

Reactive oxygen species and periodontal disease: Current biomarkers, diagnostic potential and future perspectives (Review)

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Abstract. Periodontitis is a chronic inflammatory disease primarily driven by bacterial infection, leading to the progressive destruction of periodontal tissues. Recent evidence implicates reactive oxygen species (ROS) in mediating oxidative stress and tissue damage. The present review discusses the diagnostic potential of ROS-related biomarkers in periodontal disease, focusing on key markers, such as malondialdehyde, 8-hydroxydeoxyguanosine and advanced glycation end products. Current studies highlight the correlation between elevated ROS biomarkers and the severity of periodontitis, suggesting their utility in early diagnosis and monitoring of disease progression. Furthermore, the present review discusses the variability in antioxidant levels, such as superoxide dismutase and glutathione, as well as their implications for periodontal health. Despite promising findings, challenges remain in standardizing biomarker assays and understanding the complex interplay between oxidative stress and periodontal disease. ROS-related biomarkers have potential as non-invasive diagnostic indicators for periodontitis. The standardization of assay methods and further research are required to facilitate their clinical application. Relevant literature was sourced through searches in databases including PubMed, Google Scholar, Scopus and Web of Science. The focus of the present review was placed on clinical and experimental studies published recently on ROS and periodontitis. The selection included randomized controlled trials, clinical trials and well conducted narrative reviews. The search strategy included the use of the following MeSH terms: Periodontitis, reactive oxygen species, oxidative stress, salivary biomarkers and antioxidants.

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1. Introduction

The most prevalent cause of tooth loss worldwide and the sixth most common disease affecting individuals globally is periodontitis, a chronic inflammatory disease that damages the supporting tissues of the periodontium (1). The onset of periodontitis can be influenced by several factors, including genetic predisposition, the composition of the oral microbiota and lifestyle elements, such as smoking, nutrition, stress and hormones (2). The dysbiotic environment in the genetically predisposed patient promotes the growth of pathogenic bacterial populations, which in turn stimulates the host immunological response. Reactive oxygen species (ROS), produced by polymorphonuclear leukocytes (PMNs) during the initial immunological phase, eliminate pathogens (3). In periodontitis, PMN hyperactivity creates a milieu of oxidative stress, further increasing ROS generation (4). Strong evidence has surfaced in recent years linking oxidative stress to the pathophysiology of periodontitis (5). The body contains various species, referred to as antioxidants, to defend against the increase of ROS, which maintains the level of ROS under control even though they are created in a healthy system (6). Disease occurs because of a shift in the ratio of antioxidants to reactive oxygen species. Secondary factors such as diabetes, cardiovascular diseases, tobacco use and stress all contribute to

the development of periodontitis by aggravating the oxidative stress environment in patients with periodontal species (7).

The aim of the present review was to discuss the association between ROS and periodontal disease, highlighting current biomarkers and their diagnostic value while identifying gaps and opportunities for future research in early detection and treatment.

A structured literature search was performed across PubMed, Google Scholar, Scopus and Web of Science identified ~68 articles related to ROS and periodontal disease. Following title and abstract screening, 13 studies were excluded due to irrelevance to periodontal pathology, duplication, non-clinical focus, or insufficient methodological quality. Full-text assessment was then performed, and studies specifically evaluating oxidative stress biomarkers, antioxidant status and their clinical relevance in periodontitis were included. The final set of articles was qualitatively synthesized to address the objectives of the present narrative review.

2. Reactive oxygen species: Origin, types and physiological role

ROS are chemically reactive molecules containing oxygen and include free radicals with unpaired electrons and non-radical species (8). Certain ROS function as signaling molecules at physiological levels, whereas others primarily contribute to oxidative damage when produced in excess. Both oxygen and nitrogen free radicals are part of the broader category of reactive species, which includes ROS and reactive nitrogen species. In healthy individuals, these are produced in trace amounts during biological processes (9). These reactive species originate endogenously (within the body) or from exogenous factors. In the event that a bacterial infection occurs, ROS can also function as second messengers, signaling macrophages to combat invading pathogens (10,11).

ROS are highly reactive oxygen-containing molecules, which include free radicals, such as superoxide anion (O_2^-) and hydroxyl radical ($-OH$), as well as non-radical derivatives involved in oxidative processes, such as hydrogen peroxide (H_2O_2), hypochlorous acid ($HOCl$), and singlet oxygen (1O_2) (12). ROS are constantly generated by tissue cells and are essential for maintaining normal cellular metabolism and physiological processes. However, when ROS accumulate excessively or persist over time, they can trigger oxidative stress. This stress exacerbates systemic inflammation and induces substantial tissue injury via mechanisms, such as lipid peroxidation, DNA and protein modifications, and the oxidation of critical enzymes, ultimately leading to cell death. Under physiological conditions, cellular antioxidants neutralize ROS and prevent oxidative damage. This antioxidant defense system maintains tissue integrity by effectively mitigating ROS-induced harm. Research over the past decade has increasingly linked oxidative stress to the initiation, development and the advancement of several chronic inflammatory diseases, including periodontitis (13).

The destructive processes in the periodontium observed in inflammatory periodontal diseases are considered to stem from complex interactions arising from a disruption of the balance between enzymatic and non-enzymatic breakdown pathways. ROS, produced predominantly by polymorphonuclear

leukocytes during inflammation, are particularly damaging in this context. Several lines of evidence support the involvement of ROS in the breakdown of periodontal tissue. Some examples include the presence of oxidative byproducts, elevated levels of iron and copper ions, promoting the formation of highly reactive radicals, and an imbalance between oxidants and antioxidants in the periodontal pockets. *In vitro* studies have reported that ROS can degrade various components of the extracellular matrix (ECM), such as proteoglycans, by altering amino acid side chains and fragmenting core proteins, with only minor depolymerization of glycosaminoglycan chains (14,15). Moreover, the detection of connective tissue metabolites in gingival crevicular fluid (GCF), originating from the breakdown of periodontal tissues, particularly alveolar bone, supports the key role of ROS in the tissue damage seen in inflammatory periodontal diseases (16) (Fig. 1). The commonly investigated oxidative stress and antioxidant biomarkers in periodontitis, their biological relevance, sampling matrices and methodological considerations are summarized in Table I.

3. Role of oxidative stress in the pathogenesis of periodontal disease

The presence of oxidative stress indicates an imbalance between the generation of oxidants, particularly ROS, and the antioxidant defenses of the body (11). ROS are produced by redox reactions involving oxygen and can arise from internal cellular sources, such as NADPH oxidase, cyclooxygenases, peroxisomes, xanthine oxidase and the microsomal electron transport system. Furthermore, external factors, including environmental pollution, tobacco smoke, pesticides, certain drugs, ultraviolet radiation and exposure to heavy metals, can stimulate ROS generation. The major oxygen-derived free radicals are O_2^- and $-OH$ (17,18).

ROS can function as signaling molecules in a healthy system, facilitating processes, such as regulating protein function and activating the immune response; however, excess levels lead to the development of oxidative stress (17). At low levels, ROS are part of normal physiological processes, a state known as 'oxidative eustress', in which they regulate protein function and immune responses. For instance, they can oxidize thiol groups in proteins, altering their structure and activating their functions. However, at high concentrations, ROS lead to 'oxidative distress', causing damage to cellular structures, such as DNA, RNA, lipids and proteins (9,19). To prevent this damage, cells utilize antioxidants, including enzymes and small molecules, to neutralize excess ROS and maintain homeostasis.

In periodontal disease, ROS originate from both host-derived inflammatory responses and pathogen-induced microbial mechanisms, jointly shaping the periodontal redox microenvironment. Neutrophils, gingival fibroblasts and osteoblasts generate ROS via NADPH oxidase and mitochondrial pathways as part of their antimicrobial defense. By contrast, periodontal pathogens, such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* express antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and peroxidase, thereby evading oxidative killing within biofilms. Furthermore, microbial virulence factors, heme

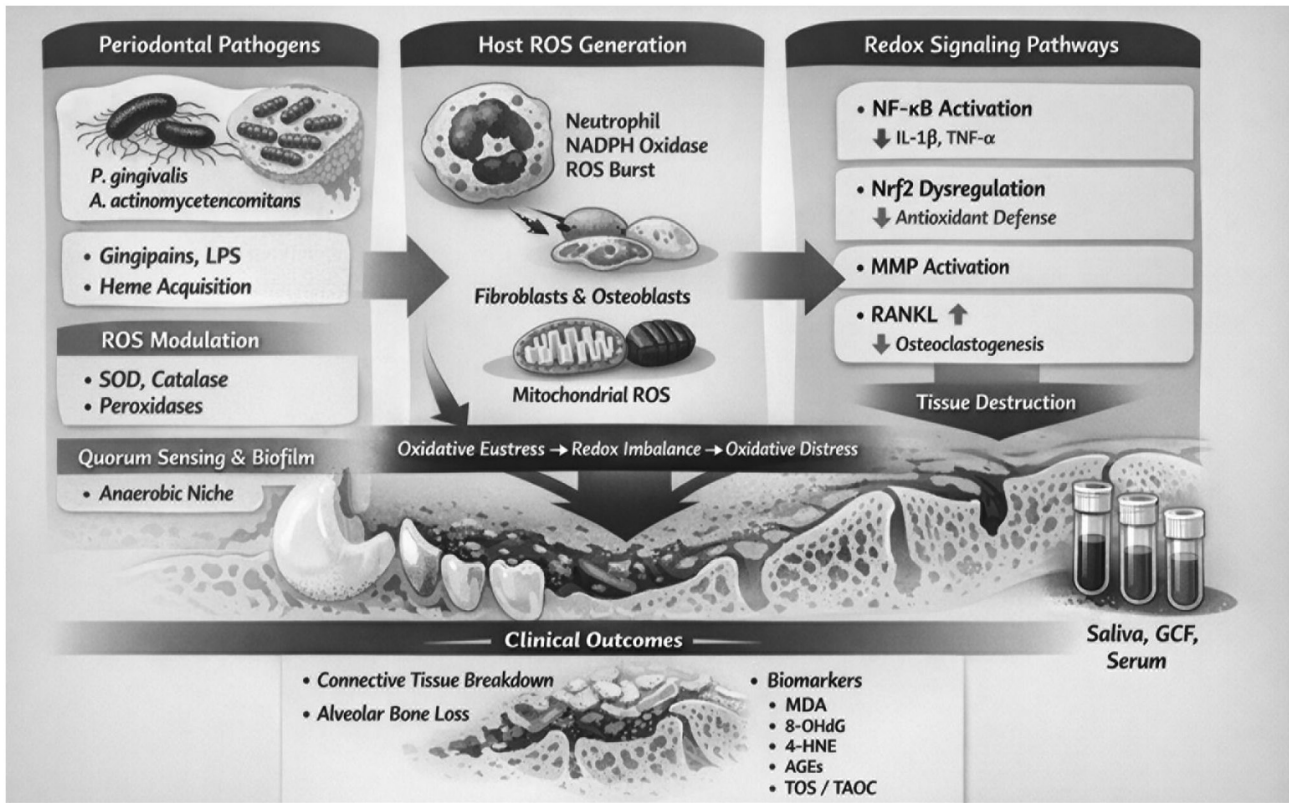


Figure 1. ROS-mediated host–microbial redox interactions in periodontal disease pathogenesis. ROS, reactive oxygen species; LPS, lipopolysaccharide; SOD, superoxide dismutase; MMP, matrix metalloproteinase; MDA, malondialdehyde; 8-OHdG, 8-hydroxydeoxyguanosine; 4-HNE, 4-hydroxy-2-nonenal; AGEs, advanced glycation end products; TOS, total oxidant status; TAOC, total antioxidant capacity; GCF, gingival crevicular fluid.

acquisition systems and quorum sensing-regulated biofilm organization modulate host-microbiome redox interactions and sustain dysbiosis. Excessive ROS activate redox-sensitive signaling pathways, such as NF-κB, promoting the release of pro-inflammatory cytokines and the expression of matrix metalloproteinases (MMPs). The dysregulation of compensatory antioxidant signaling via the Nrf2/ARE pathway may occur during chronic inflammation. In addition, mitochondrial ROS signaling amplifies inflammatory responses and apoptosis. The disruption of redox feedback loops in gingival fibroblasts, osteoblasts and neutrophils leads to progressive connective tissue degradation and alveolar bone loss (12).

4. Effects of oxidative stress in periodontal disease

Periodontal disease is an inflammatory condition that destroys tooth-supporting tissues (20). Oxidative stress plays a pivotal role in its pathogenesis. Elevated ROS levels can damage cellular structures, including DNA, RNA, lipids and proteins, leading to the development of diseases, such as cancer, diabetes, rheumatoid arthritis, neurodegeneration, asthma, reproductive disorders and periodontitis (13). Specifically, in periodontal disease, exposure to lipopolysaccharide from *Porphyromonas gingivalis* aggravates oxidative stress in human gingival and periodontal ligament fibroblasts. The accumulation of oxidative stress markers in the saliva, GCF and plasma of patients with periodontitis provides additional evidence linking oxidative stress to periodontal inflammation (21).

5. Reactive oxygen metabolites in periodontal disease

Alteration of the homeostatic axis. ROS plays a dual role in periodontal health. Under normal conditions, they function as cellular messengers, aiding in the regulation of immune responses and in maintaining homeostasis in periodontal tissues. However, when the ROS-antioxidant balance is disrupted, often due to persistent bacterial infections in the periodontal pockets, this homeostatic axis is altered (22). Excessive ROS production leads to oxidative stress, promoting inflammation and shifting the microbiota balance toward a dysbiotic state, exacerbating periodontal disease (23).

Resorption of the alveolar bone: Bone-related inflammatory illness. In periodontitis, excessive ROS production disrupts the RANKL/osteoprotegerin axis, a critical pathway for maintaining bone homeostasis, and contributes to alveolar bone resorption. Typically, this axis balances bone resorption and formation; however, oxidative stress induces the overproduction of pro-inflammatory cytokines, tipping the balance toward bone resorption. This mechanism not only leads to the destruction of alveolar bone in periodontitis, but has also been implicated in other bone-related inflammatory conditions, such as rheumatoid arthritis, osteoarthritis and osteoporosis (24).

Loss of the periodontal ligament (PDL): Clinical attachment loss (CAL). The PDL is crucial for tooth support, and its loss is a key indicator of periodontal disease progression. ROS contribute to this process by activating MMPs, particularly

Table I. Comparative overview of oxidative stress and antioxidant biomarkers in periodontitis: Biological relevance, sampling matrices and methodological considerations.

Biomarker	Biological class	Sample type(s)	Typical direction in periodontitis	Common assays	Key confounders/ caveats	Clinical utility notes	(Refs.)
MDA	Lipid peroxidation	Saliva, GCF, serum	↓ decreases after therapy	TBARS/ELISA/LC-MS	Smoking, diabetes, diet	Good for oxidative burden; limited specificity	(11)
8-OHdG	DNA oxidation	Saliva, GCF, serum	↑; decreases after therapy	ELISA/LC-MS/MS	Assay cross-reactivity; renal clearance (serum/urine)	Promising for activity/response; needs standardization	(17)
4-HNE	Reactive aldehyde	Saliva, serum	Variable; often ↑ in smokers	ELISA/LC-MS	Short half-life; protein adducts; smoking	May indicate high-risk phenotypes	(18)
AGEs/RAGE	Glycooxidation axis	Serum, saliva, tissue	↑ (particularly diabetes/smoking)	ELISA/IHC	Strongly comorbidity-driven	Links systemic-local; prognostic in diabetics	(18)
TOS	Total oxidant load	Saliva, GCF	↑; decreases after therapy	Colorimetric (Erel)	Non-specific; affected by other inflammatory diseases	Useful adjunct marker, not standalone	(18)
TAOC/TAC	Total antioxidant capacity	Saliva, GCF, serum	Often ↓ (but variable)	Colorimetric/FRAP/ABTS	Uric acid dominates saliva; diet and stimulation effects	Best interpreted with TOS and clinical data	(18)
SOD/GPx/ CAT	Enzymatic antioxidants	Saliva, GCF, serum	Variable (↑ compensatory or ↓ depletion)	Enzyme activity assays	Matrix and timing; medications; systemic status	More informative in panels	(18)

MDA, malondialdehyde; 8-OHdG, 8-hydroxydeoxyguanosine; 4-HNE, 4-hydroxy-2-nonenal; AGEs, advanced glycation end products; RAGE, receptor for advanced glycation end products; TOS, total oxidant status; TAOC/TAC, total antioxidant capacity; SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase.

under conditions of oxidative stress. These MMPs break down essential components of the PDL, including collagen, elastin, proteoglycans, and glycosaminoglycans. The breakdown of these ECM components leads to CAL, destabilizing the tooth-supporting structures (9,25).

6. Antioxidant defense systems in oral and periodontal health

Saliva contains a comprehensive array of enzymatic and non-enzymatic antioxidants that collectively protect the oral cavity from oxidative damage (9). The enzymatic components include SOD, which converts O₂⁻ into hydrogen peroxide and oxygen; CAT, which decomposes H₂O₂ into water and oxygen using manganese/iron cofactors; glutathione peroxidase (GPx), which reduces H₂O₂ to water while oxidizing glutathione; salivary lactoperoxidase, which generates antibacterial hypothiocyanite from thiocyanate and H₂O₂; myeloperoxidase, which produces germicidal hypochlorous acid from chloride and H₂O₂; and thioredoxin/peroxiredoxin systems, which facilitate redox cycling to neutralize peroxides (26). These enzymes are complemented by non-enzymatic antioxidants, which include uric acid (constituting >70% of the antioxidant capacity of saliva), melatonin (with antiaging, anti-inflammatory and osteoblast-supporting properties), glutathione (a critical GPx cofactor and direct ROS scavenger), vitamins C/A/E, albumin, lactoferrin and bilirubin. Together, these components form a synergistic defense network that neutralizes ROS, mitigates oxidative stress and maintains oral tissue homeostasis (27).

7. Biomarkers of oxidative stress in periodontitis

Malondialdehyde (MDA). MDA arises from ROS-triggered lipid peroxidation, particularly via the attack of free radicals on polyunsaturated fatty acids (PUFAs). This reaction disrupts cell membrane integrity and may induce DNA damage (28). The studies by Trivedi *et al* (29) and Veljovic *et al* (30) demonstrated that MDA levels are associated with the severity of periodontal inflammation, and decrease following scaling and root planning therapy. Furthermore, the study Veljovic *et al* (30) demonstrated that smoking increases MDA levels in patients with periodontal disease. In addition, patients with diabetes who have periodontitis exhibit elevated MDA levels, associating it with systemic oxidative stress (31).

8-Hydroxydeoxyguanosine (8-OHdG). ROS, such as hydroxyl radicals, oxidize guanine bases in DNA, forming 8-OHdG, a biomarker of oxidative DNA damage and mutagenesis. Exogenous 8-OHdG can suppress ROS production and inflammation by inhibiting Rac1-GTP binding, suggesting a feedback loop in oxidative stress regulation (32). The studies by Chen *et al* (33) and Altıngöz *et al* (31) demonstrated that 8-OHdG levels were significantly elevated in patients with periodontitis, particularly in those with increased CAL. The level of 8-OHdG decreases following non-surgical periodontal therapy, as reported by Önder *et al* (34). The strong association between 8-OHdG and CAL, particularly in patients with diabetes, renders it a promising marker for the diagnosis and monitoring of periodontal disease.

4-Hydroxy-2-nonenal (4-HNE). Generated as a result of the ROS-induced lipid peroxidation of ω -6 PUFAs, 4-HNE is a cytotoxic α,β -unsaturated aldehyde that promotes apoptosis and protein damage. In infections, 4-HNE functions as an antimicrobial agent by damaging bacterial cells, although pathogens, such as *Listeria* evolve reductases (e.g., Rha1/Rha2) to neutralize it (35). While certain researchers, such as Altıngöz *et al* (31), found a positive association between 4-HNE levels and periodontal disease indicators [bleeding on probing (BOP) and CAL], others, such as Onder *et al* (34), did not observe significant changes in 4-HNE levels in patients with periodontopathies. The concentration of 4-HNE is considerably high in smokers with periodontitis, suggesting its potential use in identifying patients at heightened risk owing to smoking-related oxidative stress.

Advanced glycation end products (AGEs). AGEs, formed via non-enzymatic glycation, amplify ROS production via receptor-mediated NADPH oxidase activation (29,36). The studies by Altıngöz *et al* (31) and Katz *et al* (37) explored the association between AGE levels and receptors for advanced glycation end products (RAGE), revealing that smoking significantly increases RAGE expression. AGEs and their receptors are linked to increased cytokine release, bone resorption and collagen degradation, particularly in patients with diabetes, emphasizing their critical role in the systemic effects of periodontitis.

8. Antioxidant levels in periodontal disease

Two key antioxidant enzymes involved in redox reactions are SOD and GPx. Chen *et al* (33) examined their levels in the saliva and GCF of patients with periodontitis and healthy controls. Their findings indicated no significant differences in enzyme levels between the two groups. However, other studies have documented inconsistent findings, with some observing variations in the levels of these enzymes among individuals with periodontal disease. For example, Trivedi *et al* (29) reported decreased activities of SOD and GPx in patients with periodontitis, suggesting that this reduction may result from antioxidant depletion during ROS neutralization. By contrast, Villa-Corea *et al* (38) observed elevated GPx activity in patients with both aggressive and chronic periodontitis compared with healthy controls. Similarly, Yang *et al* (39) noted an increased SOD activity in patients with periodontitis, with SOD levels positively being associated with clinical characteristics, such as CAL, BOP, probing depth, gingival index and plaque index.

Glutathione, another crucial antioxidant, functions as a scavenger for ROS. Kluknavska *et al* (40) observed increased glutathione levels in patients with aggressive and chronic periodontitis, consistent with earlier studies (38,41). In addition, Kluknavska *et al* (40) noted that glutathione levels were lower in patients with gingivitis than in those with periodontitis, but higher than in the healthy control group, implying that glutathione levels surge with increasing inflammation severity. Conversely, Oktay *et al* (42) reported lower glutathione levels in patients with periodontitis than in healthy individuals.

Melatonin, another antioxidant present in saliva, was studied by Balaji and Rao (43). They found lower melatonin levels in the GCF of smokers with periodontopathies and non-smokers

with periodontitis compared with healthy controls. The lowest levels were detected in smokers, likely due to tobacco-induced increase in ROS levels. Purrahmani *et al* (44) demonstrated that salivary melatonin levels, initially low in patients with periodontitis, increased following non-surgical treatment, indicating that melatonin levels may be a reliable biomarker for assessing response to periodontal therapy.

9. Total oxidant status and total antioxidant capacity

The total oxidant status (TOS) denotes the cumulative concentration of oxidants, including ROS and other free radicals, in biological samples, such as saliva or GCF. Elevated TOS levels in patients with periodontitis reflect increased oxidative stress resulting from heightened inflammatory activity and ROS production (45). The automated colorimetric assay for TOS, developed by Erel (46) in 2005, has since become a standard method in periodontitis research. Several investigations have reported high TOS values in individuals with periodontitis. For example, Zalewska *et al* (47) established that stimulated saliva exhibits higher TOS levels than non-stimulated saliva or GCF, which they attributed to the parotid glands being a significant source of oral ROS. In another study, Toczewska *et al* (48) observed a decrease in TOS levels following non-surgical periodontal treatment, suggesting that it may be a valuable indicator of oxidative stress changes associated with periodontal therapy.

Total antioxidant capacity (TAOC/TAC), also referred to as the total antioxidant status, measures the overall ability of antioxidants in a sample to neutralize oxidants, providing a comprehensive evaluation of the antioxidant defense system. In periodontitis, TAOC levels are decreased, indicating that antioxidant defenses are inadequate to counteract elevated ROS and oxidative stress (45). Numerous studies, including those by Miricescu *et al* (49) and Nguyen *et al* (50), have reported significantly lower TAOC levels in patients with periodontitis compared with healthy controls, which may be attributed to increased antioxidant consumption in response to heightened ROS.

Although TOS and TAOC have been proposed as potential biomarkers of oxidative stress in periodontitis, their diagnostic utility remains controversial. For instance, Senouci *et al* (51) reported an inverse association between TAC levels and the severity of periodontitis, whereas Behfarnia *et al* (52) observed increased TAOC following nonsurgical periodontal therapy. Toczewska *et al* (48) cautioned that various inflammatory conditions can influence TOS and TAOC levels and may not reliably distinguish between different stages of periodontitis. Consequently, while TOS and TAOC provide valuable insight into the oxidative status of the oral environment, they are not yet established as definitive biomarkers for diagnosing or staging periodontitis.

10. Challenges and future perspective of ROS biomarkers in periodontal disease

Despite considerable progress in elucidating the role of ROS in the pathogenesis of periodontal disease, several challenges remain in the clinical application of ROS biomarkers. A key limitation is the variability in the sensitivity of current

measurement techniques for oxidative stress markers, such as TOS, TAC, MDA, and 8-OHdG in biological fluids. The lack of standardized, universally accepted protocols hampers reproducibility and comparability across studies. Furthermore, the levels of these biomarkers may be affected by various confounding factors, such as age, sex, smoking, systemic health conditions, medication use and circadian rhythms, complicating their interpretation and limiting their disease specificity. Owing to the dynamic nature of ROS production, which fluctuates in response to inflammation, treatment and daily oral hygiene, single-time-point measurements may not accurately reflect chronic oxidative status or the progression of periodontal disease. In addition, the complex interplay among ROS, inflammatory mediators, host immune responses and microbial factors in periodontal disease poses difficulties in delineating the precise mechanistic pathways via which ROS contribute to tissue destruction. Although antioxidant-based adjunctive treatments have demonstrated potential to lower oxidative stress markers and enhance periodontal health, their clinical efficacy is limited by the lack of extensive, long-term randomized controlled trials.

Future research is required to focus on developing multiplex biomarker panels and integrating omics technologies, such as proteomics and metabolomics, to identify more specific and sensitive markers of oxidative stress. Moreover, ROS may be tested in combination with other adjunctive therapies, such as ozone, probiotics and photobiomodulation, to assess their potential effects on oxidative stress and periodontal tissues (53,54).

Rapid, point-of-care diagnostic assays should be developed, and longitudinal and interventional studies should be conducted. These measures will aid in validating the clinical relevance of ROS biomarkers and translating these findings into effective diagnostic and therapeutic strategies for periodontal disease.

The present review has certain limitations, which should be mentioned. At the study level, heterogeneity in study design, sample size and biomarker assessment methods, as well as inadequate control of confounding factors, may contribute to variability and potential bias. At the review level, as this is a narrative review, the incomplete retrieval of relevant literature and reporting bias cannot be entirely excluded. Furthermore, the lack of a quantitative synthesis limits the ability to draw definitive conclusions about the diagnostic utility of ROS biomarkers.

11. Conclusion

The evidence provided herein highlights the pivotal role of ROS in the pathogenesis and progression of periodontal disease, as demonstrated by increased levels of oxidative stress markers such as TOS, MDA and 8-OHdG, alongside decreased TAOC. While these biomarkers hold promise for disease monitoring and therapeutic assessment, their clinical utility is limited by methodological variability, the lack of standardization and confounding factors, such as systemic health and lifestyle habits. The multifactorial nature of periodontal disease, involving complex interactions among ROS, inflammation, immune responses and microbial factors, further complicates the interpretation and specificity of these markers. Although

antioxidant-based therapies have shown potential benefits, robust evidence from large-scale clinical trials is still needed. Future directions should focus on standardizing biomarker assessment, developing multiplex panels, integrating omics technologies, and advancing point-of-care diagnostics to validate and enhance the clinical relevance of ROS biomarkers in the management of periodontal disease.

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Ethics approval and consent for publication

Not applicable.

Patient consent for publication

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Competing interests

The authors have declared they have no competing interests.

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