ETIOPATHOGENIC MOLECULAR MECHANISMS IN THE DEVELOPMENT OF ENDO-PERIODONTAL LESIONS

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Abstract

Understanding the interaction between endodontic and periodontal lesions is of crucial importance to the clinician because of the challenges commonly encountered in the evaluation, diagnosis, treatment and prognosis of these combined diseases. Etiological factors (such as microorganisms) as well as factors contributing to the onset and development of endo-periodontal lesions (such as trauma, root resorption, perforations, fractures and dental malformations) play a role in the development and progression of such diseases. The treatment and prognosis of endo-periodontal lesions varies according to the etiology, pathogenesis and the correct recognition of each specific disease. Various molecular mechanisms underlie the onset and development of endo-periodontal lesions as a result of the host's response to molecular aggression; the inflammatory response involves the recruitment and activation of leukocytes, both non-specific and specific immune responses, with resulting osteoclastogenesis and the formation of an osteolytic lesion at the root apex. Knowing the interdependence between endodontic and periodontal diseases will enhance the clinician's ability to establish the correct diagnosis, assess the prognosis of the teeth involved, and select a treatment plan based on biological and clinical evidence.

Endo-periodontal interrelation is a unique one and can be considered a single continuous system or biological unit in which there are many ways of communication. The interdependence of these structures influences each other's health, function and disease. They can be affected individually or combined; when both systems are involved, the lesions are called true endo-periodontal syndromes. Endo-periodontal problems are responsible for more than 50% of dental mortality. They present challenges for the clinician regarding the diagnosis and prognosis of the teeth involved. It is very important to have a proper diagnosis so that appropriate treatment can be provided. The relationship between periodontium and dental pulp was first discovered by Simring and Goldberg in 1964. Since then, the term "endo-periodontal lesion" has been used to describe lesions due to inflammatory products found to varying degrees in both the periodontal tissues, and in the pulp [1].

The main etiological factors for endo-periodontal lesions are living agents (bacteria, fungi and viruses) and inert pathogens. Together with these, many factors such as trauma, root resorption, perforations and dental malformations also play an important role in the development and progression of such lesions [2] (Figure 1). The condition of the dental pulp is an important factor in susceptibility to microbial invasion. A vital pulp is very resistant to the microbial invasion. The penetration of the healthy pulp surface by the oral bacteria is relatively slow or can be completely blocked. Instead, a necrotic

pulp is rapidly invaded and colonized by bacteria. When pulp becomes necrotic, inflammatory products of pulp origin can disseminate through these pathways and initiate / trigger an inflammatory vascular response in periodontium, cause the destruction of periodontal tissue fibres, adjacent alveolar bone and cement resorption. The nature and extent of periodontal destruction depend on various factors such as the virulence of the microorganisms, the duration of the disease, and the host defence mechanism. Similarly, the effect of the necrotic pulp on the periodontal ligament was called retrograde pulpitis [3].

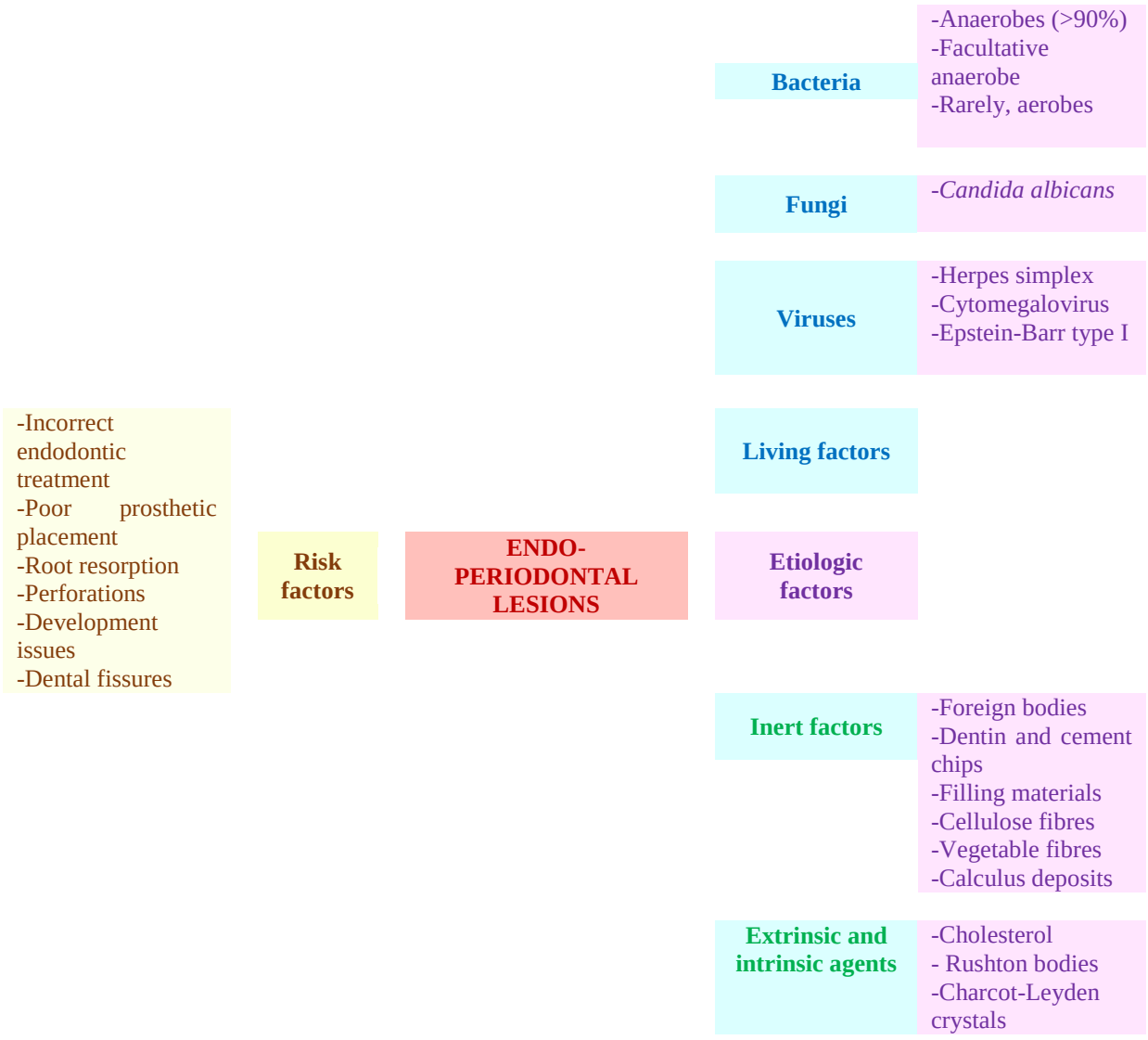

Figure 1. Etiologic and risk factors in the apparition and development of endo-periodontal lesions

In the last century, literature has consistently presented a controversy over the effect of periodontal disease on dental pulp. It has been found that the pulp has a rather complex vascular system with a capillary bed network, precapillary sphincter and arterio-venous shunts that provide significant capacity for pulp survival. From clinical observations, it is rare to find an intact tooth (without caries,

restorations, fracture, perforation) with the presence of a periapical pathology for which the cause of necrosis of the pulp can not be determined. Many studies have shown that periodontal disease or sequelae of periodontal treatment does not affect the pulp. On the other hand, studies have suggested that the effect of periodontal disease on the pulp is atrophic and degenerative in nature, including a decrease in pulp cell counts, an increase in dystrophic calcification, fibrosis, and direct inflammatory disorders [1]. Therefore, periodontal diseases and periodontal treatments should be considered as potential causes of pulpitis and pulp necrosis. However, it was claimed that periodontal disease had no effect on the pulp, unless it extends to the tooth apex, the pulp is able to survive significant aggression, and that the effects of periodontal disease and periodontal treatment on dental pulp are negligible [3].

The first classification of endo-periodontal lesions based on the pathology of origin was proposed by Simon et al. (1972), as follows: (1) endodontic primary lesions, (2) periodontal primary lesions, (3) primary endodontic lesions with secondary periodontal involvement, (4) primary periodontal lesions with secondary endodontic involvement and (5) combined real injuries.

Although Simon et al. have classified these injuries into five types, in fact, lesions of third, fourth and fifth type may be considered combined lesions. There have been numerous classifications suggested by several other authors, such as "independent periodontal and endodontic lesions" [4] or "concomitant pulp and periodontal lesions" [5] to describe endo-periodontal lesions.

There are numerous classifications for endo-periodontal lesions, but for differential diagnosis purposes, these lesions are best classified as endodontic, periodontal, or combined [6]. These lesions can also be classified according to the need for endodontic, periodontal or combined treatment. All of these classifications are mainly based on the theoretical pathways explaining how these radiographic lesions are formed. Therefore, through a comprehensive understanding of pathogenesis and investigations, the clinician can make a solid diagnosis, formulate a proper treatment plan, and evaluate the prognosis of these lesions.

Endodontic lesions usually develop through the exposure of pulp tissue to oral bacteria due to deficiencies in the integrity of a tooth. This can result from carious lesions that dissolve mineralized dental tissue, fractures of the dental structure, and iatrogenic circumstances and other circumstances that allow bacteria to penetrate into the pulp tissue. In most cases, these events lead to infection in the dental pulp, which causes the development of inflammation that spreads from the exposed area [7]. Inflammation is often followed by pulp tissue necrosis, leading to chronic infections, tooth inflammation spread and bone resorption. The inflammatory response involves the recruitment and activation of leukocytes, both non-specific and specific immune responses, with resulting osteoclastogenesis and the formation of an osteolytic lesion at the root apex.

Inflammation and bone resorption at the apical level, in most cases, is a consequence of the interaction between the microbial infection and the host response. The critical role of bacteria in the development of periapical lesions was demonstrated by mechanical exposure of dental pulp to the oral cavity of germ-free animals. In these animals, the pulp exposure is healed with an initial or transient inflammatory response in pulp tissue, followed by a reparative response from the pulp cells and leading to the

formation of a new dentin-like matrix linking to the exposed site. In contrast, mechanical pulp exposure in animals with normal oral bacteria causes an infection of the dental pulp, pulp tissue necrosis and chronic infection that prevents the repair process. The infection persists because necrotic tissue of dental pulp is inaccessible to leukocytes and therefore is a protected bacterium reservoir [8]. Chronic inflammation stimulated by bacteria and their products in the periapical area of the tooth leads to localized bone resorption, which is "decoupled", so there is no bone repair without treatment. The result consists in the formation and development of granulomas or cysts in apical tissues [9].

Understanding the pathogenic mechanisms underlying the development of endodontic lesions is confused with the persistence of a "bacterial reservoir" that exists in the pulpal canal and necrotic tissue. Bacterial presence stimulates an inflammatory response to resist infection. During this response, a number of cell types exhibit release cytokines, chemokines, leukotrienes and prostaglandins in the area. These inflammatory mediators strengthen the recruitment of polymorphonuclear leukocytes (PMN) and other leukocytes, creating an interesting dichotomy of activity and consequences in terms of essential protective or destructive roles [10].

As expected, the host response plays a critical and protective role in endodontic lesions in limiting the spread of infection. Consistent with this expectation, specific inhibitors of inflammatory cytokines tend to lead to the formation of higher osteolytic lesions, as they compromise the host's ability to protect itself from the necrotic pulp bacteria reservoir. This increase in lesion size occurs even though blocked inflammatory cytokines also play an important role in osteoclastogenesis. The use of inhibitors or targeted deletion mice can not necessarily disclose the role of a cytokine or a particular cell type that plays an important role in the activation of osteoclastogenesis because its inhibition or knockout may also increase susceptibility to bacterial infections. If the impact on resistant infection is greater, the greater lesion will be produced even if the direct effect on deletion or inhibition should reduce osteoclast formation. The reverse is also true. For example, an increased response of the host in an animal model with periapical endodontic lesions demonstrates an increased number of PMNs and monocytes with a reduction in apical bone resorption, even if the response of the host can contribute to bone resorption. In another example, the elimination of tumour necrosis factor (TNF) or IL-1 receptor signalling results in greater osteoclast lesion formation even though both cytokines stimulate bone resorption. This is because elimination of the signalling of TNF or IL-1 affects the antibacterial activity of the host response that is critical to endodontic lesions. In particular, it is necessary to signal the IL-1 receptor to prevent the spread of necrotic pulp infection and to protect the host from the significant morbidity and mortality that would result. Thus, there is considerable complexity in examining the impact of cytokine signalling, as cytokines have both destructive roles and an important protective function in antibacterial defence [11].

Periapical infection control appears to be a critical aspect of this process because the absence of the inducible pleoapropic nitric oxide synthase (iNOS) also leads to higher lesions with the recruitment of a greater number of inflammatory cells and frequently associated with the development of periapical abscesses [12]. It contrasts with periodontal disease in which there is no

protected bacterial reservoir and the use of inhibitors or targeted directed deletions of the host response typically does not sufficiently compromise antibacterial protection. Thus, lesions of endodontic origin appear to have an increased susceptibility to bacterial infection by inhibiting the host response in contrast to periodontal disease.

As a general rule, bacteria that can cause periodontitis have been classically identified as gram-negative anaerobic bacteria that survive in the gingival sulcus, the space between the tooth surface and the adjacent gingival epithelium [13]. Particular attention has been paid to *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*, which have been linked to various forms of periodontitis [14]. However, approaches to bacterial identification have suggested that a pathogen re-assessment is warranted. The presence of periodontal pathogens is necessary, but is not sufficient to initiate the disease. In fact, studies clearly demonstrate that cytokines induced by the host response play a critical role in the destruction of periodontal tissue [15]. Host-microbial interactions begin in the gingival epithelium and stimulate an inflammatory response that provides effective protection against bacteria.

However, the release of the host mediators results in a clinical outcome of the onset of gingivitis. Since gingival epithelium and underlying connective tissue are chronically exposed to bacteria or their products, both non-specific and specific immune responses are activated in connective tissue adjacent to the epithelium covering the gingival tissues. In most cases, tissue destruction caused by activation of the host response is reversible and associated with gingivitis. On the other hand, under certain conditions that are not fully understood, the disease can progress and cause the attachment of the connective tissue of the gingiva to the surface of the teeth and tooth to bone. Indeed, periodontitis differs from gingivitis by the irreversible nature of the loss of attachment. One of the most important uncertainties about periodontitis is its chronic nature. Periodontitis can be a series of short aggressions or "explosions" that accumulate and appear to be chronic over time with long periods of remission. However, the duration of exacerbation is unknown. Alternatively, there may be constant stimulation over time, but it is not known how long the chronic destructive period lasts. Despite the evidence for both models [16], the nature of the progression of periodontal disease remains uncertain. This problem has affected studies in human subjects because it is difficult to know if a person is suffering from an active periodontal destruction at some point in time. Moreover, the relative absence of longitudinal studies has made it difficult to interpret the results with human patients, because relationships between a certain variable and irreversible periodontal destruction are difficult to establish in transversal studies.

Animal models have established a clear causal relationship between bacteria and periodontitis. In an animal model, a ligature is made around the teeth, allowing bacterial plaque accumulation and bacterial penetration, resulting in subsequent inflammation and alveolar bone resorption. In fact, gnotobiotic rats treated identically do not exhibit bone periodontal loss [17], demonstrating the essential role of bacteria in this model. Additional evidence is provided by antibiotic treatment or topical application of antimicrobial agents that reduce bone resorption in the ligation model while increasing colonization by gram negative bacteria enhances bone resorption. In other animal models, inoculation of periodontal pathogens into the rodent's oral cavity induces bone loss.

In several studies, the introduction of *P. gingivalis* by oral rinsing stimulates alveolar bone resorption [18]. Similarly, the introduction of *A. actinomycetemcomitans* into rodents leads to colonization and loss of alveolar bone. Thus, experiments on animal models support human studies demonstrating the role of bacteria in triggering inflammation and periodontitis.

Since the presence of bacteria is necessary but is not sufficient to trigger the development of periodontitis, recognition of microbial components as "signalling" by host cells and subsequent production of inflammatory mediators is an essential step in the pathogenesis of periodontitis. Indeed, one of the critical components of host response to bacteria or their products is a family of receptors called Toll-like receptors (TLRs). TLR activates the binding of the non-specific immune response to various microbial components (i.e., diacyl lipopeptide, peptidoglycan, LPS, flagelin, bacterial DNA, etc.) [19].

After activation of the TLR, an intracellular signalling cascade leads to the activation of transcription factors such as nuclear factor k-B (NF-κB), activator-1 protein (AP-1) and subsequent production of various cytokines and chemokines. Studies describe a role for both TLR-2 and TLR-4 in the recognition of *A. actinomycetemcomitans*, the incidence of which ranges from stimulation of inflammatory cytokine expression and inflammatory cell migration to induction of osteoclastogenesis and alveolar bone loss [20]. In addition to TLR, nucleotide linker oligomerization (NOD) receptors and inflammatory systems have been identified as potential accessory molecules that trigger the host response to periodontal pathogens [21].

Animal models have also provided clear initial evidence for the role of inflammatory factors of host immunity in the progression of periodontal disease. When the response of the host is modified by treatment with specific inflammatory inhibitors or genetic manipulation, the severity of periodontal connective tissue loss and bone loss stimulated by periodontal bacteria is clearly reduced. The first clear evidence that inhibition of an inflammatory response reduces periodontal disease was performed in a canine model [17], where inhibition of prostaglandins significantly reduced the loss of alveolar bone. Subsequent studies have used a number of techniques to demonstrate that cytokines play an important role in periodontitis.

Non-human primates treated with inhibitors of two major proinflammatory cytokines, IL-1, IL-17 and TNF, exhibit reduced periodontal bone loss and loss of attachment compared to control animals [22, 23]. Similarly, RANKL inhibition reduces alveolar bone loss in several periodontal disease models [24]. It is estimated that the progression of periodontal disease is due to a combination of several factors, including the presence of periodontal pathogenic bacteria, high levels of cytokines and proinflammatory prostaglandins, the production and activation of MMP and RANKL and relatively low levels of interleukin-10 (IL-10) transformer-b (TGF-b), metalloproteinase tissue inhibitors (TIMP) and osteoprotegrin (OPG) [18].

Thus, polymicrobial infection in lesions of endodontic origin stimulates bone resorption by interacting with non-specific and specific immune response leukocytes. In endodontic lesions, the presence of inflammation suppresses bone formation so that lesion resolution does not occur until causal bacteria are eliminated by treatment and inflammation disappears. Periodontitis is caused by the host's response to the presence of bacteria or their invading connective tissue products. Therefore, in the case of patients

affected by endo-periodontal lesions, a correct diagnosis is absolutely necessary, with the establishment of an adequate, complete and complex treatment plan involving the interdisciplinary collaboration, but also a profound knowledge of the mechanisms underlying the occurrence and evolution of such lesions.

References

1. Parolia A, Gait TC, Porto IC, Mala K. Endo-perio lesion: A dilemma from 19th until 21st century. J Interdiscip Dentistry 2013; 3:2-11.
2. Sunitha VR, et al. The periodontal-endodontic continuum: A review. J Conserv Dent 2008; 11:54-62.
3. Rotstein I. Interaction between endodontics and periodontics. Periodontol 2000 2017; 74: 11–39.
4. Harrington GW, Steiner DR. Periodontal-endodontic considerations. In: Walton RE, Torabinejad M, editors. Principles and Practice of Endodontics. 3rd ed. Philadelphia: Saunders Co.; 2002.
5. Wang HL, Glickman GN. Endodotic and periodontic interelationships. In: Cohen S, Burns RC, editors., Pathways of the Pulp. 8th ed. St. Louis: Mosby; 2002. p. 651-664.
6. Rotstein I, Simon JH. The endo-perio lesion: A critical appraisal of the disease condition. Endod Top 2006; 13:34-56.
7. Zaharescu A et al. Quantification of proinflammatory molecules (IL1-α, IL1-β, IL2, IL12, IFN-γ, TNF-α) in crevicular fluid and serum in patients with endo-periodontal lesions. Rev Chim (Bucharest) 2019; 70:2252-2255.
8. Ricucci D, Siqueira JF Jr, Bate AL, Pitt Ford TR. Histologic investigation of root canal-treated teeth with apical periodontitis: a retrospective study from twenty-four patients. J Endod 2009; 35: 493-502.
9. Liapatas S, Nakou M, Rontogianni D. Inflammatory infiltrate of chronic periradicular lesions: an immunohistochemical study. Int Endod J 2003; 36: 464-471.
10. Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med 2004; 15: 348-381.
11. Graves D, Chen C, Douville C, Jiang Y. Interleukin-1 receptor signaling rather than that of tumor necrosis factor is critical in protecting the host from the severe consequences of a polymicrobe anaerobic infection. Infect Immun 2000; 8: 4746-4751.
12. Fukada SY et al. Factors involved in the T helper type 1 and type 2 cell commitment and osteoclast regulation in inflammatory apical diseases. Oral Microbiol Immunol 2009; 24: 25–31.
13. Haffajee AD, Teles RP, Socransky SS. The effect of periodontal therapy on the composition of the subgingival microbiota. Periodontol 2000 2006; 42: 219-258.
14. Fine DH, Kaplan JB, Kachlany SC, Schreiner HC. How we got attached to Actinobacillus actinomycetemcomitans: a model for infectious diseases. Periodontol 2000 2006; 42: 114-157.
15. Delima A, Spyros K, Amar S, Graves DT. Inflammation and tissue loss caused by periodontal pathogens is reduced by IL-1 antagonists. J Infect Dis 2002; 186: 511-516.
16. Gilthorpe MS, et al. Unification of the ‘‘burst’’ and ‘‘linear’’ theories of periodontal disease progression: a multilevel manifestation of the same phenomenon. J Dent Res 2003; 82: 200-205.
17. Graves DT, Oates T, Garlet GP. Review of Osteoimmunology and the Host Response in Endodontic and Periodontal Lesions. J Oral Microbiol 2011; 3: 5304.
18. Graves DT, et al. The use of rodent models to investigate host-bacteria interactions related to periodontal diseases. J Clin Periodontol 2008; 35: 89-105.
19. Mahanonda R, Pichyangkul S. Toll-like receptors and their role in periodontal health and disease. Periodontol 2000 2007; 43: 41-55.

20. Lima HR, et al. The essential role of TLR4 in the control of Aggregatibacter actinomycetemcomitans infection in mice. J Clin Periodontol 2010; 37: 248-254.
21. Uehara A, Takada H. Functional TLRs and NODs in human gingival fibroblasts. J Dent Res 2007; 86: 249-254.
22. Delima A, et al. Soluble antagonists to interleukin-1 (IL-1) and tumor necrosis factor (TNF) inhibits loss of tissue attachment in experimental periodontitis. J Clin Periodontol 2001; 28: 233-240.
23. H. Xiong , L. Wei & B. Peng The Presence and involvement of interleukin-17 in apical periodontitis. International Endodontic Journal, 2019; 52:1128–1137.
24. Jin Q, et al. RANKL inhibition through osteoprotegerin blocks bone loss in experimental periodontitis. J Periodontol 2007; 78: 1300-1308.