

**Title:** Inflammatory mediators' essence in apical periodontitis

**Running title:** Mediators in apical periodontitis

**Article type:** Invited review

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**Received:** 18.01.2024

**Revised:** 08.02.2024

**Accepted:** 15.02.2024

**DOI:** 10.26650/eor.20241423117

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**How to cite:** Milojevic N, Lazic Krdzovic E, Lukic E, Puresevic D, Markovic M, Jakovljevic A. Inflammatory mediators' essence in apical periodontitis. Eur Oral Res 2024. Advance online publication.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record

## **Abstract**

Apical periodontitis (AP) represents chronic inflammatory reaction of periradicular tissues of teeth with necrotic pulp. Although AP has been considered as a multifactorial disease, different microorganisms and their virulence factors from infected root canals are considered to be the primary cause of periradicular inflammatory process. The interplay between microbes and host leads to an inflammatory cascade of events that includes activation of innate and adaptive components of immunity. Activation of different immune cells in AP is intermediated by different molecules known as mediators of inflammation. These molecules establish various network interrelationships in the inflamed periapical area and induce alveolar bone resorption. This narrative review aimed to explore and present the current knowledge of selected inflammatory mediators, including cytokines, matrix metalloproteinases, bone resorption regulators and components of oxidative stress involved in the alveolar bone resorption in AP.

**Key words:** *periapical periodontitis, cytokines, oxidative stress, matrix metalloproteinases, bone resorption*

**Türkçe öz:** Apikal periodontitis (AP), dişlerin periradiküler dokularının nekrotik pulpa ile kronik inflamatuvar reaksiyonunu ifade eder. AP çok faktörlü bir hastalık olarak kabul edilmesine rağmen, enfekte kök kanallarından farklı mikroorganizmalar ve bunların virülans faktörlerinin periradiküler inflamatuvar sürecin birincil nedeni olduğu düşünülmektedir. Mikroplar ve konakçı arasındaki etkileşim, bağışıklığın doğuştan gelen ve adaptif bileşenlerinin aktivasyonunu içeren inflamatuvar bir olaylar dizisine yol açar. AP'de farklı bağışıklık hücrelerinin aktivasyonuna, inflamasyon araçları olarak bilinen farklı moleküller aracılık eder. Bu moleküller iltihaplı periapikal bölgede çeşitli ağ ilişkileri kurar ve alveolar kemik emilimini indükler. Bu derleme makalesi, sitokinler, matriks metalloproteinazlar, kemik rezorpsiyon düzenleyicileri ve AP'de alveolar kemik rezorpsiyonunda rol oynayan oksidatif stres bileşenleri dahil olmak üzere bazı inflamatuvar mediatörlerle ilgili mevcut bilgileri sunmayı amaçlamaktadır. Anahtar kelimeler: periapikal periodontitis, sitokinler, oksidatif stres, matriks metalloproteinazlar, kemik rezorpsiyonu

## **Introduction**

Apical periodontitis (AP) represents chronic inflammatory reaction of periradicular tissues of teeth with necrotic pulp (1). Although AP has been considered as a multifactorial disease, in most of the cases it is a consequence of dental caries. Therefore, different microorganisms and their virulence factors from infected root canals are considered to be the primary cause of periradicular inflammatory process (2). The leading radiographic characteristic of AP is the destruction of periradicular tissues, manifested as a radiolucency surrounding the apex of the roots of the affected tooth (1). Infectious agents, along with the toxins they produce, and metabolic waste products mediate an array of immunological responses within the host's dental pulp and periradicular tissues. Such kind of interplay between microbes and the host leads to an inflammatory sequence of events, including the activation of innate (polymorphonuclear leukocytes, macrophages, and endothelial cells) and adaptive components (T – and B – lymphocytes) of immunity (3). Activation of different immune cells in AP is intermediated by different molecules known as mediators of inflammation. It is due to these molecules that different network interrelationships are established in the inflamed periapical area. However, the arrangement and extent of their gene expression are different among individuals and determined by the origin of the stimulatory agent (4). Bearing in mind the importance of proinflammatory mediators' essence in the pathogenesis of AP this narrative review aimed to explore and present the current knowledge of selected inflammatory mediators, including cytokines, matrix metalloproteinases, bone resorption regulators and components of oxidative stress involved in the alveolar bone resorption in AP.

### *Cytokines*

Cytokines are low molecular weight polypeptides or glycoproteins originating from hematopoietic and/or structural cells. They regulate many important events in human body including cell growth, differentiation, inflammation, immune defense, tissue remodeling and repair, etc. (5).

Cytokines exhibit a pleiotropic effect on the target cells and can function through autocrine, paracrine, and/or endocrine pathways. They can act as pro- and anti-inflammatory mediators showing their synergistic and/or antagonistic effects as well. Cytokines encompass a wide range of categories, i.e., interleukins (ILs), tumor necrosis factors (TNFs), colony stimulating factors (CSFs), monokines, lymphokines interferons (IFNs), and transforming growth factors (TGFs) (5). Making the complex interrelationship networks in periradicular tissues proinflammatory cytokines are able to stimulate osteoblasts differentiation into osteoclasts and promote alveolar bone resorption (3, 6). This subsection will present a brief overview of the most important proinflammatory cytokines involved in that process.

#### *Tumor necrosis factor – alpha*

Tumor necrosis factor – alpha (TNF- $\alpha$ ), which is also known as cachexin or cachectin, belongs to the TNF superfamily, which comprises various transmembrane proteins with a homologous TNF domain. TNF- $\alpha$  represents an inflammatory cytokine generated during acute inflammation as a byproduct of macrophages/monocytes. It induces a variety of signaling events within cells, eventually resulting in either necrosis or apoptosis (7). TNF- $\alpha$ , as an endogenous pyrogen, can cause fever, apoptotic cell death, cachexia, inflammation, inhibit cancer genesis and viral replication, and respond to septic conditions (7). An extensive range of human conditions has been associated with dysregulation of TNF- $\alpha$  production. These include cancer, inflammatory bowel disease, Alzheimer's disease, psoriasis, major depression, etc. (8). Moreover, TNF- $\alpha$  belongs to the group of pro-inflammatory cytokines that have a prominent role in alveolar bone resorption in AP (3). Several *in vitro* studies showed that inflammatory cells of harvested periapical lesions produced TNF- $\alpha$  (9, 10). Gazivoda and co-workers (9) documented a heightened level of TNF- $\alpha$  within the inflammatory cells of larger periapical lesions when compared to smaller ones. The authors also revealed a positive relationship when it comes to the levels of TNF- $\alpha$  and increased presence of inflammatory cells, e.g.,

monocytes, macrophages and dendritic cells in periapical lesions (9). In addition, Artese *et al.* (10) showed that mononuclear cells cultivated from harvested AP lesions are able to secrete TNF- $\alpha$ . Furthermore, the involvement of TNF- $\alpha$  in the pathogenesis of AP has been shown in animal experimental model. In 2001, Graves *et al.* (11) notified that TNF- $\alpha$  modulates fibroblast apoptosis, polymorphonuclear recruitment and osteoclast formation in mice as a consequence of *Porphyromonas gingivalis* (*P. gingivalis*) infection. The authors employed *in vivo* calvarial model in mice with targeted deletion of TNF receptors p55 and p75 and matched wild-type mice. In conclusion, the authors stated that TNF represents a major mediator when it comes to *P. gingivalis*-induced apoptosis and inflammation in AP (11). Moreover, Samuel *et al.* (12) noted that the multiple AP in rats can affect overall health by increasing lymphocyte and TNF- $\alpha$  levels in the blood. Noteworthy, 30 years ago, Safavi and Rossomando (13) identified detectable levels of TNF in periapical tissue exudates in chronic AP. Most recently, Nunez *et al.* (14) also identified significantly elevated concentrations of TNF- $\alpha$  in gingival crevicular fluid (GCF) from diseased teeth with AP compared to the healthy controls. Previously conducted studies in humans analyzed the association between the levels of TNF- $\alpha$  and different clinical, radiographic, and pathohistological features of AP (15-19). Although some studies reported that the levels of TNF- $\alpha$  were elevated in larger in comparison with smaller lesions (15), and in the case of radicular cysts compared to periapical granulomas (16), there were no significant differences between different clinical presentations of analyzed AP (17-19).

#### *Interleukin – 1 beta*

Interleukin – 1 beta (IL-1 $\beta$ ), which is also known as a leukocytic pyrogen, lymphocyte activating factor, leukocytic endogenous mediator, and mononuclear cell factor, represents a proinflammatory cytokine that is encoded by the *IL1B* gene in humans (20, 21). This cytokine serves as a salient mediator in the inflammatory response and has a role in various cellular activities, including cell proliferation, differentiation, and apoptosis (20, 21). Earlier *in vitro* studies

demonstrated that inflammatory cells from symptomatic AP lesions, which harbored a higher percentage of granulocytes, produced elevated concentrations of IL-1 $\beta$  in comparison with asymptomatic lesions (9). In another *in vitro* study Artese et al. (10) observed IL-1 $\beta$  positive cells were present in human periapical granulomas to a small extent, and the morphology of positive cells corresponded to monocytes/macrophages. Moreover, some animal studies also examined the role of IL-1 $\beta$  in alveolar bone resorption (22, 23). In 1995, Hamachi and co-workers demonstrated cells expressing IL-1 $\beta$  mRNA by *in situ* hybridization in periapical lesions in rats (22). They concluded that macrophages could play a role in IL-1 $\beta$  production and that they could significantly contribute to activating osteoclastic bone resorption in AP (22). This was also confirmed by Matsumoto *et al.* (23) who showed that that macrophages expressing IL-1 $\beta$  might have a considerable influence on the activation and recrudescence of osteoclastic bone resorption in an AP rat model. In 1992, for the first time, IL-1 $\beta$  was detected in human AP lesions (24). An IL-1 $\beta$  enzyme-linked immunosorbent assay (ELISA), which relied on monoclonal antibodies specific for IL-1 $\beta$ , was used to measure its activity. . In this study, AP samples demonstrated a considerable activity of IL-1 $\beta$  whereas healthy pulp tissue had no activity (24). The authors concluded that IL-1 $\beta$  is locally generated and released in inflammatory AP lesions and that it probably mediates alveolar bone loss (24). More recent studies investigated the correlation between IL-1 $\beta$  concentrations in human AP lesions and their clinical, radiographic and histopathological presentation (17, 19, 25, 26). Jakovljevic *et al.* (19) reported significantly elevated IL-1 $\beta$  concentrations in symptomatic lesions compared to asymptomatic lesions and control tissue samples (19). This is in line with previous investigations that also observed increased IL-1 $\beta$  concentrations symptomatic lesions exudates (25, 26). Moreover, Jakovljevic *et al.* (19) revealed that IL-1 $\beta$  levels were significantly increased in radicular cysts in comparison with periapical granulomas. These results were partly in line with the investigation performed by Ataoğlu *et al.* (17) who also noted a significant increase of IL-1 $\beta$  levels in canals with larger compared to

those with smaller radiolucent areas. Based on the reported data, it can be established that AP development is in close association with IL-1 $\beta$  expression and that IL-1 $\beta$  represents a powerful bone-resorptive cytokine that triggers osteoclast formation and activation (27).

### *Interleukin-6*

Interleukin-6 (IL-6) can act as both pro- and anti-inflammatory cytokine (28). This interleukin is thought to act like a hormone that mobilizes extracellular substances and/or alters substrate delivery during physical activity. In addition, it is generated in the body on the site of either acute or chronic inflammation and may act as a pyrogen that can cause fever in autoimmune, infectious, or non-infectious diseases (28). Several *in vitro* studies investigated how IL-6 affects AP pathogenesis (9, 29). Namely, IL-6 has been detected in AP and IL-6 concentrations are proportional to the size of periapical lesions. Gazivoda *et al.* (9) reported that inflammatory cells from symptomatic and large-size lesions secreted higher concentrations of IL-6 compared to asymptomatic and small-size AP lesions. It is also important to stress that neutrophils and macrophages present in AP lesions can secrete IL-6 *in vitro* after different bacterial stimuli. Thus, Matsushita *et al.* (29) showed that *Prevotella melaninogenica* and *P. gingivalis* may be involved in the pathogenesis of AP by increasing levels of IL-6. In 1999, IL-6 was detected in experimentally induced murine AP lesions (30). Thereafter, several animal investigations confirmed its role in the development of AP (31, 32). Huang *et al.* (31) have reported that it took far less time for large AP lesions to develop in mice in whom IL-6 deletion was detected than in healthy one. On the other hand, it has been noted that the increased bone resorption in IL-6-deficient animals was in a correlation with an increase in osteoclast numbers and elevated expression of other bone-resorptive cytokines in AP lesions (32). IL-6 in human AP lesions was first described in an investigation by Barkhodar *et al.* (33). The authors reported the mean IL-6 concentrations were significantly higher in AP lesions when compared to healthy pulp tissue (33). Moreover, several clinical investigations observed significantly increased IL-6 concentrations



in symptomatic lesions in comparison with asymptomatic lesions and the control group (18, 19, 34). Furthermore, a different investigation showed that IL-6 could be potentially used as a marker of pathologic inflammatory activities in chronic AP lesions (35). Therefore, it could be implied that pro-inflammatory cytokines are strongly involved in AP development and alveolar bone resorption.

#### *Bone resorption regulators*

Molecular mechanisms involved in alveolar bone loss in AP are regulated by the interaction of a group of molecules entitled bone resorption regulators (36-38). This group of molecules is presented by: receptor activator of NF- $\kappa$ B ligand (RANKL), its cellular receptor – RANK, and the decoy receptor osteoprotegerin (OPG) (36-38). They belong to the TNF receptor and ligand superfamilies (36-38). Initially, RANKL was recognized as a cell membrane-bound ligand capable of triggering osteoclast formation and bone resorption. Presently, it has been acknowledged that RANKL production can be attributed to different cells, including osteoblasts, fibroblasts, and activated T and B lymphocytes (36-38). By binding to its receptor which is found on the surface of precursor cells, i.e. the cells belonging to the lineage of monocytes/macrophages, RANKL initiates their transformation into fully developed osteoclasts (36-38). Besides, OPG acts as a soluble decoy receptor that interferes with RANKL, prevents it from binding with RANK, and thus inhibits osteoclast activation (36-38). Different cells produce RANKL and OPG and this is regulated by both systemic and local stimuli. These include cytokines from IL-1 family, virulence factors of different microorganisms, etc. (36-38). In 2005, Zhang and Peng examined the presence of RANKL in periapical areas and its role in alveolar bone loss using a rat model (39). In this study, osteoclast-like cells, which were identified owing to their tartrate-resistant acid phosphatase (TRAP) positivity, and cells positive for RANKL were found in the periapical region as early as one week after exposing the dental pulp. By the end of the second week, a notable increase in inflammatory cells was detected and bone resorption around the periapical area became evident. This was accompanied by a peak in

the number of cells positive for RANKL and osteoclast-like cells. Following a four-week exposure, even though chronic inflammation persisted, the levels of these osteoclast-like and RANKL positive cells returned to their initial values, and the periapical bone resorption rate decelerated (39). Thereafter, Kawashima *et al.* (40) confirmed these findings at the mRNA expression level. Namely, the authors reported that the expression of RANKL in the periapical area reached its highest levels following a pulp exposure of 2 to 3 weeks and remained elevated above the baseline values for up to 8 weeks. The expressions of RANK and OPG also increased, although not as prominently as RANKL. The increase in OPG expression was seen as a response that was supposed to counteract the effects of an abrupt rise in RANKL levels. The relative expression ratio of RANKL/OPG reached its peak after 3 weeks and remained high throughout the 8 weeks of observation, indicating considerable potential in terms of bone resorption. During the 2- to 3-week period, when the RANKL/OPG ratio was at its highest, the expression of pro-inflammatory cytokines like IL-1 $\alpha$ , IL-1 $\beta$ , and TNF- $\alpha$ , which can stimulate RANKL production, was also increased (40). In addition, Chuang *et al.* (41) also revealed that level of bone resorption regulators in animal experimental models can be stimulated by lipopolysaccharides from *Escherichia coli*. Following a 3-week exposure, the authors noticed a significant increase in RANKL expression, reaching a peak of 208% in comparison with the control group with unexposed pulp, by the end of the 8-week period. Moreover, the expression of OPG varied from week 1 to week 8; yet it consistently remained lower when compared to the control group (41). In 2004, Tay *et al.* (42) using immunohistochemistry, reported that RANKL was present in radicular cysts. The same findings were verified by Sabeti *et al.* (43) who showed an increased expression of RANKL in human AP lesions. Moreover, some investigations confirmed that the presence of RANKL to OPG predominant ratio was in periapical granulomas compared to radicular cysts (44, 45). In addition, recent investigations reported significant difference in RANKL and OPG expression between AP lesions with different clinical presentation suggesting that these molecules could serve

as discriminating biomarkers (46, 47). These findings are in accordance with Nikolic *et al.* (48) who reported that symptomatic AP lesions were more frequently detected in RANKL-predominant AP lesions than in OPG-predominant ones. The authors also revealed significant positive correlation between investigated pro-inflammatory cytokines and bone resorption regulators in AP lesions, suggesting their concomitant role in complex process of alveolar bone resorption (48).

### *Matrix metalloproteinases*

Matrix metalloproteinases (MMPs) represent a broad category of calcium dependent zinc containing endopeptidases which contribute to remodeling and degradation of extracellular matrix components. These proteolytic enzymes are also involved in different physiological and pathological processes regulated by hormones, growth factors and cytokines (49).

According to their substrate specificity and domain structure, MMPs can be classified into 6 groups (50): 1) collagenases (MMP-1, MMP-8 and MMP-13, mostly digesting collagen types I, II, III, soluble proteins and extracellular matrix components); 2) gelatinases (MMP-2 and MMP-9, cleaving collagen types IV, V, XI, laminin); 3) stromelysins (MMP-3, MMP-10 and MMP-11, whose characteristics are similar to those of collagenases, but not to degrading interstitial collagen); 4) matrilysins (MMP-7 and MMP-26, interacting with cell surface proteins); 5) membrane-type MMPs (MMP-14, with collagenolytic action) and 6) others (MMP-12).

The domain structure of MMPs is common. There are four such domains and they include the signal domain, pro-peptide, catalytic domain, and hemopexin-like C terminal domain, which is connected to the catalytic domain by a flexible hinge region. Under normal physiological conditions, the catalytic activity of MMPs is closely monitored and this is achieved at four different levels : 1) gene expression with transcriptional and post-transcriptional regulation; 2) extracellular localisation and tissue or cell type of MMP release, called compartmentalization; 3) pro-enzyme activation by

pro-domain removal and 4) inhibition by specific inhibitors, i.e. tissue inhibitors of matrix metalloproteinases (TIMPs), and by non-specific proteinase inhibitors (e.g.  $\alpha$ 2-macroglobulin) (51). MMPs, providing they have become active, can modulate the global proteolytic potential in the extracellular milieu through zymogen (i.e. MMP pro-form) activation and inhibitor degradation or inactivation of other proteases (49, 51). MMPs and TIMPs are normally expressed in low concentrations under physiological conditions. Nevertheless, it has been noted that during the developmental stages of various human diseases, diverse types of MMPs are overexpressed in particular tissues and distinct processes, such as malignancy and inflammation (49, 51). Alveolar bone resorption in AP is encompassed by MMPs as one of their modulators (49, 52). Breakdown of the extracellular matrix by MMPs is initiated and even further enhanced by low pH level caused by endodontic pathogens (49, 52 - 54). A number of *in vitro* studies investigated MMPs production by human pulp cells cultures (55-57). Panagakos *et al.* (55) treated pulp cells of humans and pulp cells RPC-C2A of clonal rats with IL-1 $\alpha$ , IL-1 $\beta$ , TNF- $\alpha$ , and LPS for 24 hours, while conditioned medium and cell lysates were gathered and examined by gelatin zymography method. The authors revealed that pulp cells of humans that had been treated with either cytokines or LPS's did not demonstrate any changes concerning the MMP pattern that was generated or secreted in either cellular or conditioned medium fractions (55). A few years later, O'Boskey and Panagakos (56) investigated MMP production by human pulp cells both in the presence and absence of IL-1 $\alpha$  and TNF- $\alpha$  in long-term cultures (2 to 16 days) applying the same method. This time, the authors concluded that MMP production in human pulp cells in long-term cultures is stimulated by cytokines. Another conclusion implied that these MMPs could contribute to pulp inflammation (56). Thereafter, it has been reported that MMPs are also produced by mast cells of human AP lesions (57). Further experiments on animals showed a moderate expression of MMP-2 and MMP-9 during the chronic stage of the AP lesion in rats suggesting that a decreasing number of polymorphonuclear cells during

the chronic stage may interfere with IL-1 $\alpha$  and IL-6 expression (58). Based on these findings the authors concluded that MMP-2 and MMP-9 have an essential role regarding the development of AP lesions. This could be accounted by extracellular matrix degradation that occurs during the beginning stage of lesion development (58). Having explored the levels of different MMPs and their tissue inhibitors in the process of AP lesion development, Wan *et al.* (59) reported that the MMP-1, MMP-2, MMP-9, TIMP-1, and TIMP-2 mRNA and protein expression values rose in the acute and chronic phases of AP lesions, with the values of MMP-2 and MMP-9 expression being lower during the chronic phase, which supports previous results. In 2002, Shin *et al.* (60) using the ELISA method showed that MMP-3 values were significantly higher in the periapical lesion than in healthy pulps. Further study demonstrated that MMP-13 expression pattern contributes to a periapical granuloma with epithelium transforming into a radicular cyst (61). Martinho *et al.* (62) investigated the significance of MMPs and TIMPs in clinical settings with regard to AP lesions. The authors reported that in teeth with larger-size radiolucent lesions higher mean values of MMP1, -2, and -9 were recorded in comparison with the smaller ones. They also elucidated the association of MMP-9 with higher risk of pain on palpation, while MMP-1 was correlated with lower chance of tenderness to percussion (62). Investigating the clinical relevance of MMPs and their tissue inhibitors Letra *et al.* (63) showed that significantly higher TIMP-1 was observed in asymptomatic AP cases than in the cases with a chronic apical abscess, while, in turn, the cases with a chronic apical abscess demonstrated higher MMP-2, MMP-7, and MMP-9 mRNA values. Finally, Hadziabdic *et al.* (64) found no significant difference in the mRNA expression of MMP-1, MMP-2, TIMP-1, and TIMP-2 between periapical granulomas and radicular cysts. On the other hand, Pereira Faustino *et al.* (65) reported that in periapical granuloma and in cases associated with pain MMP-2 expression is increased. All these data stress the significance of MMPs and their tissue inhibitors in relation to AP pathogenesis.

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### *Oxidative stress*

Reactive oxygen species (ROS) represent highly reactive by-products of oxygen metabolism, serving as essential signaling molecules in various cellular processes (66). ROS, including oxygen-derived free radicals (e.g. superoxide ( $O_2^-$ ) and hydroxyl anion ( $\bullet OH$ )), as along with non-radicals that can easily convert into radicals (e.g. nitric oxide ( $NO\bullet$ ), hydrogen peroxide ( $H_2O_2$ ), and hypochlorous acid ( $HOCl$ )), have a key role in cell signaling, metabolism, and the regulation of cellular functions, e.g., in gene expression, proliferation, cell death, migration, and inflammation (67-70). In normal cellular physiology, ROS and antioxidant mechanisms maintain a delicate balance to sustain physiological processes. However, during inflammation, there is an excessive production of ROS that overwhelms the available antioxidants, leading to oxidative stress (OS) (66). OS is defined as a disturbance in the pro-oxidant-antioxidant balance in favor of the former, resulting in disruptions to redox signaling and possible molecular damage (71). Those alterations in oxidative metabolism are often referred to as the “respiratory burst” (72).

It has been reported that OS could be a contributing factor in a few chronic inflammatory diseases, including cancer, rheumatoid arthritis, diabetes, atherosclerosis, etc. (73). Additionally, recent investigations indicated its role in the pathophysiology of AP (74). Frazão and co-workers (75) investigated whether AP alters systemic values of the antioxidant and pro-oxidant parameters in different periods in Wistar rats. The authors revealed that glutathione (GSH) and Trolox equivalent antioxidant capacity (TEAC) were increased after 14 days and lipid peroxidation (TBARS) was significantly elevated following 28 days of AP induction. Thus, they concluded that the oxidative biochemistry response was modulated based on the progression of alveolar bone resorption (75). Moreover, several studies explored the influence of AP on OS parameters in animals with general health impairment (76-78). Prieto *et al.* (76) revealed that uric acid, and malondialdehyde (MDA)

levels, as well as inflammatory infiltration were more increased in the periapical region of diabetic rats with induced AP than in rats with AP but without diabetes mellitus (DM) suggesting that DM may change the antioxidant status. Also, Milojevic Samanovic *et al.* (77) conducted an investigation involving rats which showed a correlation between AP and impaired cardiodynamics, disturbed cardiac OS, antioxidant defense, and cardiac pathologic alterations in the conditions of hypertension. Finally, Tsosura *et al.* (78) presented that maternal AP modulates the antioxidant defense system (i.e. attenuating lipid peroxidation) in the investigated tissues of their adult offspring. These results suggest that a maternal chronic oral inflammatory process may aggravate the damage of oxidative tissue in their offspring during the postnatal phase.

In parallel, numerous studies in humans have been conducted to investigate the impact of OS on AP (66). These investigations have explored OS both systemically in blood and saliva (79 - 83) and locally in GCF and root canal contents (83, 84).

Another recent investigation conducted by Cotti *et al.* (79) explored the role of ROS in the pathogenesis of AP systematically in blood. Their findings not only revealed significantly elevated ROS levels in AP patients overall compared to healthy patients, but also highlighted a notably greater increase in ROS levels among female patients (79). This observation aligns with the outcomes of a separate study that investigated potential sex differences in prooxidant and antioxidant status (85). In study performed by Miller *et al.* (86) it was proposed the existence of sex-dependent variations in both the production and metabolic deactivation of ROS. In the pioneering study by Vengerfeldt *et al.* (83) that comprehensively investigated both local and systemic levels of OS in various endodontic pathologies, it was established that OS not only serves as a crucial pathogenetic mechanism in several endodontic conditions including AP, but also exhibits a significant association with certain clinical indicators, including pain and bone destruction (83). Similar results, indicating alveolar bone loss, were obtained by Dezerega *et al.* (84) which examined ROS in both, GCF and AP tissue. This study



revealed an imbalance favoring ROS in both apical lesions and GCF from AP-affected teeth when compared to healthy controls and teeth that underwent endodontic treatment (84), once again establishing a clear connection between ROS and degradation of extracellular matrix components that occurs in connective tissue during inflammatory conditions (87). Furthermore, the study found a positive association between the Total Oxidant Status (TOS) and the extent of bone resorption, as well as a negative association between TOS and Total Antioxidant Status (TAS) in AP lesions, which was not the case in healthy periodontal ligaments (PDL). Such an imbalance favoring ROS could potentially encourage the loss of alveolar bone. Latest findings also suggest that ROS may contribute to osteolysis by inhibiting bone formation by means of suppressing osteoblastic differentiation and through promoting osteoclast differentiation and bone resorption (88), primarily by inducing the RANKL. Additionally, increased MMP-2 expression and activation in response to ROS have been reported highlighting a critical association between the production of ROS, MMP-mediated proteolysis, and bone resorption, which may have a vital role when it comes to the progression of apical lesions (89, 90).

The evidence presented underscores the pivotal role of ROS in the intricate cascade of immunologic responses, highlighting their significance in both the initiation and perpetuation of inflammatory reactions. Notably, phagocytic cells produce ROS in response to bacterial pathogens, which serves as a critical host defense mechanism (74). AP, with its microbial etiology, including viruses (91) further accentuates the relevance of ROS. Certain endodontic pathogens, e.g., *E. faecalis* and/or *Epstein-Bar virus*, have been identified as inducers of ROS production (92) and certain microorganisms are also capable of independently generating ROS (93). Consequently, locally produced ROS in the context of AP can emanate from either human or microbial sources. Nevertheless, the current limitations prevent the definitive discrimination between the origins of these ROS. It is important to stress that AP can also influence both local and systemic antioxidant activities.



Markers assessing antioxidant status in the blood have shown significantly lower levels in individuals with AP compared to healthy control groups (94). Inchingolo *et al.* (95) reported that, in comparison with the posttreatment values, higher concentrations of blood ROS and lower blood antioxidant levels were recorded prior to treatment.

In light of the aforementioned considerations, a potential strategy to disrupt this cycle of oxidative stress and inflammation lies in the systemic administration of antioxidants. Insights from animal studies suggest that systemic antioxidants, such as vitamin C, have the capacity to mitigate oxidative stress triggered by injuries (96). Beyond their role in pain reduction, antioxidants may also serve as a valuable tool for enhancing and expediting the scale of the inflammatory response. Specifically, once the microbial infection within the root canals has been effectively eradicated, the administration of antioxidants could potentially contribute to fostering the resolution of AP.

### **Conclusion**

This narrative review presents state-of-the-art related to the role of pro-inflammatory cytokines, bone resorption regulators, matrix metalloproteinases and oxidative stress parameters concerning alveolar bone resorption in apical periodontitis. It is evident that all of these molecules make a complex interrelationship network, triggered by different stimuli (mostly by different microorganisms), induce osteoclastogenesis and promote alveolar bone loss. Interaction between inflammatory mediators and activated cells is regulated by different molecular pathways. The potential clinical implications of these findings may be further explored in future investigations. These studies could examine the effects of inhibiting these mediators and their associated pathways in the context of preventing alveolar bone loss. Acquiring knowledge about all these processes will allow scientists to understand the pathogenesis of apical periodontitis and therefore guide and shape future investigations in this field.

**Ethics Committee Approval:** Not required.

**Informed Consent:** Not required.

**Peer-review:** This invited review article has not undergone formal peer review; however, it has been evaluated by the Endodontics section editor, Prof. Dr. Meriç Karapınar Kazandağ.

**Author contributions:** NM, EKL, LL, DP, MM, AJ participated in designing the study. NM, EKL, LL, DP, MM, AJ participated in generating the data for the study. NM, EKL, LL, DP, MM, AJ participated in gathering the data for the study. NM, EKL, LL, DP, MM, AJ participated in the analysis of the data. NM, EKL, LL, DP, MM, AJ wrote the majority of the original draft of the paper. NM, EKL, LL, DP, MM, AJ participated in writing the paper. NM, EKL, LL, DP, MM, AJ has had access to all of the raw data of the study. NM, EKL, LL, DP, MM, AJ has reviewed the pertinent raw data on which the results and conclusions of this study are based. NM, EKL, LL, DP, MM, AJ have approved the final version of this paper. NM, EKL, LL, DP, MM, AJ guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

**Conflict of interest:** The authors declared that they have no conflict of interest.

**Financial disclosure:** The authors declared that they have received no financial support.

**Acknowledgments:** The authors thank to Irena Hajdukovic Aleksic for improving the English language of the manuscript.

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