxidants in saliva do not always match with the values found in peripheral blood, showing a diagnostic and selective specificity of these diagnostic parameters in relation to pathologies affecting the oral cavity. This makes the method even more important in the dental surgeon's hands. With the ex juvantibus experience of antioxidant products already existing, and the ones coming shortly, there is a sound hope to get long-lasting recoveries and more efficacy in technical-odontoiatric results and prevent the development of leukoplakias and tumours that critically afflict each specialist's clinical experience.

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Authors' contribution

The authors hereby certify that all work contained in this review is original. The authors claim full responsibility for the contents of the article.

Conflict of interest

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.