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Review

Periodontitis and mechanisms of cardiometabolic risk: Novel insights and future perspectives

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ABSTRACT

Periodontitis is an infectious and inflammatory disease of the tooth-supporting tissues caused by the accumulation of subgingival plaque and the action of specific periodontopathogenic bacteria. Periodontitis has been associated with cardiovascular diseases and considered a cardiovascular risk factor. Several mechanisms have been proposed to explain this association, such as the infection of atherosclerotic plaques by periodontal pathogens, the pro-atherogenic effect on the lipid profile, the systemic dissemination of pro-inflammatory mediators or the contribution to type 2 diabetes mellitus. Periodontal treatment has also been related to improvement in cardiometabolic risk variables, and oral hygiene techniques may be useful in reducing cardiometabolic risk. The aim of this review is to provide new and recent insights on the relationship between periodontitis and cardiometabolic risk, focusing on recent evidence. Comments on shared potential therapeutic targets, such as the role of glucagon-like peptide 1, are also highlighted.

1. Introduction

Periodontitis is an infectious and inflammatory disease that affects the tooth-supporting tissues, and exhibits a wide range of clinical, microbiological, and immunological manifestations. It is associated with and probably caused by a multifaceted dynamic interaction among specific subgingival microbes, host immune responses, hazardous environmental exposure, and genetic propensity [1]. A recent hypothesis about the pathogenesis of periodontitis states that the disease is initiated by a synergistic bacterial community, instead of specific pathogens. This community modulates the composition of the rest of the biofilm and can affect and be affected by the host response, generating a condition of dysbiosis in the periodontal environment. According to this model, certain species of this community, called “Keystone pathogens” [2], have essential roles in initiating and contributing to this dysbiosis. These species do not even require high levels of colonization in the biofilm. This hypothesis would explain the low counts (< 0.01% of the total bacterial count) of a main periodontopathogen, such as *Porphyromonas gingivalis (P. gingivalis)*, that have been reported in periodontitis in previous studies [3]. Recently, Archaea, viruses and protozoa have also been implicated as potentially playing a role in the pathogenesis of periodontitis. However, their role as periodontal pathogens still needs confirmation [4,5].

Periodontal inflammation is characterized by a chronic inflammatory infiltrate of varying intensity. This infiltrate is mainly composed of lymphocytes, plasmatic cells and macrophages distributed in patches on the lamina propria, frequently surrounding vascular structures [6]. Chronic inflammation causes the destruction of the connective tissue of the periodontal ligament, the resorption of the alveolar bone, causing mobility and eventually the loss of the tooth. The mean estimated alveolar bone destruction rate in millimeters is of 0.05–0.1 mm/year [7]. Periodontitis is considered as the main cause of tooth loss in people older than 40 years, having a higher prevalence than caries [8]. A high prevalence of periodontitis has been reported, with > 47% of adults (> 60 million) in the USA affected and the prevalence still grows every year [9,10].

Initially it was thought that the main consequence of periodontitis was tooth loss, which was solved by prosthetic rehabilitation of the lost teeth. However, a link between periodontitis and other systemic pathologies began to be established later. The findings reported by Mattila et al. [11] and Offenbacher et al. [12], related for the first time periodontitis with myocardial infarction and preterm and/or low

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birthweight neonates, respectively. These are pathologies of great impact and social relevance, and the discovery brought back again the concept of focal infection. Since then, the research involving systemic implications of periodontitis has grown exponentially over the years, with epidemiological studies now relating it to at least 57 different systemic pathologies [13]. As a result, the scientific community is accepting periodontitis as an infection focus from which oral bacteria, bacterial byproducts or inflammatory mediators can interact with other parts of the organism through blood dissemination. Periodontal pathogen components such as DNA, RNA or specific antigens from *P. gingivalis, Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans)*, *Treponema denticola (T. denticola)*, *Fusobacterium nucleatum* (*F. nucleatum*) or *Campylobacter rectus* have been isolated in different tissues outside their natural ecological niche, like atheroma plaques, placenta, amniotic sac, respiratory tract, pancreas, appendix or colon [13,14]. However, the presence of bacterial components cannot distinguish if the bacterial species are dead or alive [15,16]. It has been shown that periodontitis can contribute to endothelial dysfunction (ED), a surrogate marker of atherosclerosis. It has been associated with lower values of flow-mediated dilation of the brachial artery, probably due to an increased thickening of the intima-media layer of the vessel. Periodontal bacteria, bacterial components or inflammatory mediators could cause a dysregulation in the endothelium and hence contribute to atheroma plaque formation [17,18]. Cardiovascular disease (CVD) is one of the leading worldwide causes of death, with 16.7 million deaths each year worldwide [19]. Some papers suggest that periodontitis should now be considered a public health problem [20], due to its high prevalence and its consideration as a risk factor for CVD, now included in statements from both the European Society of Cardiology and the American Heart Association [19,21].

In this review, we aim to summarize the current available knowledge, and to integrate and summarize novel insights about periodontitis and their metabolic implications as a risk factor for CVD, as well as novel future perspectives to follow in this topic, regarding innovative therapies.

2. Epidemiologic evidence on the association or causality between periodontitis and CVD

18 systematic reviews and/or meta-analysis, mostly performed on observational studies, have addressed this topic since 2001. Although the association between the two diseases has been confirmed, clinicians should be aware that not all systematic reviews of the periodontitis-CVD association have been conducted in a rigorous manner. Some of them exhibit significant structural and methodological variability [22]. The most recent meta-analysis, performed on 15 observational studies and including a total of 17,330 patients, showed that the presence of periodontal disease was associated with carotid atherosclerosis (OR: 1.27, 95% CI: 1.14–1.41), but the authors found substantial statistical heterogeneity ($I^2 = 78.90\%$, $p < 0.0001$). The inclusion of studies performed on different ethnicities may be one of the causes of this heterogeneity [23].

Three recent observational studies of different designs should also be highlighted. The PAROKRANK study, one of the biggest epidemiologic studies performed to date, followed a case-control design in > 1600 Swedish patients. The results showed a significant increased risk for first acute myocardial infarction (AMI) among those diagnosed with periodontitis (OR 1.28, 95% CI 1.03–1.60) after adjusting for confounding variables (smoking habits, diabetes mellitus, years of education, and marital status). 43% of the cases were diagnosed with periodontitis, verified by radiographic bone loss, and the risk for first AMI was near 30% higher than controls and independent of traditional risk factors [24]. This CVD risk for periodontitis patients compared to healthy subjects is on the range of 25–50%, also reported by similar studies [25]. Beukers et al. reported an association of periodontitis with atherosclerotic disease independent of other confounders in a cross-sectional analysis of 60,174 participants, stratified by sex age and sex, in Dutch population (OR 1.59, 95% CI 1.39–1.81) [26]. The size of AMI is one of the determinants of its severity, as it indicates the degree of myocardial necrosis. The peak troponin I level in blood is used a marker of AMI size. In the study by Marfil-Alvarez et al., among 112 AMI patients that needed revascularization therapy (primary angioplasty), multiple mediated regression analysis showed higher levels of troponin I, and hence greater infarct size, in those patients diagnosed with periodontitis. According to their results, 65.18% of the AMI patients were diagnosed with periodontitis. This number was higher compared to previous studies, maybe attributable also to the higher severity that presented patients enrolled in this study compared to previous ones [27].

As stated previously, the main consequence of periodontitis is tooth loss. Bahekar et al. studied the relationship between number of teeth and incidence of coronary heart disease. Their results showed that patients with < 10 teeth presented a risk of 1.24 (95% CI 1.14–1.36, $p < 0.0001$) for coronary heart disease compared to patients with > 10 teeth [28]. Also, another recent article showed that severe tooth loss, defined as > 10 teeth, showed to be a predictor variable of cerebrovascular disease/silent cerebral infarction. Patients that lost > 10 teeth showed an adjusted OR of 3.9 (95% CI 1.27–5.02, $p < 0.001$) compared to patients with tooth loss between 0 and 5 teeth [29]. A reduction in the number of posterior teeth was associated with a greater prevalence of atherosclerosis in a cross-sectional study on Japanese population [30]. Hence, periodontitis acting as a chronic inflammatory process or as a cause of tooth loss, seems to be clearly associated with a greater prevalence or incidence of CVD events.

Only 8 articles to date have addressed the link between periodontitis and endothelial dysfunction (ED), one of the first steps of CVD pathology, and a surrogate marker of early subclinical atherosclerosis. ED was mostly assessed measuring flow-mediated dilation (FMD) of the brachial artery, considered the gold standard test. The results clearly conclude that periodontitis is associated with an impaired endothelial function [17,18,31,32], and that periodontal treatment can improve it [33–36]. Desvarieux et al. reported for the first time that an improvement in the periodontal status, assessing it both clinically (probing pocket depth and attachment loss measurements at 6 sites per tooth using a UNC-15 periodontal probe) and microbiologically (quantitative assessment of 11 known periodontal pathogens by DNA-DNA checkerboard hybridization) was associated with less atherosclerosis progression (assessed by carotid intima-medial thickness (CIMT)). These findings were observed on a relatively short period (3-year median follow-up), supporting the hypothesis that faster atherosclerosis progression could be the mechanistic model to explain previously published results associating periodontitis and CVD, and also emphasizing the possibility of primary periodontal care as a potential preventive measure for CVD [37]. It was also showed previously after a 5-year follow-up period that the diagnosis of periodontitis at the beginning of the study was associated with 4-times more probability of developing carotid atherosclerosis and 2.3-times for atherosclerosis progression if already diagnosed [38].

Although in the recent years there has been an increasing number of intervention studies, mainly focusing on the effect of periodontal treatment on endothelial function, the available evidence is still insufficient to establish a causal relationship. The multi-factorial nature of periodontitis and atherosclerosis (Fig. 1), the chronic nature of both diseases, and other facts such as the identification of fatty streaks and foam cells in children/teenagers, and the limited sample sizes and short follow-up periods, make difficult nowadays to confirm a causal relationship, since some of the published results are contradictory.

3. The emerging role of periodontal microbiome and immune dysregulation in atherosclerosis

Dental biofilm is characterized by its biodiversity. > 700 bacterial

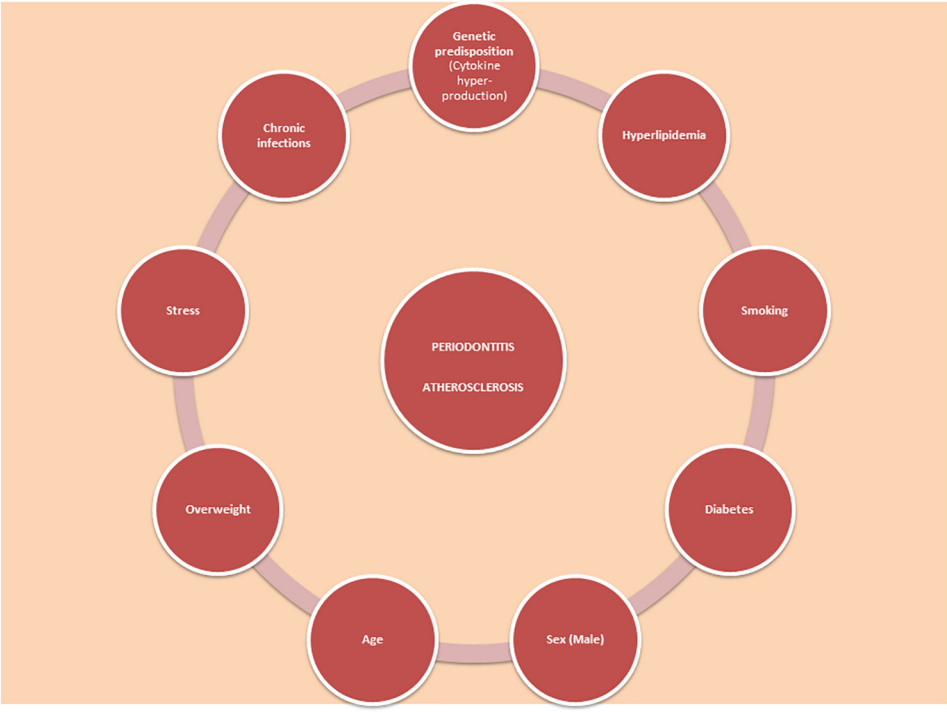

Fig. 1. Common risk factors between periodontitis and atherosclerosis.

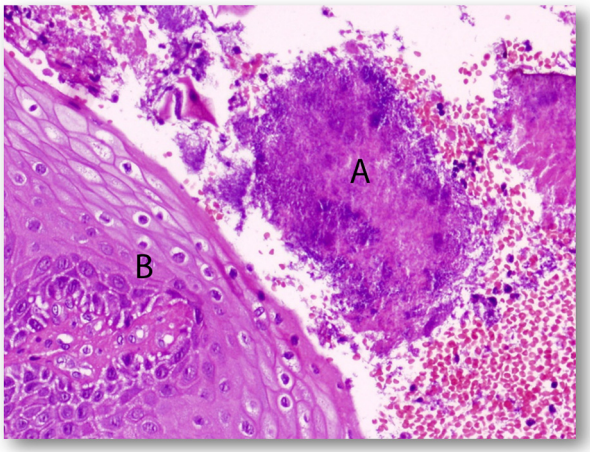

Fig. 2. Optical microscopy image from a gingival biopsy. Presence of bacteria in the gingival sulcus, forming the subgingival biofilm (A). This biofilm is in constant interaction with the sulcular epithelium (B) (Hematoxylin-eosin, magnification ×20). Image courtesy of Prof. F. O'Valle (Pathology department, University of Granada).

species have been isolated [39]. It presents a high cell density (10^{11} cells/g wet weight), but only a few anaerobic gram negative bacteria would play a role in the pathogenesis and progression of the periodontal inflammatory process [40] (Fig. 2). It has been documented that frequent bacteremia occur in patients with periodontitis, after daily regular oral hygiene procedures such as tooth brushing, that would implicate a chronic insult on the vasculature [41].

In the 2000s, 4 independent clinical trials were conducted in order to demonstrate that infection also contributes to the etiology of atherosclerosis. They included in total > 4000 patients with CVD that were treated with azithromycin and clarithromycin. After follow-up, results showed treatment with antibiotics did not add any benefits, when compared to untreated controls. It should be highlighted though, that the antibiotic that was specific to treat *Chlamydia pneumoniae*, and not periodontal pathogens [42–45]. In a meta-analysis on systemic markers of bacterial exposure in periodontitis and CVD risk, Mustapha et al. reported a poor correlation between systemic bacterial involvement in periodontitis and clinical results, and proposed the use of markers of bacterial exposure for a more correct study of this association. For this reason, their meta-analysis only included studies that assessed levels of C-reactive protein, antibodies against specific periodontopathogens and CIMT. Their results showed that periodontitis with elevated bacterial exposure was associated with early atheroma plaque formation, showing a significantly increased CIMT (0.03 mm, 95% CI 0.02–0.04) [46].

3.1. Periodontal bacterial infection in atherosclerosis

P. gingivalis is the most studied periodontal pathogen in terms of its interactions with cells from cardiovascular system. Chiu et al. published 19 years ago the first *in vivo* evidence of *P. gingivalis* in macrophages of human carotid atherosclerotic plaques by immunostaining [47]. A year later, Haraszthy et al. isolated in samples from 50 atheromatous plaques RNA from *A. actinomycetemcomitans*, *P. gingivalis, Tannerella forsythia (T. Forsythia)* and *Prevotella intermedia*, 4 of the main periodontopathogens, and also *Chlamydia pneumoniae* and human cytomegalovirus, determined by 16S rRNA PCR. 22 of these plaques had at least one periodontal species and 13 of them had 2 or more periodontopathogens [48]. Since then, several studies have demonstrated the presence of DNA, RNA or oral bacterial antigens in samples from human atheroma plaques. More recently, presence of 16S rRNA of *P. gingivalis, T. forsythia, T. denticola and Prevotella nigrescens* was found both in samples from subgingival biofilm and atheromatous plaques [49]. These findings may be explained by the presence of bacterial compounds from dead species that arrive and infiltrate the atheroma plaque or the endothelial wall by blood dissemination. This fact may point to a more passive mechanism instead of active infection of vascular structures by these species. To our knowledge, only one study has successfully isolated alive species of *A. actinomycetemcomitans* and *P. gingivalis*, by culture of atheroma plaque samples with human endothelial cells

from coronary arteries [50]. In periodontitis, with an ulcerated sulcular epithelium, bacteria from the subgingival biofilm can easily go through the lamina propria, due to the lack of resistance of the tissue. They could also easily go through the endothelial cells of the subgingival inflamed vessels and access the blood stream. It is well documented that *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* have the ability to invade epithelial, endothelial and immune cells, such as dendritic cells and monocytes [51]. *P. gingivalis* has been isolated in circulating dendritic cells in patients with periodontitis and atheroma plaques [52]. This supports the hypothesis that periodontal pathogens could be phagocyted by immune cells and stay alive inside them, using them for dissemination through the blood flow and being able to arrive to the atheroma plaques.

F. nucleatum is a bacterial species from the subgingival microbiome that has similar properties to *P. gingivalis*. The stimulation of interleukin-8 by *F. nucleatum* is 10 times more lasting than the one caused by *E. coli* [53]. Several characteristics make *F. nucleatum* a relevant and pathogenic bacterium. Its component fusobacterium adhesin A (FadA), a main virulence factor, is expressed on the surface and allows the bacteria to easily adhere and invade epithelial and endothelial cells by binding to E-cadherin. FadA has also shown high proinflammatory properties alone and together with *F. nucleatum* [54].

3.2. Indirect role of bacterial compounds and other mediators in systemic inflammation

The cell wall of anaerobic gram-negative bacteria is composed of peptidoglycans, polysaccharides, proteins, lipids, lipoproteins and lipopolysaccharide (LPS). LPS is a glycolipid endotoxin and the main component of the membrane of gram-negative bacteria, considered to be the most important surface antigen. It has a wide variety of functions that include maintaining the membrane structure, molecular mimetism, antibody inhibition, antigenic variations, immune system activation and mediation of the adherence to host cells. The exact mechanism of adherence of LPS is still unknown, but physicochemical interactions and lectin receptors on the cell surface have been postulated as possible mechanisms [55]. LPS-induced endotoxemia has been proposed as possible molecular mediator between periodontitis and CVD. In a recent study, 3 groups of patients were divided according to the grade of coronary stenosis assessed by coronary angiography (stenosis $< 50\%$, stenosis $\geq 50\%$ and acute coronary syndrome) and serum and salivary LPS levels were correlated with periodontitis and periodontal pathogens. Serum LPS were associated with periodontitis and periodontopathogen count and also with a greater risk for having a grade of stenosis $\geq 50\%$. The authors conclude that periodontitis is associated with a low-grade systemic inflammation that, together with other risk factors, would contribute to a higher risk for CVD [56].

LPS is released by bacteria as a result of division or lysis and contacts with several host proteins and co-receptors, such as LPS binding protein (LBP), CD14, toll-like receptors (TLR) and MD-2. LBP needs to bind bacterial LPS in order to be presented to CD14, expressed in the surface of macrophages, neutrophils and endothelial cells. This receptor intervenes and mediates in the transduction and recognition process, mediated by the TLR4/MD-2 complex [57]. A genetic alteration of this receptor, conditioned by bacterial stimuli, may also play a role in the pathogenesis of atherosclerosis. Patients who survived to AMI showed a higher presence of a single nucleotide polymorphism in the promoter of the CD14 gene, compared to controls [58]. Human umbilical veins infected with *P. gingivalis* or stimulated with LPS, have shown exacerbated levels of oxidized LDL (ox-LDL) and tumor necrosis factor α (TNF-α), and inducing endothelial injury through increased endothelial cell death [59]. Other results have shown that *P. gingivalis*-infected endothelial cells have a higher expression of surface adhesion molecules, such as ICAM-1, VCAM-1, P-selectin and *E*-selectin, which also contributes to the pathogenesis of atherosclerosis and other inflammatory diseases [60].

Brain natriuretic peptide (BNP) is a 32-amino-acid peptide, released mainly from myocardial cells as response to stress on the ventricle wall. BNP is produced as a pro-hormone that is divided in two: the active BNP and the inactive N-terminal-pro-BNP. Elevated levels of BNP could act as a biomarker of atherosclerosis progression. Increased levels of microRNA from BNP have been found in coronary arteries with atherosclerotic lesions, and serum levels of BNP have been associated with coronary atherosclerosis burden measured by electron beam tomography [61]. Plasma levels of N-terminal-pro-BNP increased as response to LPS and patients with periodontitis have higher serum N-terminal-pro-BNP levels compared to healthy individuals. In addition, N-terminal-pro-BNP levels increased as periodontitis severity progressed [62].

Periodontal pathogens can produce toxins of different origin, such as proteases, adhesins, lectins and other molecules that regulate bacterial biofilm. These toxins can inhibit host immune response like the protease gingipain from *P. gingivalis*, which has the ability to degrade and/or inactivate different interleukins (1β, 6 and 8) by a process called "localized chemokine paralysis", or surface receptors from both immune (CD14 in monocytes) and non-immune cells (Intercellular Adhesion molecule 1 in epithelial cells and fibroblasts) [63,64]. Platelets play a key role in the pathogenesis of thrombosis, and facilitate the adhesion of monocytes due to an interaction between CD40 receptors in both cells [65]. Interestingly, increased number of circulating platelets have recently been reported in Korean patients with periodontitis compared with controls [66]. Another documented action of gingipain from *P. gingivalis* is the activation of protease-activated receptors type 1 and 4, highly expressed in the platelet surface, inducing platelet aggregation in a similar way than thrombin. An increased platelet aggregation induced by *P. gingivalis* has been also observed in studies performed on platelet-rich plasma, which also supports this hypothesis [67]. Gingipain from *P. gingivalis* has also shown to affect vascular smooth muscle cells (VSMC) and contribute to the development of atherosclerosis through this mechanism. A phenotypic transformation and greater proliferation were observed in VSMCs exposed to gingipain in a rat and *in vitro* models [68,69]. This action on VSMCs may be mediated by the effect of gingipain on the expression of Angiopoietin 1 and 2, regulators of vascular maturation, stability and remodeling [70].

Cross-reactivity or molecular mimicry between bacterial components and the host have been described. The homology between human and bacterial heat shock proteins (HSPs) could activate atherosclerotic changes [71]. This hypothesis states that the host immune system is not able to differentiate between self-HSPs and bacterial HSPs. Bacterial HSPs can be recognized as host HSPs and generate an autoimmune response that contributes to the progression of atherosclerosis. Endothelial cells express HSPs in atherosclerosis, and cross-reactive T-cells are present in the arteries and peripheral blood of patients with atherosclerosis [72]. Cross-reactivity between HSP60 from *P. gingivalis* and human HSP60 from endothelial has been determined by specific antibodies. Also, T lymphocytes reactive against HSP60 from *P. gingivalis* have been found in peripheral blood from patients with atherosclerosis [73]. Results from a study published by our group, showed that untreated patients with mild periodontitis presented increased serum HSP60 and small, dense LDL concentrations, in comparison with controls matched by age and body mass index. Our findings indicated that atherogenic dyslipidemia and elevated circulating levels of HSP60 were associated with periodontitis [74].

4. Periodontal microbiome and periodontitis: relationship with lipid profile

The link between periodontitis and atherosclerosis has been described by two mechanisms, directly by the periodontal pathogens or indirectly by bacterial components or inflammatory mediators. A third mechanism that would also support this link, is the relationship between periodontitis and the lipid profile. Atherosclerosis is defined as the progressive accumulation of lipids, macrophages, calcium, fibrotic

tissue and other components in the coronary artery wall, and is considered to be one of the main causes of CVD [75]. Nowadays an immunologic mechanism is considered instead to be responsible for its progression [76].

Previous studies have shown that certain bacterial infections may elevate plasma concentrations of very-low-density lipoprotein and small dense LDL levels in humans [77,78]. Gingipain from *P. gingivalis* caused a selective proteolysis of apoB-100, a main component of LDL particles, and necessary for the binding of LDL to cell surface receptors, which is a crucial step for the promotion of atherosclerosis [79]. Gingipain has also shown to modify vascular LDL/VLDL and HDL through proteolytic effects to an atherogenic form (mainly lipid peroxidation). This evidence supports a role of lipoproteins as a key link between periodontal disease and the development of atherosclerosis [80]. LDL present differences between particles in term of size and density. These subclasses present variations in surface lipid content and conformational changes in apoB-100, with increased exposure on the particle surface [81]. Small, dense low-density lipoproteins are an LDL subclass that have shown to be particularly atherogenic and have little affinity for the apoB/E receptor of hepatocytes, delaying their blood clearance. They also have a high affinity for vascular glycosaminoglycans, which keeps them in contact with the vascular artery wall for a longer time. They are taken up more easily by arterial tissue and also present a greater oxidative and glycation susceptibility [82]. Recent evidence has indicated that small, dense LDLs play an important role in atherosclerosis, may have a predictive role in different cardiometabolic states and interact with several traditional cardiovascular markers [83]. This LDL subclass levels have also been found increased in patients with periodontitis [74].

Recently, a systematic review has been published investigating serum lipid levels (total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol) in patients with and without periodontitis. They found that the studies cannot provide consistent information, due to the great variability and the lack of consensus in the diagnosis and case definition of periodontitis, as also confirmed by the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. Important shortcomings are identified in this workshop (lack of clear pathobiology distinction between categories, diagnostic imprecision, and implementation difficulties) and a new framework for a new case definition and classification of periodontitis is proposed to improve the methodology of assessment and the data quality for future studies [84]. The authors performed a meta-analysis with meta-regression of 19 studies, with a total of 2104 participants. Authors performed the analysis dividing the articles in those with secure diagnosis of periodontitis and those with insecure diagnosis of periodontitis. They conclude that periodontitis was significantly associated with a reduction of HDL-cholesterol and elevations of LDL-cholesterol and triglycerides. They state that periodontal inflammation may negatively affect serum lipid control, and also contributing to a higher risk for CVD. Lipid dysregulation would also increase the susceptibility to periodontitis, since dyslipidemia is associated with a state of systemic inflammation [85]. This suggests a possible bi-directional relationship between dyslipidemia and periodontitis (Fig. 3) [86].

5. Glucagon-like peptide-1, cardiovascular risk and periodontitis

5.1. Diabetes, periodontitis and cardiovascular risk

A primary metabolic condition with an elevated impact on cardiovascular risk is Type 2 diabetes mellitus (T2DM) [87]. It has been reported that severe periodontal disease often coexists with severe diabetes mellitus and that diabetes is a risk factor for severe periodontal disease; thus, their “two-way” relationship is recognized [88]. This link is further strengthened by evidence of improvement in HbA1c levels of about 0.4–0.7% following periodontal treatment in patients with combined periodontitis and T2DM coming from different meta-analyses of randomized clinical trials [89,90]. A pathogenic model links inflammation to diabetes and periodontal infections, in which chronic systemic inflammation caused by periodontitis elevates the plasma concentrations of proinflammatory cytokines, such as TNF-α and interleukin 1β (IL-1β). This interacts and increases the generation of advanced glycation end-products and insulin resistance [89]. Exposure to high-glucose levels can also affect cellular functions through increased reactive oxygen species activity [91]. These pro-inflammatory mediators are important cardiovascular risk factors and may affect both the control of diabetes and the development of CVD as a complication [92]. This inflammatory state would also have an effect in gingival tissues with diabetes play a role with an increased periodontal destruction. A different subgingival microbial population has been found in patients with periodontitis and T2DM compared to periodontitis only. These results showed that periodontal bacterial species commonly associated with periodontitis, such as *P. gingivalis, T.forsythia* or *A. actinomycetemcomitans,* were found less frequently in patient with both diseases and hence present different characteristics.

Regarding the risk for complications, T2DM patients with periodontitis are more likely to develop diabetes complications (OR 2.6 for cardiovascular complications). Also, the overall CVD mortality is significantly elevated in patients with T2DM and periodontitis [93]. There is no direct evidence for specific mechanisms arising from periodontitis impacting upon the complications of diabetes. However, indirect evidence exists for common mechanistic pathways (oxidative stress pathways, dyslipidaemia, elevated CRP, endothelial dysfunction) that may act synergistically in worsening cardiovascular complications in diabetes [92].

Recent joint guidelines from the European Federation of Periodontology and the International Diabetes federation have proposed that periodontal examination should be an essential part of the diabetes visit. The discovery of signs of periodontitis through interview by the physician is crucial and the patient should be referred and encouraged to receive periodontal treatment, as part of the global management of the disease [93]. In this context, the dental profession may have an important role in diagnosis, as the condition may be first identified by the dental practitioner, and be also a part in the treatment of the diabetic patient [94].

5.2. Role of incretin hormones in cardiovascular risk and periodontitis

The vast majority of T2DM patients will eventually die from cardiovascular causes including AMI and heart failure [95]. Antidiabetic treatments may represent a novel therapeutic strategy in the treatment of each patient who needs better control of cardiovascular risk [87]. New drug-classes approved for the treatment of T2DM, include glucagon-like peptide-1 (GLP-1) receptor agonists and dipeptidypeptidase-4 (DPP-4) inhibitors, also known as incretin-based drugs [96]. Many CV outcome trials with these novel antidiabetic drugs have been completed or are currently ongoing [97], and the available data demonstrates positive effects of various GLP-1 receptor agonists on different cardiometabolic parameters [98]. Another incretin hormone, Glucose-dependent insulinotropic polypeptide (GIP), has shown to increase fat deposition in adipose tissue and promote bone formation [99]. In addition to pancreatic cells, GIP receptor is expressed in many other organs, such as the nervous system, eyes, adipose tissue and bone, as well as immune cells [100]. GIP and GLP-1 were found upregulated in T2DM patients with periodontitis compared with T2DM only, and GIP was correlated with the presence of periodontitis, defined by pocket depth > 4 mm [101]. DPP-4, the enzyme that degrades incretin hormones, is also present in periodontal bacterial species. It has been theorized that DPP-4 from bacterial origin could also be recognized and act as human DPP-4 by mechanisms of molecular mimicry. Through this mechanism, periodontal bacteria could degrade human incretins and play a role in the impairment of the glycemic control in the periodontal patient [102].

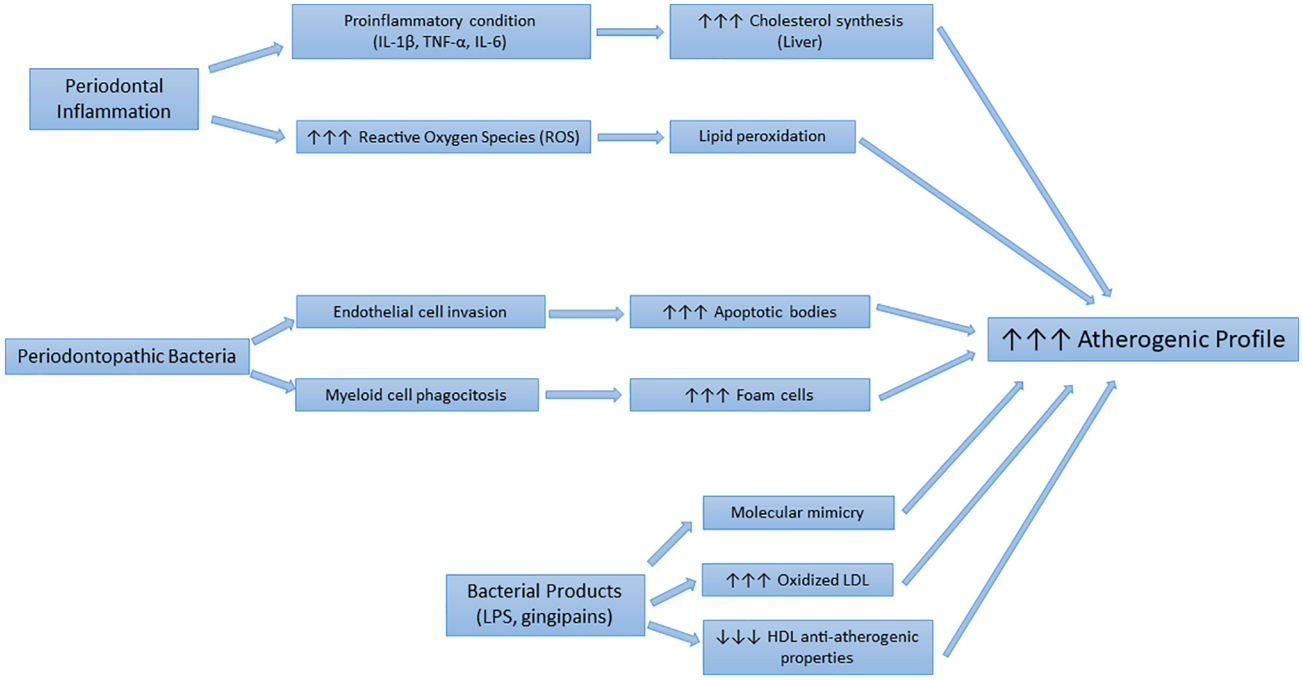

Fig. 3. Model of the interaction between periodontitis and atherosclerosis.

5.3. Role of incretin-based therapies in periodontitis

In this context, drugs with an anti-inflammatory activity, or a protective effect on bone tissue can suppose an alternative and complementary therapy for periodontitis. Considering the immune-inflammatory pathogenesis of periodontal disease, it has been hypothesized that these drugs could contribute to control bone and connective tissue degradation during the progression of periodontal disease. A recent *in vitro* article showed that liraglutide improves proliferation, migration, and osteogenic differentiation of human periodontal ligament cells, and inhibited the inflammation induced by LPS. These effects were independent of the hypoglycemic activity [103]. A decrease in inflammatory mediators was observed in a non-diabetic murine model, but no effect of bone was observed [104]. However, the evidence of GLP-1 receptor agonists on bone metabolism and bone markers in obese nondiabetic individuals after a larger weight loss is still scarce and needs further investigation [105]. This question is highly relevant since these drugs are now being indicated in higher doses for the treatment of obesity. Obese women treated with liraglutide showed an increased bone formation of 16% and a prevention of bone loss [106]. Several studies support the hypothesis that the gut hormones glucose-dependent insulinotropic peptide and GLP-2 are modulators of bone growth [105,107], and remodeling [108]. Treatment with the GLP-1 receptor agonist exendin-4 in rats with ovariectomy-induced osteoporosis has been shown to improve bone strength; to prevent deterioration of trabecular bone and to increase bone formation markers [109]. In a recent *in vitro* study on periodontal ligament stem cells, the GLP-1 receptor agonist Exendin-4 prevented dysfunction caused by glucose on cells and also promoted osteoblast differentiation of these stem cells. These results suggested the potential use of these drugs in diabetic patients with periodontitis [110]. Other studies verified that Exendin-4 inhibited the inducible nitric oxide synthase expression induced by lipopolysaccharide [111], and also was associated with a decreased expression of IL-1β and metalloproteinases such as MMP-2 and 3, which also proves a potential utility in inflammatory diseases [112].

Unfortunately, the currently available evidence evaluating the influence of DPP-4 inhibitors, another type of incretin-based therapy, in the pathogenesis of periodontal disease is still scarce and controversial [113].

6. Conclusion

The association between periodontitis and CVD has been reassured by recent evidence. However, longitudinal studies are still needed get a deeper knowledge about theses hypothesis and mechanistic links proposed, as well as a causal relationship between these two diseases. Well-designed interventional studies are also needed to prove the effect of periodontal treatment on CVD-related variables. The identification of these mechanism has also brought novel therapies that may have effects and be of potential use in both diseases, such as the novel GLP-1 receptor agonists. A new drive towards a personalized medicine approach to treatment, based on a specific biomarker-based risk profile, may bear benefits for the treatment of both conditions. Also, a great effort is needed to achieve standardize definitions of periodontitis, in order to permit meta-analysis of better quality. Widely accepted diagnostic criteria, such as the new classification of periodontal diseases of 2018, will be needed to obtain evidence of greater quality.

A better understanding of the link between periodontitis and CVD may make necessary the integration of periodontal treatment and maintenance into the public health system, as another aspect of regular CVD prevention and treatment. A constant and clear relationship between the practitioner and the dentist may be useful considering and addressing these associations. Even more important, it can also help to give a complete and personalized preventive medical approach.

Conflict of interest

Authors declare having no conflict of interest, either directly or indirectly, in any of the companies or products listed in the study.

Transparency document

The Transparency document associated with this article can be found, in online version.

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