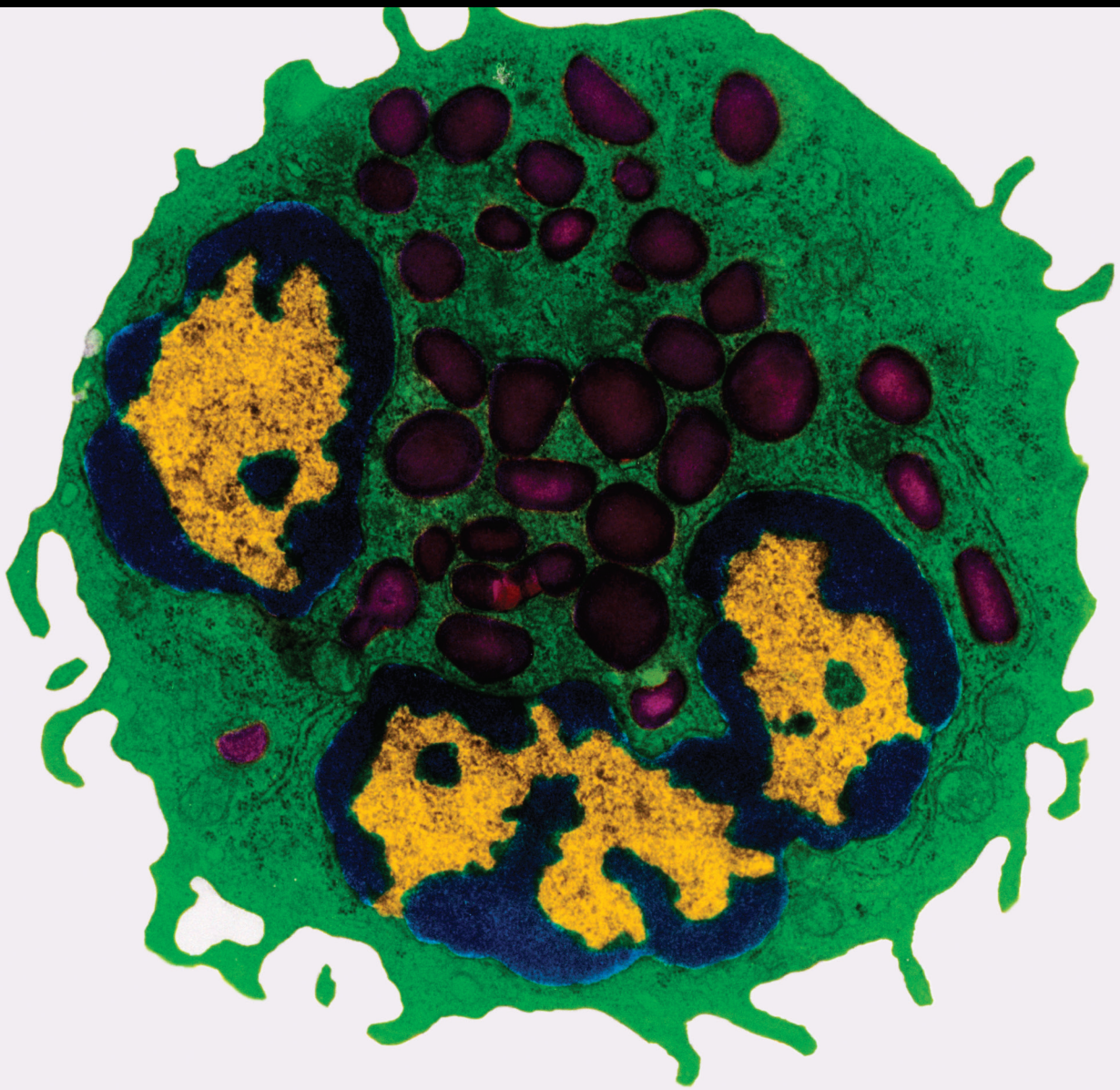
Mediators of Inflammation

The Role of Inflammatory Mediators in the Pathophysiology of Periodontal and Oral Diseases

Lead Guest Editor: Gaetano Isola
Guest Editors: Ray C. Williams and Kristina Bertl



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Review Article

Periodontal Health and Disease in the Context of Systemic Diseases

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During recent years, considerable progress has been made in understanding the etiopathogenesis of periodontitis in its various forms and their interactions with the host. Furthermore, a number of reports have highlighted the importance of oral health and disease in systemic conditions, especially cardiovascular diseases and diabetes. In this regard, research has attempted to explain the role of periodontitis in promoting alteration in distant sites and organs. Recently, DNA sequencing studies have revealed how oral infections can occur in distant sites such as the colon, reproductive tissues, metabolic diseases, and atheromas. The objective of this review is to describe and update the emerging evidence and knowledge regarding the association between periodontitis and systemic disease and to analyse the evidence that has reported periodontitis as a risk factor for the development of various forms of systemic diseases in order to provide a better understanding of the possible shared etiopathogenetic pathways between periodontitis and the different forms of systemic diseases.

1. Introduction

In recent decades, significant progress has been made in the comprehension of the pathogenesis and pathophysiology in periodontal diseases (PD), their interaction with the host, and their relationships with systemic diseases [1].

Periodontal research has identified specific microbial pathogens (consisting of anaerobic Gram-negative bacteria such as *Aggregatibacter Actinomycetemcomitans* (*A. a.*), *Porphyromonas gingivalis* (*P. gingivalis*), *Tannerella forsythia* (*T. forsythia*), *Treponema denticola* (*T. denticola*), and *Spirochetes*) which, in conjunction with mildly virulent organisms, highly organised complex communities in the form of biofilms, is presented first at the supragingival level and, in the more advanced stages, the subgingival [2]. The presence of periodontal pathogenic biofilm induces inflammatory and immune mechanisms of the host with the following inflammatory response and tissue alteration that occurs in the gingival tissues and tooth support apparatus (including alveolar bone) [3, 4]. An important role in the inflammatory response by the host immune system, which can change from one individual to another, is represented by exposure to certain risk factors [4, 5]. Multiple host risk factors associated with the multifactorial etiology of periodontitis are recognised. In recent decades, these risk factors have been studied in-depth and include genetic predisposition to the disease, associated with certain environmental stimuli (diet, diabetes, smoking, poor oral hygiene, etc.) that have been closely associated with the development and progression of periodontitis. In the new classification of periodontal diseases, drawn up in 2017, risk factors assume a relevant role in both diagnosis and prognosis definition for each patient. It has introduced the concept of "risk stratification," which is based on well-validated risk factors, smoking

and uncontrolled type II diabetes. The presence of which in the patient's history increases the probability of the case progressing at a faster rate than is typical for the majority of the population or responding less predictably to standard therapy. Therefore, diabetes and smoking represent "grade modifiers" in that, if present, they are responsible for the progression to a higher degree, independently of the grade resulting from the analysis of direct evidence loss of clinical attachment loss (CAL) in the last 5 years and indirect evidence of progression (age-related bone loss and phenotype) [6].

From there, the inflammatory response during periodontitis has been shown to be marked by the local release of specific proinflammatory mediators and enzymes, including C-reactive protein (CRP); metalloproteinases (MMPs); interleukin- (IL-) 1β, IL-6, and IL-10; and tumor necrosis factor (TNF-α). It has been widely demonstrated that the periodontal damage associated with periodontitis, with the development of deep periodontal pockets, represents an extremely favourable microenvironment for anaerobiosis with a relative increase in inflammatory cytokine levels associated with periodontal destruction. Given the cumulative increase in inflammatory cytokines, periodontitis usually evolves into acute and chronic inflammatory status at the gingival level, which can represent a negative factor and possible risk factor for developing various systemic diseases, including diabetes, endocrine diseases, metabolic syndromes, and endothelial and cardiovascular diseases. Based on these associations, the concept of "periodontal medicine," coined by Stephen Offenbacher, has developed in recent years.

In the last twenty years, several epidemiological, experimental, and interventional studies on large-scale populations have shown how periodontitis may negatively impact systemic health, both in healthy patients and in those suffering from pathologies. Specifically, periodontitis has been independently associated with a large number of chronic noncommunicable diseases related to ageing, premature death, and low quality of life. In the study conducted by Romandini et al. in 2020 [7], the results have also indicated that periodontitis and all types of edentulism are associated with an increased risk of mortality from cardiovascular disease, cancer, coronary disease heart disease, and cerebrovascular disease, but not pneumonia.

Diabetes or cardiovascular diseases are still the most studied systemic diseases associated with periodontitis. There have been more and more trials that have attempted to evaluate periodontitis as a risk factor for the development of different diseases including metabolic syndromes, obesity, rheumatoid arthritis, autoimmune diseases, cognitive disorders (e.g., Alzheimer's disease), and even some forms of cancer, all of which are independently associated with or aggravated by the presence of periodontitis. Only more recently, however, a connection was observed between PD, reproductive health, and fertility problems in men and women, as well as negative pregnancy outcomes such as preterm delivery, preeclampsia, miscarriage, and low birth weight of babies [8]. Although it has been reported that periodontitis is linked to about 60 systemic diseases, there are still many aspects that are not known or sufficiently understood, creating therefore discordant thinking within the scientific community.

In light of the above, the aim of this review is to update the knowledge regarding the association between periodontitis and systemic diseases, trying to improve the understanding of possible shared etiopathogenetic pathways between periodontal disease and systemic diseases in relation to the evidence that has emerged in recent years.

For this purpose, a series of searches were carried out using the main scientific databases, such as PubMed, Scopus, and Google Scholar. The keywords of the searches performed were the following: periodontitis, periodontitis and systemic diseases, periodontitis and cardiovascular diseases, periodontitis and diabetes, periodontitis and osteoporosis, periodontitis and obesity, periodontitis and neurodegeneration, periodontitis and infertility, and periodontitis and adverse pregnancy outcomes. The included studies were selected independently by the authors.

2. Periodontitis and Cardiovascular Disease

Globally, noncommunicable diseases (NCDs) are increasing in both prevalence and incidence due to the increasing average age of the population, mainly due to unhealthy diets and lifestyles accounting for over 41 million deaths per year in the world or 71% of all global deaths [9]. Surveys carried out over the last decade have shown that 80% of people over the age of 65 in the USA have one or more NCDs and about 7777% have at least two forms of NCD, with a significant burden of disease that negatively affects both the individual and the economy of the health system. In fact, the presence of the comorbidity of at least two NCDs represents an important challenge for a nation's health system, which can cover over 60% of total health costs [10]; in this regard, in recent years, healthcare spending has mainly focused on general health prevention programs.

Among the main NCDs, cardiovascular diseases (CVDs) represent one of the main causes of death in the world, with an estimated average annual death of 17.7 million people; about 31% of these deaths were CVDs, including stroke, myocardial infarction, or valvular diseases. In order to significantly reduce the incidence of CVD diseases and deaths by 2025, the World Health Organization has already introduced a specific worldwide plan of action [11].

CVD is used as an acronym encompassing atherosclerotic disease, mainly coronary, peripheral, and cerebrovascular diseases. In the medical field, there is now evidence that demonstrates a close association between the presences of specific gene polymorphisms that play a key role in the process of atherogenesis and, in general, in the development of CVD [12]. However, a major stimulus in CVD development is several environmental risk factors, including lifestyle factors, primarily smoking, alcohol, dyslipidemia, impaired glucose metabolism, and hypertension. Among other things, there is a marked association between CVD and metabolic diseases with a bilateral relationship, especially in the presence of diets based on processed carbohydrates, salt, and saturated fats which contribute to obesity, type II diabetes

mellitus, and CVD, representing the main risk factors for myocardial infarction [13] and stroke [14]. In this regard, one of the cardinal principles of every form of NCD, especially CVD, is represented by identifying individuals at greater or lesser risk of developing the disease through a preventive reduction of risk factors to reduce the disease burden. The risk factors described above are all modifiable through the initiative of improved lifestyles, including physical activity, proper diet, intake of antioxidants and vegetables, and moderate consumption of alcohol [13].

However, CVDs have also been associated with several chronic inflammatory, infectious, or multifactorial diseases, related to a sharp increase in CVD development, which include preterm birth, psoriasis, systemic lupus erythematosus, rheumatoid arthritis, and also periodontitis [15].

More specifically, an increasing body of evidence has supported the close existence of an independent correlation between periodontitis and different NCDs, including diabetes [16, 17], CVD [3, 18], lung diseases [19], and acute and chronic nephropathies [20]. Indeed, periodontitis is independently associated with the same causes and risk factors as CVD. Among the various mechanisms proposed for CVD development, there are also bacteremia and associated systemic inflammatory sequelae, which are associated with an increase in the host response with the release of C-reactive protein, inflammatory mediators, and associated oxidative stress [21, 22]. In patients with multimorbidity, including diabetes and chronic kidney disease, periodontitis is related to reduced survival with a significant increase in CVD and associated pathologies [23]. Therefore, periodontal disease may not be an easily modifiable risk factor for the development of CVD.

In this regard, a joint workshop was held in 2012 between the American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP) and a subsequent perio-cardio workshop in 2019 (in order to reevaluate the evidence relating to the association between CVD and periodontal disease) [24]. The workshops highlighted the significant epidemiological evidence that periodontal disease represented a real risk factor for the increased development of atherosclerotic CVD through several mechanisms, including the presence of oral dysbiotic microbiota that can directly or indirectly induce a potential negative systemic inflammation with an impact on the development of atherothrombogenesis [25]. However, even if the inflammation of the periodontal tissue has been correlated in the last twenty years with a higher possible incidence of CVD events, different mechanisms have been hypothesised to explain the correlation between CVD and periodontal disease. Among these are the common status of systemic inflammation that determines these pathologies, the molecular mimicry, and the direct vascular damage associated with the various stages of the disease and mediated by the release of similar inflammatory and microbial agents [1, 26] (Figure 1). Although there are classic risk factors for CVD and periodontitis such as age, sex, hormonal factors, diabetes, hypertension, and hypercholesterolemia, there is nevertheless an important number of acquired factors that can be decisive as a risk factor. In fact, the initial evidence that suggested a correlation between periodontitis and CVD was that hospitalised individuals for acute CVD presented worse dental hygiene more often than healthy patients. Therefore, according to a recent study by Dembowska et al., the prevention and treatment of periodontitis, especially in patients in the so-called high-risk group for cardiovascular disease, are of crucial importance [27].

Since then, further studies and meta-analyses have been carried out, suggesting that the link between CVD and periodontal disease is very high and linked to more complex risk factors and patterns. Among the various studies, Humphrey et al. [28] showed that the presence of moderate to severe periodontitis caused a proportional enhanced risk of coronary heart disease (CHD) approximately three times greater. Specifically, the relative risk (RR) demonstrated that developing CDH in individuals affected by the periodontal disease was between 1.24 and 1.34, while it was also not significantly elevated in patients with gingivitis alone [28]. Other authors have suggested that there is a low correlation between periodontitis and CVD and that an abundant supra- and subgingival biofilm was the primary factor in the development of CVD and associated endothelial damage (Figure 1).

The studies on the microbiome made it possible to determine better which periodontal pathogens, among the bacteria of the biofilm, were strictly associated with the development of CVD and endothelial damage. Among these, the presence of Gram-negative anaerobic bacteria such as *T. forsythia* and *P. gingivalis* has been related to an enhanced risk of myocardial infarction and endothelial diseases with a 2.52- and 2.99 times higher probability compared to control patients. In fact, the increase in the levels of Gram-negative bacteria during periodontitis determines the capacity to unleash a sustained immune reaction through own pathogenic mechanism, such as lipopolysaccharide (LPS) [2] at the vascular and endothelial level, triggering a subsequent systemic host immune response closest to the deep vascular lumen [30]. Numerous publications showed that periodontal bacteria related to chronic inflammation common among periodontitis and CVD can alter the barrier function of the vascular endothelium by means of a specific epithelial-mesenchymal transition that results in vascular damage [30, 31]. This transition includes cellular events that begin with the loss of cellular structure, adhesion proteins, the extracellular matrix and the epithelial phenotype, and the mesenchymal-like nature of the vascular epithelium which is the basis of endothelial damage and dysfunction. This results in the loss of the epithelial layer's traditional morphological and physiological structure with the formation of vascular microulcerations that facilitate the penetration of periodontal pathogens and associated virulence factors into the vascular circulation [32, 33]. On the other hand, oral biofilm bacteria have also been shown to be present in the formation of atheroma with a high capacity to evade the host's immune response. Various explanations have been provided to explain how periodontal disease adversely affects the development of CVD. The first phase is linked to the direct invasion of the host's endothelial tissues by periodontal pathogens. This theory is supported by polymerase chain reaction studies on atherosclerotic

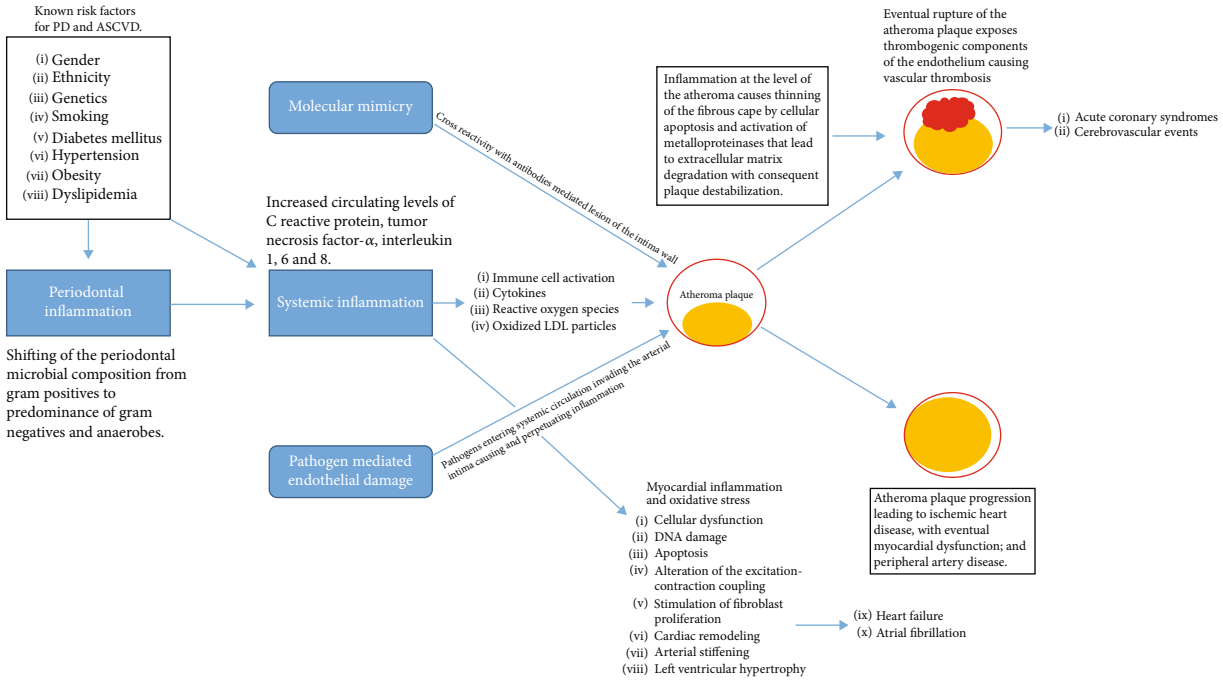

Figure 1: Proposed model for the correlation between CVD and periodontitis. From "Periodontal Disease, Systemic Inflammation and the Risk of Cardiovascular Disease," Carrizales-Sepulveda [29], Heart, Lung and Circulation 2018, Vol 27 (11): 1327-34, reproduced with permission from Elsevier.

plaques, which demonstrated the presence of Streptococcus mutans (78%), and *A. a.*, *Tannerella forsythia* (*T. forsythia*), *Prevotella intermedia* (*P. intermedia*), and *P. gingivalis* in atheromatous plaques and vascular tissues of patients affected by CVD episodes. However, at present, it has not been established how these bacteria modulate atherosclerosis, possibly due to the capacity of some bacteria, including *P. gingivalis*, to trigger specific T cells or cause a state of secondary inflammation leading to endothelial dysfunction. The most accepted theory of CVD development is that which associates the increase in systemic levels of inflammatory cytokines through an indirect pathway mediated specifically by some periodontal pathogenic bacteria. In fact, a pathogenic biofilm has been associated with the strong release of factors stimulating atherosclerotic vascular diseases, such as cytokines such as IL-1β, IL-6, IL-8, and TNF-α and the chemotactic proteins of monocytes. Some of these can lead to increased hepatic production and the release of plasma proteins such as fibrinogen and CRP. Furthermore, bacterial components like LPS associated with periodontitis are associated with the strong immune response that could trigger atherosclerosis through their influence on the endothelium with altered lipid metabolism and increased oxidative stress. This was pointed out by the results of various sets of evidence that demonstrated high endothelial dysfunction in individuals with periodontal disease [34, 35] (Figure 2).

Furthermore, the possible criticality of blood sedimentation of bacteria following nonsurgical periodontal therapy was raised. It has been observed that bacteremia frequently occurs immediately after scaling and root planing (SRP). *P. gingivalis* showed the highest frequency of incremented levels in the blood. However, after 30 minutes, bacteria levels in the blood are already decreased. Interestingly, flossing induced higher viridans streptococcal bacteremia compared to SRP, but this difference was not significant. However, it should be noted that this bacteremia condition is transient and the primary cause is oral microbiota. Nonsurgical periodontal therapy is safe in healthy patients. In subjects at risk, prophylaxis with oral antibiotics or irrigation with antiseptic may be indicated to reduce the incidence of bacteremia [36].

However, based on the current evidence, it is difficult to assert with any certainty that periodontitis represents a direct route of inflammation for an enhanced risk of CVD; this in part is due to the fact that patients with periodontitis also have also significant other risk factors such as diabetes and tobacco use, which can be a confounder in the study results [37, 38]. However, data is emerging that periodontitis, even excluding confounders, can significantly determine increased systemic inflammation related to the risk of developing CVD. In this regard, a recent meta-analysis showed increased concentrations of CRP plasma in individuals affected by periodontal disease compared to healthy patients. Also, the treatment of periodontitis induced a significant decrease in CRP levels [39]. Moreover, although several lots of evidence demonstrated a correlation between periodontitis, systemic inflammation, and CVD, some data suggests how periodontitis could be linked to CVD by genetic factors. In this regard, Czesnikiewicz-Guzik et al. [40] evaluated the effect of periodontal disease on blood pressure levels by means of Mendelian randomisation on about 750,000 participants (UK-Biobank/International Consortium of Blood Pressure-Genome-Wide Association Studies), highlighting that some similar single-nucleotide polymorphisms related

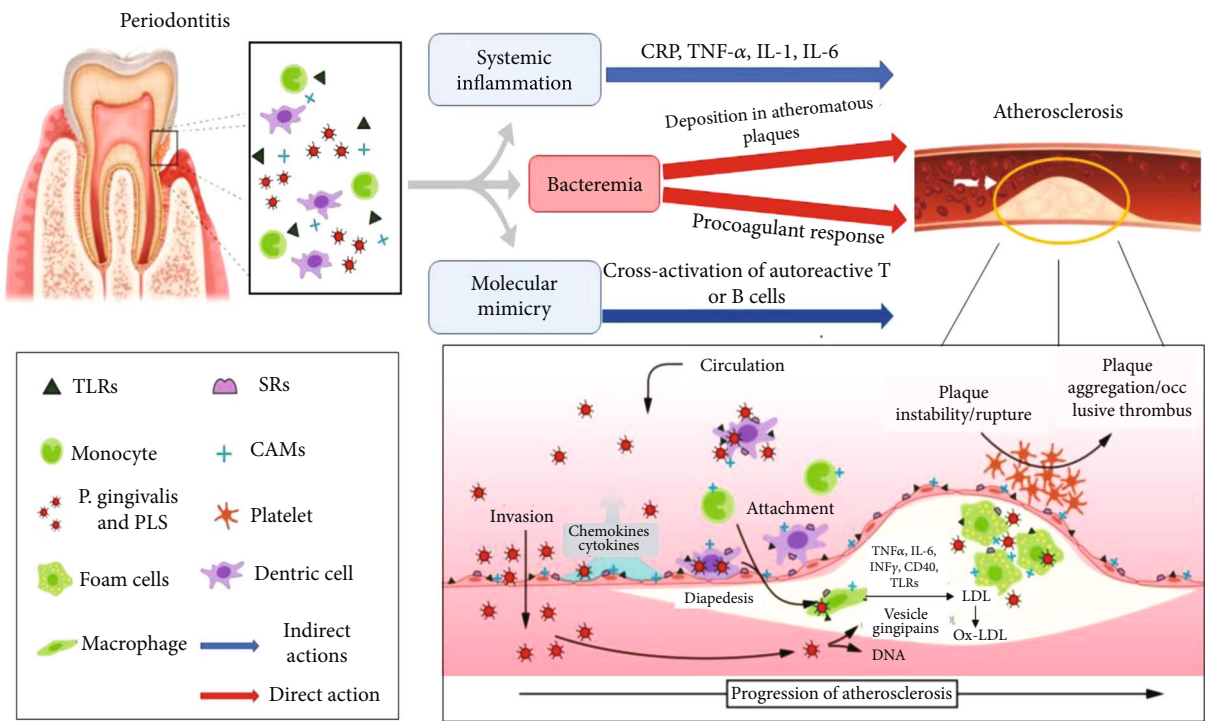

Figure 2: From "Association between Periodontal Disease and Atherosclerotic Cardiovascular Diseases: Relationship between PD and ACVD Induced by Endothelial Dysfunction."

to periodontitis cause increased blood pressure. In addition, this study in hypertensive patients with moderate to severe periodontitis proved that an active treatment of periodontitis for 2 months resulted in a significant decrease in systolic and diastolic blood pressure of 7.5 and 5.8 mmHg over 24 hours, an inflammatory mediator reduction in association with CVD, and an improvement in flow-mediated dilation, also demonstrating an influence of periodontitis on CVD through specific oxidative stress pathways [40].

2.1. Role of Oxidative Stress during PD and Cardiovascular Disease. Oxidative stress is a highly defined mechanism that promotes several inflammatory diseases, including CVD and periodontitis [41–43]. Reactive oxygen species (ROS) cause dysfunction at the cellular and extracellular matrix levels. The oxidation of proteins and lipids can result in specific damage to deoxyribonucleic acid (DNA), causing diffuse cellular apoptosis and necrosis. Furthermore, the various ROS forms cause alteration of the contractile function of muscle cells by modifying specific proteins in muscle contraction, favouring the proliferation of fibroblasts and metalloproteinases with the result of a strong remodeling of cardiac muscle fibers [44]. In this regard, experimental studies on animal models observed a close correlation between periodontitis oxidative stress and cardiac stress since rats that had been induced with periodontitis showed significantly high levels of markers of oxidative heart damage in the left ventricle when compared to rats without periodontitis.

Specifically, the ROS and associated oxidative stress are primarily involved in the onset and progression of various pathological pictures such as periodontitis, Parkinson's disease, and CVD through different modalities [45]. First, as a result of infection from the oral biofilm associated with an important inflammatory reaction on the part of the host, there is the production of ROS proteins. In this regard, evidence has shown that subjects affected by the periodontal disease had significant levels of polymorphonuclear cells that determined a local and systemic increase in ROS when compared to healthy patients [46]. Furthermore, the high oxidative stress status identified in specific phenotypes of patients with periodontitis showed that the high oxidative stress due to the release of ROS at the local (gingival) level due to the periodontopathogenic biofilm could increase the oxidative stress levels and systemic inflammation. Moreover, patients with periodontitis have been shown to have low antioxidant status in crevicular gingival fluid (GCF), with an association between this status and high ROS levels, a key factor in oxidative damage and gingival tissue destruction very similar to tissue damage associated in both patients with periodontitis and CVD. Specifically, the release of ROS induced by infection and oxidative stress due to periodontitis causes greater systemic inflammation, especially at gingival level, making it more susceptible to infections and to the transfer of risk mediators of endothelial damage into the systemic vascular circulation. Furthermore, even a state of chronic inflammation due to altered oxidative stress determines the favouring of highly predictive events of CVD [47], a primary connection between the induction of tissue damage during periodontal disease and systemic inflammation typical of CVD and endothelial damage. At the same time, conditions associated with an unregulated lifestyle together with other comorbidities (e.g., obesity and diabetes) can determine high

oxidative stress with an increase in ROS and lipid peroxidation mediators, which may also enhance the individual's susceptibility to the development of periodontitis [48]. Therefore, in patients susceptible to periodontitis, when exposed to LPS and bacterial antigen, infection promotes the recruitment of neutrophils and the synthesis of proteolytic enzymes, which further release ROS from which the gingiva stimulates a negative action of oxidative stress at a local level with consequent tissue damage. However, as moderate to severe periodontitis progresses, the gingival inflammatory status results in ROS production and inflammatory mediators, which thus spread into the systemic bloodstream. For these reasons, there is an increase in oxidative stress on vascular tissue and other districts, causing circulating oxidative stress [49]. This action has been demonstrated by the specific ability of some species of periodontal pathogenic bacteria (e.g., *P. gingivalis*), which suppress ROS detoxification by consuming large quantities of antioxidants present in the gingival tissue, accelerating the inflammatory status and causing oxidative damage and decay of the tooth support tissue [37]. This is related to a progressive change in food intake with a decrease, especially in adolescents, of fruit and vegetable intake related to a concomitant growing intake of soft drinks, which has led to an increase, over time, in the risk of developing periodontitis and CVD [50]. However, associated with altered oxidative stress status, the primary risk factor for CVD in patients with periodontitis appears to be determined by increased CRP levels. CRP plasma concentrations are increased in subjects affected by the periodontal disease due to several studies carried out on populations even on a large scale in different continents; CRP has several biological functions relevant to the pathogenesis of CVD. It is widely demonstrated that CRP is involved in atherogenesis thanks to its capacity to bind to modified low-density lipoproteins promoting endothelial dysfunction, with harmful consequences in the instability of the atheromatous plaque and associated thrombosis. However, although subjects affected by the periodontal disease have increased CRP levels, the effects of periodontal therapy on this marker are variable, especially in patients with concomitant conditions (i.e., diabetes and obesity) which act as strong comorbid factors that contribute to increasing CRP levels [51], even if with not well-defined mechanisms. Conversely, a study conducted on an Indian population showed significant increased CRP plasma concentration in subjects with CVD and periodontal disease than in patients with periodontitis alone, suggesting a synergistic mechanism of increase of this marker by patients with both periodontitis and CVD [52, 53].

Finally, some recent evidence has analysed the thrombotic potential and CVD risk due to platelets and some coagulation mediators. Among these, in addition to platelets, elevated levels of fibrinogen (a highly predictive risk factor for atherosclerosis) and its degradation products are able to stimulate the release of inflammatory mediators and reduce the synthesis of the plasminogen activator inhibitor, which is a major marker of fibrinolysis inhibition, with the risk of thrombus development [54]. Furthermore, recent evidence has proved increased fibrinogen levels in subjects affected by the periodontal disease [55, 56], also noting that the platelet count in patients with periodontitis was elevated compared to that of healthy individuals [57] and that periodontal therapy significantly reduced the platelet count [58].

Therefore, the current evidence established that subjects affected by periodontal disease present a high risk of developing CVD through various pathways; however, the increased risk of developing adverse CVD events occurs in individuals with combined periodontitis and CVD. It is also recommended, on the basis of the current evidence, that patients with periodontitis or with CVD should be included in specific periodontal treatment maintenance programs that could reduce the risk of CVD through the significant reduction of oxidative stress, release mediators associated with cardiovascular risk or factors related to thrombogenesis or atherosclerosis. Recently, Marfil-Álvarez et al. conducted a cross-sectional and analytical study that observed that the extent and severity of periodontitis are positively associated with the extent of AMI (acute myocardial infarction) as measured by serum troponin I and myoglobin levels. However, this is still a preliminary study that needs to be further investigated, which through its preliminary data provides further confirmation of the association between PD and AMI [59].

3. Periodontal Health and Disease, Diabetes and Chronic Inflammatory Conditions, and Obesity

Periodontal disease is characterised by periodontal tissue destruction, which is a combined result of a deranged immune response to an organised dysbiotic biofilm, and it has been associated with numerous systemic conditions and diseases [37].

For many years, the correlation between diabetes and periodontitis has been established. Patients affected by diabetes (both type 1 and type 2) are more susceptible to developing periodontitis, and, vice versa, people with periodontitis show an increased predisposition to diabetes, constituting a "two-way" relationship [60]. In detail, both type 1 diabetes and type 2 diabetes present the same bidirectional relationship with periodontal disease. In fact, whatever the pathogenetic cause of diabetes, autoimmune, or insulin resistance, the result is that there are high glucose concentrations in the bloodstream, with all the consequences that this implies for the correct function of the organism. In 2013, during the European Federation of Periodontology (EFP) workshop, a review explaining the biological plausibility of the bidirectional interrelationship was published [60]. It has been shown that multiple mechanisms may be involved in this sense (Figure 3). Recently, a meta-analysis found that diabetes complications are more frequent in subjects affected by periodontal disease than those without comorbidity. The authors also concluded that patients with periodontal disease have an increased risk of developing type 2 diabetes compared to healthy subjects [61].

Furthermore, several systematic reviews and meta-analyses revealed moderate evidence supporting that

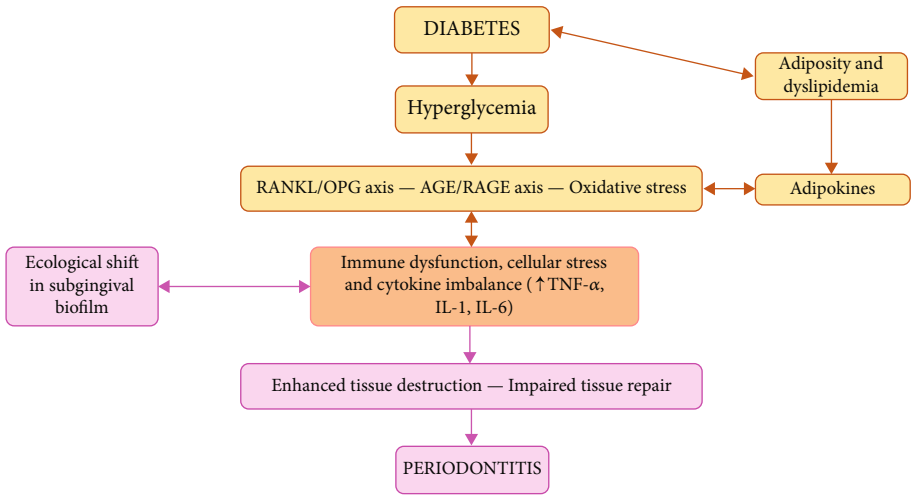

Figure 3: The hyperglycaemic state in diabetic patients induces: (1) the expression of irreversible advanced glycation end products (AGEs) and related receptors (RAGE) and (2) enhancement of oxidative stress and (3) modulation of the RANKL/OPG ratio both directly and indirectly through the AGE/RAGE axis. The result is immune cell dysfunction and cytokine imbalance. All the above, complemented by the effects of subgingival dysbiosis and the circulating adipokines produced due to diabetes-associated adiposity and dyslipidemia, induce a vicious cycle of enhanced periodontal destruction and impaired tissue repair, leading to acceleration and worsening of periodontal disease. Of course, a significant interindividual variation in these processes must be considered (genetics, age, smoking, and stress). Adapted from "A Review of the Evidence for Pathogenic Mechanisms that May Link Periodontitis and Diabetes," Taylor, J.J. [60], Journal of Clinical Periodontology, 2013, 40: S113-S134, Copyright (2013), reproduced with permission from Wiley.

periodontal treatment may modestly improve the levels of circulating mediators related to glycemic control in diabetic subjects [55–57, 62–66]. The potential mechanisms are illustrated in Figure 4.

Periodontitis is also influenced by other lifestyle factors such as diet, physical activity, and obesity [4]. Obesity is defined by the World Health Organization (WHO) as an abnormal fat accumulation which constitutes a risk factor for well-being, and it is primarily diagnosed through the body mass index. It is considered a chronic metabolic disease defined by an inflammatory response of the adipocytes associated with the release of hormones and cytokines (adipokines) which can cause changes in blood pressure, dyslipidemia, insulin resistance, and a continuous state of oxidative stress [67]. Obesity has been strongly associated with diabetes, CVD, osteoarthritis, and periodontitis [67, 68].

Many pathogenic mechanisms may link obesity and periodontitis. The promoted proinflammatory state in obese patients may increase the susceptibility to pathogenic bacteria in periodontal tissues. Therefore, obesity can be a modifying factor for periodontitis [68, 69].

Moreover, gingival inflammation may be induced/aggravated by the increased levels of circulating ROS in obese individuals [70]. Two recent systematic reviews supported the negative influence of obesity on periodontitis onset, progression, and response to therapy [71, 72]. Conversely, periodontitis is also associated with proinflammatory cytokines release and, consequently, with other chronic diseases, such as obesity [67]. A recent systematic review found that compromised masticatory function (tooth loss, a principal consequence of periodontal disease) is associated with obesity [72].

Furthermore, the results of an experimental study showed that a combination of obesity and periodontitis could have a negative synergistic effect on systematic inflammation resulting in metabolic dysregulation [73]. Therefore, obesity may be a risk factor for periodontitis via the induction of an inflammatory and hyperoxidative state. On the other hand, the destruction of periodontal tissues promotes the release of bacterial antigens and proinflammatory cytokines into the bloodstream, concurring to the development of both inflammatory diseases. Figure 5 resumes the potential reciprocal mechanisms of the interrelationship between obesity and periodontitis. Finally, interesting studies have shown a decrease in the risk of periodontitis in adults who perform high levels of physical activity. However, further prospective studies are needed to evaluate these aspects better.

Diabetes can be preceded by metabolic syndrome, a condition defined by a spectrum of metabolic abnormalities (obesity, dyslipidemia, dysglycemia, and hypertension) and associated with an enhanced risk for diabetes and CVD [74, 75]. Metabolic syndrome induces a proinflammatory state and has been plausibly correlated to obesity and periodontitis [75]. Three systematic reviews agreed on the possibility of a positive correlation between periodontal disease and metabolic syndrome, but the extent is not clear [76–78]. Further studies are needed to establish causality or to characterise the direction of this association much better.

4. Periodontal Health and Disease, Osteopenia, Osteoporosis, and Alveolar Bone Loss

In recent years, many studies have been conducted to understand whether there is a link between periodontal disease and osteoporosis. Both are slowly progressive diseases that share several common features: bone loss. In detail,

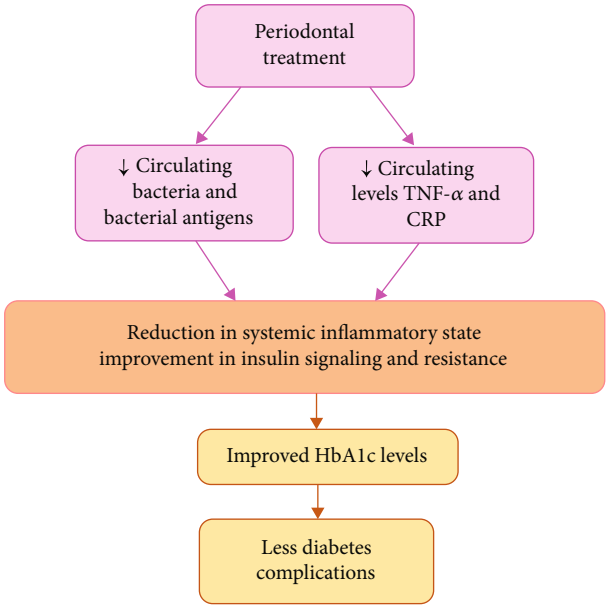

Figure 4: Scaling and root planing determines a reduction of circulating levels of proinflammatory proteins, cytokines, bacteria, and their antigens, thus inducing a lowering of the systemic inflammation and an improvement in insulin signaling and resistance. The reduction in HbA1c levels is a protective factor against the complications of diabetes. Adapted from "An Update on the Evidence for Pathogenic Mechanisms that May Link Periodontitis and Diabetes," Shapira L. & Polak D. [62], Journal of Clinical Periodontology, 2017, 45: 150-166, Copyright (2017), with permission from Wiley.

osteoporosis is a disease characterised by a weakening of the microarchitecture of bone tissue and low bone mineral density (BDM), which induces excessive bone fragility and, consequently, an increased risk of fracture [79]. Currently, the scientific community does not have a unanimous view on the possible link between the two diseases. Choi et al. [80] and Mongkornkarn et al. [81] showed a positive relationship between osteoporosis and periodontitis, while Marjanovic et al. [82] did not observe any clear link. According to several authors, systemic bone loss can significantly influence periodontal destruction. In fact, it is known that oral dysbiosis can induce a rapid resorption of alveolar bone and, consequently, periodontal destruction. However, changes in local tissue responses induced by systemic mediators involved in bone remodeling would also play an important role. RANKL, TNF-α, IL1-β, and other cytokines, implicated in both the pathogenesis of periodontal disease and osteoporosis, would stimulate the continuous production of osteoclasts by progenitor cells, initiating bone destruction and inflammation. The systemic increases in these cytokines stimulate local osteoclast activity, promote clinical loss of adhesion and destruction of alveolar bone, and accelerate the development of periodontal disease. In addition, poor smoking habits, low calcium intake, vitamin D deficiency, sex, genetics, lifestyle, menopause, and inflammation could increase BMD reduction and the risk of periodontal disease. This has been confirmed by a recent review, which considers that, from the analysis of the current literature, osteoporosis can be listed as one of the risk factors for periodontitis [79]. Osteoporosis and osteopenia are very common conditions in postmenopausal women (with a prevalence of up to 50%), and the postmenopausal state is associated with an increase in severity of periodontitis, with a prevalence of up to 30%. The promotion of alveolar bone resorption and, therefore, the increased severity of periodontal disease in affected women is associated with the role of oestrogen. Oestrogen deficiency promotes both the development of osteoporosis and periodontal disease. More specifically, gum tissue affected by periodontal disease expresses higher levels of RANKL and lower levels of OPG. Furthermore, confocal microscopy showed that 50% of T-lymphocytes and 90% of B-lymphocytes expressed RANKL in diseased gingival tissue and that these percentages were lower in healthy gingival tissue. Oestrogen reduces cytokine production by T cells (TNFα and RANKL), monocytes (IL-1 and TNF-α), and bone marrow stromal cells (IL-6, RANKL, GM-CSF, and M-CSF); increases TGF-β production by osteoblasts; and decreases osteoclast activity and differentiation. In menopause, oestrogen deficiency increases TNF-α and RANKL production by T cells, inducing increased osteoclast differentiation; it promotes osteoblast apoptosis. Therefore, periodontal disease and alveolar bone loss are likely to be more severe in menopause, and hormone replacement therapy may prevent or slow the course of periodontal disease and osteoporosis [83].

Furthermore, in a pathological condition, such as periodontal disease, the onset of a disturbance in the homeostasis of bone turnover results in destructive osteolytic processes. These mechanisms are mediated by both bacterial and host-derived factors. More specifically, proinflammatory cytokines such as TNF-α, IL-1, and IL-6 have been implicated in the activation of osteoclastic bone resorption in periodontitis. However, many other mediators have been identified that can stimulate osteoclast-mediated bone resorption: IL-11, IL-17, TNF-β, TGF-β, kinins, and thrombin. Furthermore, studies demonstrated that gingival crevicular fluid contains mediators such as IL-1α, IL-1β, and PGE_2 capable of stimulating bone resorption. This fluid is a potential source of extracellular matrix-derived biological markers of alveolar bone resorption in periodontitis [84].

5. Periodontal Health and Disease and Cancer

In the field of oncology, an innovative field of research is the possible relationships between dysbiosis, chronic inflammation, and tumors [85, 86]. Many tumors have been related to chronic periodontitis, such as head and neck cancers, colorectal cancer, breast cancer, and pancreatic cancer. Although the biological mechanisms are still not understood, according to the most accepted hypotheses that explain the correlation between periodontal disease and cancer, persistent infection and inflammation related to periodontal disease may cause a critical stimulus to chronic systemic inflammation [87–89].

Head and neck cancers (HNC) may involve different surfaces such as nasal cavities, paranasal sinuses, the pharynx, oral cavity, and salivary glands and are the sixth most

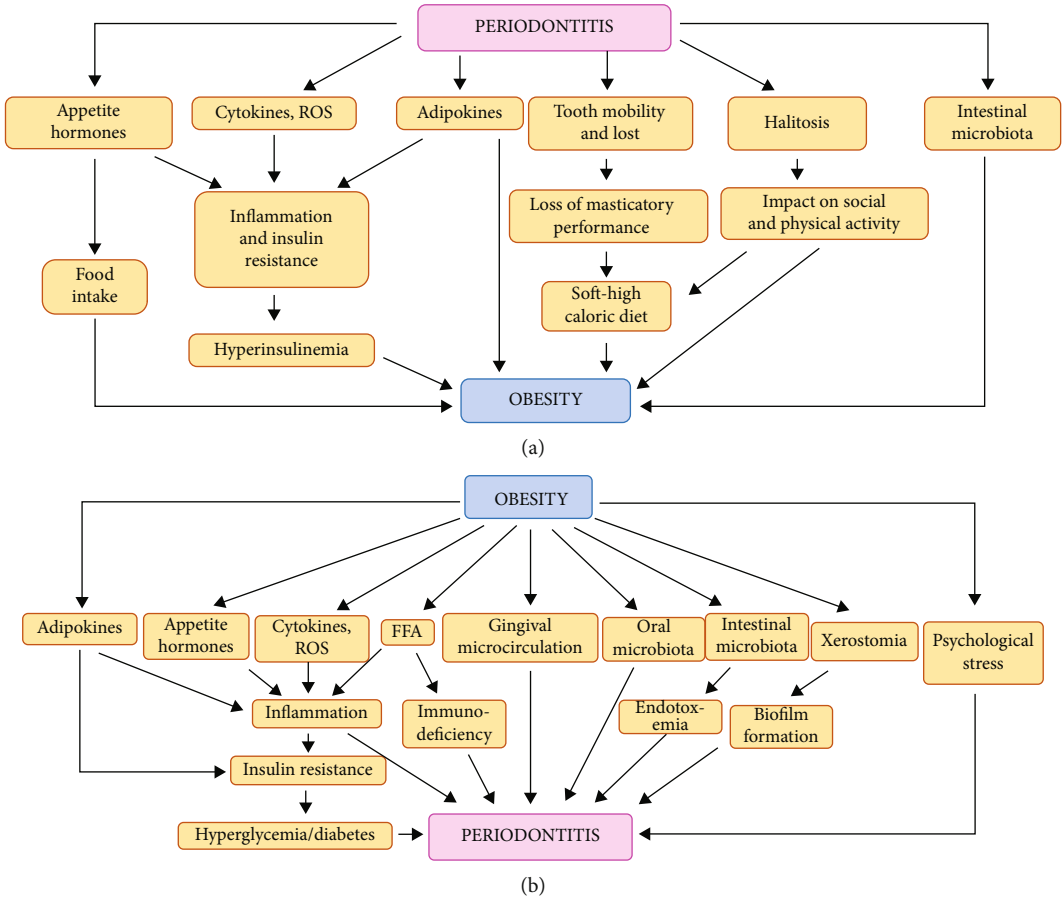

Figure 5: Potential mechanisms linking periodontal disease to obesity (a) and vice versa (b). FFA: free fatty acids; ROS: reactive oxygen species. Modified from "The Association of PD with Metabolic Syndrome and Obesity," Jepsen S. [74], Periodontology 2000, 2020, 83: 125-153, Copyright (2020), with permission from Wiley.

malignant tumors [90, 91]. Currently, the connection between the oral biofilm and the up- and downregulation of prooncogenic pathways is poorly understood [85, 86, 92]. However, oral microbiome is involved in establishing and evolving potentially malignant oral and malignant disorders [92]. For example, oral microbiome contributes to ethanol metabolism: the consequent formation of acetaldehyde has toxic effects for epithelial cells, increasing the risk of HNC and especially oral squamous cell carcinoma (OSCC) [86]. Another mechanism might involve Gram-negative bacteria lipopolysaccharides (LPS) that showed higher levels in cancerous conditions [93]. The LPS/TLR4 (Toll-like receptor 4) interaction induces inflammation and type 1 polarisation in T helper cells, suppressing IL-10 expression whose signaling deficiency increases the risk of carcinogenesis [94–96]. Furthermore, oral microbiota composition is different between healthy and OSCC patients. Genera such as *Streptococcus*, *Veillonella*, and *Rothia* are less present in cancerous tissues, while different commensal species including *Fusobacterium nucleatum* (*F. nucleatum*), *P. intermedia*, *Aggregatibacter segnis*, *Peptostreptococcus stomatis*, and *Catonella morbi* are expanded, indicating that they might be opportunistic bacteria with possible relationships with OSCC [93]. Even salivary counts of *S. mitis*, *Prevotella melaninogenica*, and *Capnocytophaga gingivalis* are increased in OSCC patients compared to healthy ones. Recent studies showed that important bacterial species involved in periodontal diseases, such as *F. nucleatum* and *P. gingivalis*, may be correlated to OSCC pathogenesis through different mechanisms: the transformation of normal epithelial cells into cancerous cells through FadA, Fap2, and LPS [97], the promotion of OSCC cell invasiveness via the upregulation of IL-8 and MMPs [98, 99], the induction of epithelial-mesenchymal transition (EMT) of oral keratinocytes by increasing phospho-GSK3ß [100], and the promotion of autophagy processes [100, 101]. Two hypotheses may explain oral microbiota alterations in neoplastic tissues: (1) neoplastic tissues drive alterations in the oral biofilm, or (2) oral microbiota is able to shift towards facilitating carcinogenesis [102, 103]. A meta-analysis that considered five eligible studies evaluated the risk of OSCC in subjects affected by periodontitis, concluding that these individuals showed enhanced susceptibility to oral cancer [104]. A similar systematic review, which selected 12 case-control studies, found that periodontitis is related to a small but significantly higher risk of OSCC; however, this correlation was attenuated after adjusting for smoking and alcohol use [105]. Therefore, it is possible to affirm a plausible

correlation between periodontal disease and OSCC; however, the strength of this correlation and the molecular pathways underlying this correlation are not fully elucidated.

With regard to management, recent studies indicate that malignant cells exposed to inflammatory signals develop chemoresistance and more aggressive biological behaviours, promoting tumor progression [106]. Therefore, dentists must look after patients affected by periodontal disease, especially smokers and consumers of alcohol and must also be encouraged to improve domiciliary and professional oral hygiene (scaling and root planing), food hygiene, and in smoking cessation [107].

Breast cancer is the most widespread malignant tumor in women and the sixth cause of cancer-related deaths [108]. The incidence is increasing because of the shorter periods of breastfeeding, later age of first pregnancy, later menopause, an earlier age of menarche, fewer pregnancies, lack of physical activity, alcohol consumption, and obesity [109, 110]. Two longitudinal studies, using data, respectively, from 1676 and 7373 women for a follow-up period of 18 and 6.7 years, reported an enhanced risk of breast cancer in patients affected by periodontitis [111, 112]. A case-control study adjusted for age and smoking status, but with a small sample, found a significant association between periodontal disease and breast cancer. However, other important studies did not find this association [113, 114]. Two recent meta-analyses concluded that periodontitis may increase the risk of breast cancer; therefore, periodontal therapy should be encouraged in the prevention of this tumor [115, 116]. However, additional studies are recommended to confirm these results and reach a consensus.

Some other cancers possibly related to periodontitis are pancreatic cancer and colorectal cancer (CRC). The gut microbiota of colorectal cancer-affected patients show a different composition compared to healthy patients, partially due to ectopic colonisation from bacterial species of the oral microbiota. Evidence for *F. nucleatum* (Fn) involvement in CRC stands out [117, 118]. In particular, this bacterial species is able to favour the growth, migration, and invasion of colorectal cancerous cells, thereby increasing IL-8/chemokine secretion [119, 120] and their resistance to chemotherapy by modulating autophagy [121]. Furthermore, a 10-year follow-up study based on 68273 adults evaluated the role of periodontal disease as a risk factor for cancer mortality [122]. However, from the study conducted by Mesa et al. [123], there are still unknown aspects that need to be clarified regarding the association between PD and CRC. Certainly, understanding how it colonises the gastrointestinal tract remains a hotly debated topic. There are currently two theories: a direct route through the oro-gastrointestinal tract or an indirect route through the blood. Certainly, what is known is that, in the intestine, bacteria would find an ideal microenvironment to disrupt the balance between the local microbiota and the immune system, resulting in dysbiosis. A dysbiotic gut biofilm can cause intestinal diseases such as inflammatory bowel disease, irritable bowel syndrome, and CRC. Finally, the perpetuation of a proinflammatory environment would lead to a chronic exposure to mediators of inflammation, activation of oncogenes, and development of CRC. The authors found an increased pancreatic cancer mortality in patients affected by periodontal diseases.

Cancer currently represents one of the most challenging worldwide health problems in the contemporary age. Primary prevention, and therefore the knowledge of possible etiological factors, is of fundamental importance prognostically. Therefore, delving into the biological mechanisms and the real impact on cancer of an extremely widespread disease such as periodontitis will be a major challenge for research.

6. Periodontal Health and Disease, Infertility, and Adverse Pregnancy Outcomes

In recent years, research has observed how periodontal disease and the bacteremia it causes can have a negative impact on reproduction health and fertility issues in men and women and on adverse pregnancy outcomes, such as preterm birth, preeclampsia, miscarriage, and low birth weight in children [124, 125]. Although little information shows a direct relationship between bad periodontal health and fertility problems, it is well known that systemic bacteremia, caused by subclinical infections, can hamper reproductive function in both sexes [126–128]. Gingival tissues contain receptors for the sex hormones, oestrogen and progesterone, which are susceptible to hormonal imbalances that occur in women during the menstrual cycle, pregnancy, menopause, hormone, and contraceptive therapies. The interplay between receptors and ligands and high metabolic activities induces important modifications in the permeability and underlying microcirculation of gingival capillary vessels.

Such changes may result in an increased inflammatory reaction, suppression of cell-mediated immunity, and changes in the periodontal milieu's microflora, a mechanism which can worsen existing periodontitis [129, 130]. During pregnancy, if there is preexisting gingival inflammation, the metabolism of these hormones in the gum is higher than in normal periodontal tissues [131]. It has been observed that maternal bacteremia, induced by periodontal infection outbreaks, can hinder fetal development and the achievement of pregnancy, as endotoxins and bacterial intermediates in the bloodstream result in bacteremia in the uterus [132]. Therefore, oral bacteria can penetrate and colonise the maternal-fetal unit, thus leading to infertility and gestational disorders [133]. Moreover, a study conducted by Lafaurie et al. recently showed that periodontal disease is an independent risk factor from other important risk factors for an adverse pregnancy outcome (preterm delivery, low birth weight, and preeclampsia), indicating that periodontal disease prevention should be included in preconception and antenatal care programs [134]. Two different mechanisms have been suggested to describe the link between periodontal disease and adverse pregnancy outcomes. The first proposed mechanism is that periodontal bacteria, generated in the gingival biofilm, can move from the oral cavity and enter the intra-amniotic fluid and fetal circulation, through the bloodstream and placenta, directly influencing the fetoplacental unit and thus causing bacteremia. The second mechanism involves the systemic spread of endotoxins and/or

inflammatory mediators originating from periodontal bacteria and released from the subgingival inflammatory site, which are transported to the fetoplacental unit via the bloodstream, stimulating an inflammatory response, capable of affecting fetal growth or leading to pregnancy complications (Figure 6) [135].

In a study carried out in Australia by Hart et al., it was observed that women with periodontitis needed about two months more time to reach the required gestation (7.1 months) than women without periodontitis (5 months) [126]. Preterm birth in women with periodontitis is thought to be induced by the systemic spread of oral bacteria and elevated levels of proinflammatory cytokines produced, which are delivered to the systemic circulation in inflammation and cause myocyte contraction and preterm pregnancy. To confirm this, *P. gingivalis* and *F. nucleatum* have been discovered in amniotic fluid samples or in the placenta of mothers with premature birth and periodontitis, while *P. gingivalis* and *A. a.* have been detected in the amniotic fluid of pregnant women with periodontitis [136]. Studies on mice have demonstrated that *P. gingivalis* can have a negative impact on pregnancy: LPS from *P. gingivalis* caused placental and fetal growth restriction and placental resorption [137]. Another study observed that antibodies against *P. gingivalis* caused fetal loss when administered passively [138]. Furthermore, inflammatory mediators produced by periodontal pathogens, released from the circulation or produced by infected endometrial and placental tissues, play a crucial role in the pathogenesis of preterm low birth weight [133]. In several high-quality randomised controlled trials, it has been observed that nonsurgical periodontal therapy carried out during the second trimester of gestation does not improve pregnancy outcomes, because therapy carried out from the fourth to the sixth month of pregnancy is late in preventing placental colonisation by periodontal pathogens and is not able to act on pathogen-induced lesions to the fetoplacental unit sufficiently. This would suggest that intervention during the preconception period could induce more significant improvements [139]. In contrast, a randomised controlled trial found that periodontal treatment was not linked to an elevated risk of preterm labour and could significantly improve periodontal status and lower the risk of preterm birth [140]. Several studies have observed an important connection between periodontitis and the risk of developing preeclampsia, a multisystem gestational disorder characterised by proteinuria and maternal hypertension after 20 weeks of gestation. The connection between the two diseases would again be attributable to the placental inflammatory response resulting from the bacteremia induced by periodontal pathogens migrating from periodontal tissues through the bloodstream. Thus, during infection, oral microorganisms release cytokines and immunoglobulins that limit the growth of pathogens and, at the same time, increase the inflammatory reaction, probably leading to embryonic or fetal lesions [141].

While the association between periodontitis and preterm birth with low birth weight and female infertility is well researched and established, the connection between male infertility and periodontitis is not well understood. Currently, there are few and often conflicting studies available. A possible link between male infertility and periodontal disease has only recently drawn attention. Male infertility can be associated with multiple factors, such as sexual dysfunction or altered sperm quantity and quality. Infections can affect sperm quality and quantity, so periodontitis can affect male fertility. In a 2011 study by Klinger et al. [142], it was observed that profound periodontal pockets and loss of clinical attachment are linked to the submotility of sperm. This finding is supported by the observation of Zhu et al. [143] showing that sperm quality deteriorated with worsening chronic periodontitis. Práger et al. [144] observed that the percentage of participants with dental tartar and bleeding on probing (BOP) is significantly higher among men with idiopathic infertility. A recent case-control study showed a significantly higher prevalence of moderate/severe periodontitis in men with semen abnormalities (case group) compared to the control group of men with normospermia [145]. In contrast to the above-mentioned studies, a study by Pásztor et al. showed that adverse periodontal conditions are not linked to any abnormalities in seminal parameters [146]. The relationship between erectile dysfunction (ED) and PD is endothelial dysfunction. Recently, a study evaluated the prevalence of periodontitis in patients with ED, observing that patients with ED showed a greater extent of moderate or severe chronic periodontitis than the control group [147]. A prospective study, also conducted by the same research group, observed that patients with periodontitis and ED showed a 3.7-fold increased risk of suffering major cardiovascular adverse events after a mean follow-up of 4.2 years [148]. In conclusion, the literature review suggests that preventive actions against periodontal disease in both men and women are justified in order to avoid problems such as infertility and preterm birth.

7. Periodontal Health and Disease, Neurological Diseases, and Alzheimer's Disease

Alzheimer's disease is a gradual neurodegenerative disease that results in a progressive and irreversible decline in memory, cognition, language, and learning ability. Cognitive impairment has been associated with the generation of synaptotoxic β-amyloid plaques and hyperphosphorylated tau proteins in brain areas linked to enhanced cognitive functions [149]. β-Amyloid plaques and neurofibrillary tangles (NFTs) are not pathognomonic of Alzheimer's disease but can be found in other pathological conditions typical of the central nervous system (CNS), including chronic infections, which have these specific histopathological hallmarks. Recent studies also show that β-amyloid has significant antimicrobial activity, prompting the idea that infections can prompt its generation and deposition as plaques in the brain [150, 151]. Until a few years ago, the brain was thought to have "immunological privilege" status, but it has been discovered that it can undergo various inflammatory processes, such as the activation of complement, glial cells (microglia and astrocytes) and lymphocytes and the

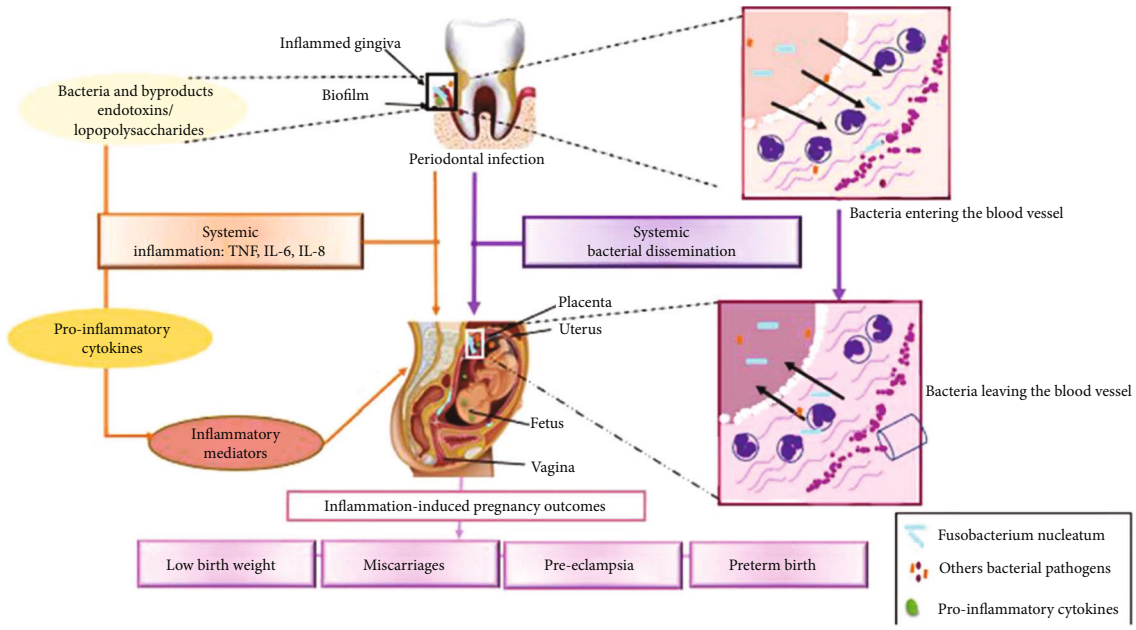

Figure 6: Periodontal disease and adverse pregnancy outcomes. From "Oral Microbiome and Pregnancy: A Bidirectional Relationship," Saadaoui [135], Journal of Reproductive Immunology, 2021, 2021: 103293, Copyright (2021), with permission from Elsevier.

expression of cytokines, chemokines, and reactive oxygen species (ROS), which lead to neuronal apoptosis and dysfunction of the blood-brain barrier (BBB), essential for the integrity and proper working of the CNS, promoting the development of Alzheimer's disease [152]. Thus, neurodegeneration is due both to direct damage caused by β-amyloid plaques and tau aggregates and to the innate immune reaction activated to remove these clusters from the brain, which negatively affects neurodegeneration. Inflammation is the connection between periodontitis and Alzheimer's disease, so the inflammatory process associated with periodontal disease could influence the pathogenesis and prognosis of Alzheimer's disease [153]. This has been shown by an elevation of proinflammatory cytokines in older patients with Alzheimer's disease and periodontitis [154]. In a study on mice, it was noted that chronic systemic infection induced *P. gingivalis* led to β-amyloid accumulation in the brain, in middle-aged mice, and it also prompted β-amyloid accumulation in inflammatory monocytes/macrophages through the activation of CatB/NF-κB signaling [155]. This result is in accordance with findings from a further study in which it was observed that oral infection induced by *P. gingivalis* leads to neurodegeneration and extracellular deposition of β-amyloid 42 in the brains of young adult wild-type (WT) mice demonstrating how chronic low-grade periodontal pathogenic infection resulted in the appearance and development of AD-like neuropathology [156]. In 2019, several studies demonstrated a correlation between periodontitis and early cognitive impairment and Alzheimer's disease [149, 157]. In the same year, another study was carried out by Gaur and Agnihotri who observed that *A. a.* activates the secretion of proinflammatory cytokines from microglia [149]. In a cohort study of 219 individuals (110 AD patients and 109 healthy volunteers), conducted by Noble et al. [158], it was found that patients with elevated serum IgG against *Actinomyces naeslundii* (which is linked to periodontal disease) were at increased risk of developing Alzheimer's disease. The authors concluded that periodontitis bacteria are connected to Alzheimer's disease via microbial toxins, inflammatory agents, and serum antibodies. Thus, chronic inflammation developed by these bacteria is a susceptible predictor for the onset of Alzheimer's disease. Lipopolysaccharides of *P. gingivalis* and *T. denticola* have been isolated from the human brain of Alzheimer's disease sufferers, further supporting the hypothesis that virulent elements of these pathogens could have a role in the development of brain inflammation and Alzheimer's disease. Ueda et al. [159] suggest that leptomeningeal cells, which are implicated in the transmission of systemic inflammatory signs between brain-resident macrophages and microglia, secrete inflammatory mediators during periodontitis. In this regard, Kamer et al. [153] observed that Alzheimer's disease patients with periodontitis have an increased level of specific antibodies against periodontal bacteria and TNF-α. Similar results have also been reported from further studies with a higher level of TNF-α in the serum of patients with AD and periodontal disease [160]. Although the literature review indicates that there is an association between periodontal pathogens and Alzheimer's disease, more longitudinal studies need to be conducted to confirm with certainty that periodontal pathogens and antibodies directed against them are directly involved in neurodegeneration in Alzheimer's disease. However, it should be underlined that bad oral hygiene plays a role in the development of chronic periodontitis and may indirectly enhance the risk of Alzheimer's disease. Conversely, patients with Alzheimer's disease have a limited ability to maintain even little, if any proper oral hygiene or even to see a dentist for the treatment of oral hygiene, which increases the risk of periodontitis but also the progression of Alzheimer's disease [155, 161]. In conclusion, evidence

suggests that preserving good oral health can have a preventive effect against Alzheimer's disease.

8. Periodontal Health and Disease and Respiratory Disease

During the last decade, the association between periodontal disease and respiratory disorders has been much discussed, and researchers have not yet come to a unanimous opinion. Although several studies claim that proper oral hygiene prevents the development of respiratory diseases, more evidence is needed to verify this [19, 162, 163]. Respiratory diseases and periodontitis are the most common human diseases in the world [164]. Pneumonia, an infectious disease of the lung parenchyma, asthma, and chronic obstructive pulmonary disease (COPD), a chronic condition that includes both bronchitis and emphysema and characterised by airflow blockage caused by an intensified chronic inflammatory response within the airways, may share several immunological processes and etiopathological aspects with periodontal disease [165, 166]. It has been observed that the course of lung disease may be influenced by infective and inflammatory processes like periodontitis [167]. This relationship would be determined by microorganisms in periodontal pockets, especially anaerobic bacteria, which can be drawn into the lower airways, which act as a further inflammatory load in lung tissues. COPD and periodontal disease have comparable pathophysiology, which includes inflammation, recruitment of neutrophils, and release of proteolytic enzymes, leading to pulmonary alveolar disruption or progression of periodontal disease. More studies are needed to confirm a direct connection between COPD and periodontal disease, although several studies report a significant relationship between the two diseases. One study showed that lack of dental care may also be associated with an increased risk of COPD [168]. There have been various systematic reviews evaluating the association between poor oral health and pulmonary diseases—including the one conducted by Ferreira et al. [169] which evaluated the association between periodontal clinical parameters and asthma, the one conducted by Shi et al. [116] who analysing the relationship between worse periodontal conditions and COPD; and the one conducted by Cagnani et al. [162] regarding the association between periodontal disease and pneumonia; these authors concluded that there is an association between periodontal disease and respiratory diseases. These results only consider the association between periodontal clinical parameters, like probing depth (PD), clinical attachment level (CAL), bleeding on probing (BOP) or plaque index (PI), and respiratory disease. In contrast, a recent systematic review found that there is a weak correlation between periodontitis and pulmonary disease [170]. A recent study by Dembowska et al. indicated how inhaled antiasthmatic drugs affect both general and oral health. ICSs (inhaled corticosteroid therapy) worsened BOP at the anterior sextant in patients with asthma but no periodontal disease [171]. In conclusion, further investigation will be required to validate a true association between periodontal diseases and to be able to state with certainty that preventive oral hygiene practices and periodontal care may have a potential future role in decreasing exacerbations of respiratory disease and increasing patients' quality of life.

9. Conclusions and Future Directions

Periodontitis is a multifactorial etiology whose pathogenesis depends on the complex interactions between the individual's immune reaction and periodontal pathogens that can evolve in association with specific environmental factors. Although it is agreed that the primary etiology of periodontal disease is infectious in nature due to the supra- and subgingival biofilm, the comprehension of the etiopathogenesis and progression of periodontitis in its various forms has evolved over the years. It has also been well established that factors such as genetics and the role of the immune system contribute significantly to the resistance and susceptibility of the individual to periodontal disease as well as to its rapid or slow evolution.

Recently, research and clinical studies in the periodontal field have made steps forward, with the demonstration of the concept of "periodontal medicine" and which have strongly demonstrated a strict association between periodontitis and oral and systemic health. It is clear that the outcomes of the impact of periodontitis are increasingly considered relevant by health stakeholders and beyond. In this regard, the analysis of oral health-related quality of life measures employed in recent years has highlighted important adequate psychometric properties in terms of reliability and validity to record a change in oral health-related quality in patients with periodontitis and how periodontal treatment is useful for improving both patients' oral and systemic well-being.

Definitive evidence for the role of periodontitis in most chronic systemic diseases is lacking since there have been few well-conducted clinical trials. Moreover, from a careful and in-depth analysis of the various studies in literature, it is not possible to define a strict bilateral association between systemic and periodontal diseases well. However, the research carried out in recent decades in this regard has clearly defined that the comprehension of the etiopathogenesis of periodontitis is translated from the consideration of an exclusively bacterial origin to a more holistic consideration, with a true cause-effect relationship of a multifactorial nature in which genetic, exogenous, and lifestyle factors in general can significantly affect oral health. However, with the ever-increasing understanding of periodontal disease becoming known, our ability to provide "personalised periodontal treatment" is now a fundamental term to address. In the coming years, in order to obtain an effective treatment of periodontal disease, especially in the long term, it will increasingly require a careful analysis of the individual modifiable risk factors such as smoking, diabetes control, and diet, which, through a personalised approach, will surely improve in time the long-term outcomes of the therapies and the quality of patient care at a local and systemic level at the same time. However, large-scale studies in different types of populations will be required to understand the correlation between systemic and PD better and also the impact

that periodontitis plays in the etiology, progression, and therapeutic success of systemic diseases.

Data Availability

Data of the present manuscript is available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions

Conceptualization was made by G.I. and E.M. Methodology was made by S.S. and A.P. Validation was made by S.M.L. and E.M. Resources was made by R.P. and R.S. Manuscript revision was made by R.S. Writing—reviewing and editing—the manuscript was made by G.I., S.S., and A.P. All authors have read and agreed to the published version of the manuscript. Gaetano Isola and Simona Santonocito contributed equally and share the first authorship.

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Research Article

NLRP3 Inflammasome Expression in Gingival Crevicular Fluid of Patients with Periodontitis and Chronic Hepatitis C

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The study is aimed at assessing the impact that periodontal disease and chronic hepatitis C could have on gingival crevicular fluid levels of the NLRP3 inflammasome, caspase-1 (CASP-1), and interleukin-18 (IL-18) and at evaluating whether the increased local inflammatory reaction with clinical periodontal consequences is correlated to their upregulation. Patients were divided into four groups, according to their periodontal status and previously diagnosed hepatitis C, as follows: (i) CHC group, chronic hepatitis C patients; (ii) P group, periodontal disease patients, systemically healthy; (iii) CHC + P group, patients suffering from both conditions; and (iv) H group, systemically and periodontally healthy controls. Gingival crevicular samples were collected for quantitative analysis of the NLRP3 inflammasome, CASP-1, and IL-18. CHC + P patients expressed the worse periodontal status and the highest NLRP3, CASP-1, and IL-18 levels, the difference being statistically significant ($p < 0.05$). The P group patients also expressed significantly more elevated NLRP3, CASP-1, and IL-18 levels, as compared to nonperiodontal patients (CHC and H groups). Chronic hepatitis C and periodontal disease could have a significant influence on the upregulation of NLRP3 inflammasome and its components, possibly contributing to an increased local inflammatory reaction and clinical periodontal consequences.

1. Introduction

Recent research on the complex molecular mechanisms of the inflammatory reaction has led to the development of the "inflammasome" concept, a multiprotein, oligomer compound, governing inflammation in its early stages [1, 2]. One of these inflammasomes, the NLRP3 complex, has a crucial role in innate immunity and inflammatory mechanisms [3, 4]. It consists of a Nod-like receptor (NLR) that mediates the activation of protease enzymes (Caspase 1 (CASP-1)) and further regulates the expression of pioneer, key, proinflammatory cytokines, such as interleukin-18 (IL-18) [3].

The periodontal inflammatory process, which defines periodontitis (P), can interact with the chronic inflammatory reaction generated by viral hepatic infection, resulting in systemically important pathogenic implications [5]. The activation of the NLRP3 is caused by bacterial stimuli, as lipopolysaccharides (from *Porphyromonas gingivalis*) and

bacterial RNA or by endogenous ones, as extracellular ATP, uric acid, or cholesterol crystals [3]. Despite its elevated concentrations within epithelial tissues, such as the gingival epithelium [6], the NLRP3 inflammasome has received little scientific attention from periodontal researchers, as part of the pathogenic mechanisms governing P alone, or in association with systemic diseases [7–9]. A recent study by Hernandez et al. highlighted the upregulation of the NLPR3 inflammasome in patients with periodontitis and uncontrolled type 2 diabetes [8]. This could also be the case in patients with periodontitis and cardiovascular diseases, as highlighted by Mahendra et al. [10]. The activation of the NLRP3 inflammasome during periodontal inflammation has been illustrated by using samples of serum and saliva, by Isola et al. [11]. Thus, the study of this particular inflammasome (NLRP3) and its components (CASP-1 and IL-18) was chosen as a focus point of our research, considering the promising results of its assessment as a potential biomarker for the periodontal clinical status [2] and its upregulation in periodontal patients with uncontrolled type 2 diabetes [6, 8, 11].

Chronic hepatitis C (CHC) patients can often manifest important oral health issues, that can have a negative impact on their life's quality, adding to the pathological manifestations of the liver disease and its complications [12]. When seeking dental treatments, CHC patients may face various elements of difficulty, such as high personal anxiety or modified healing and recovery processes after dental and periodontal surgery, that limit the complexity of therapeutical options [13]. Corroborated with possible behavioral particularities, CHC patients may comprise more risk factors for the onset of P, leading to its clinical manifestation, triggered by the accumulation of subgingival bacterial plaque deposits [5, 14].

In essence, both CHC and P generate a chronic inflammatory reaction, such a pathologic event being driven by proinflammatory mediators that control its extent and intensity [15, 16]. Our previously published study, focusing on the gingival crevicular fluid (GCF) assessment of interleukin-1alpha's and interleukin-1beta's involvement in the pathogenic process of periodontitis patients with chronic hepatitis C, highlighted a significantly worsened periodontal status and increased levels of these cytokines in patients with both diseases, as compared to those of nonhepatitis C patients suffering from periodontitis [17]. This suggests the negative impact that hepatic pathology may have on local periodontal inflammation.

Hence, the purpose of the present study was to evaluate the GCF levels of the NLRP3 inflammasome and its components in patients with P and CHC and to determine if its upregulation is influenced by these joined pathologies, periodontitis, and chronic hepatitis C, leading to a more exacerbated manifestation of periodontal disease in CHC patients.

2. Materials and Methods

2.1. Study Design. The study's design was approved by the Ethical Research Committees at the University of Medicine and Pharmacy of Craiova, Romania, and at the Craiova Emergency County Hospital fulfilling the requirements of the European Union's General Data Protection Regulation (GDPR) on patient data protection and discretion and the 1975-2013 Declaration of Helsinki. The study approached two main research directions: the first one, clinical and metabolic, consisting of assessment of the patient's periodontal status and certain metabolic parameters, which reflected their systemic and hepatic status; the second direction, immunological, consisting of a quantitative determination of the targeted inflammatory markers within the patients' gingival crevicular samples. The data resulting from the two study directions was subjected to intra- and intergroup statistical analysis for significance and correlation identification.

2.2. Patient Selection. Chronic hepatitis C participants were selected from patients attending the Gastroenterology Clinic of the Craiova Emergency County Hospital, while nonhepatitis C participants were selected from the patients addressing the Periodontology Department of the Dental Medicine Faculty of the University of Medicine and Pharmacy of Craiova. For inclusion, all hepatitis C patients had asymptomatic forms of disease. All periodontal patients had to meet the diagnosis criteria issued during the 1996 World Workshop in Clinical Periodontics: (i) minimum of 20 existing natural teeth, (ii) minimum of six periodontal pockets in two different quadrants (probing depth ≥ 5 mm), (iii) bleeding on probing, and (iv) minimum one mm clinical attachment loss [18]. According to the 2018 new classification system of periodontal diseases [19], this would correspond to Stage II (moderate) and Stage III (severe) periodontitis, in terms of severity and Grade A (slow) in terms of rate of progression. For inclusion in the healthy control group, patients had to show no symptoms and history of periodontal or gingival disease (no periodontal pockets/bone loss, no gingival bleeding) and no declared systemic diseases.

The exclusion criteria consisted of (i) anti-inflammatory or other type of medication taken in the last 30 days prior to examination, (ii) previous antiviral anti-HCV therapy, (iii) antibiotic treatment taken in the last 90 days prior to examination, (iv) pregnancy, (v) active smoking status, and (vi) other associated systemic diseases.

Upon applying all of the above inclusion/exclusion criteria and obtaining the informed and written individual consent for entering the study, the 62 participating patients were divided into four study groups: (i) CHC + P group: 18 patients (aged from 54 to 79 years) suffering from both CHC and P; (ii) CHC group: 14 patients (aged from 50 to 61 years), suffering only from CHC and being periodontally healthy; (iii) P group: 15 patients (aged from 42 to 63 years), suffering only from P and being systemically healthy; and (iv) H group: 15 controls (aged from 40 to 61 years), periodontally and systemically healthy patients.

2.3. Dental and Periodontal Assessment. All participating patients were subjected to a complete oral and periodontal examination, which was used for the diagnosis of possible periodontal conditions. During this examination, the level of dental hygiene was evaluated, by using the O'Leary Index [20].

After the assessment of the oral hygiene level, an ultrasonic scaling procedure of calculus and plaque deposits' removal was conducted when required, in order to enable unbiased and undisturbed periodontal probing. The periodontal measurements were performed by using manual University of North Carolina probes (Hu-Friedy, Chicago, Illinois, USA). The periodontal probing provided periodontal parameters such as (i) periodontal probing depth (PD)—in six points for each tooth (messial, central, and distal for the buccal and oral aspects of the teeth), (ii) clinical attachment loss (AL), and (iii) bleeding on probing index (BPI). All periodontal probing measurements were performed by a single, calibrated, examiner. The attachment loss was calculated for each probing site by using the formula: periodontal probing depth (mms) – gingival margin (mms) = attachment level (AL, mms).

2.4. Metabolic and Hepatic Assessment. From each patient, a blood sample was collected for the laboratory tests required for the assessment of their metabolic status. This included standard laboratory blood tests such as (i) level of serum glucose—glycemia, as indicator for the glucidic metabolism (reference range 90-110 mg/dL) [21]; (ii) level of total cholesterol, as indicator for the lipidic metabolism (reference range 140–180 mg/dL) [21]; and (iii) level of triglycerides, as a complementary indicator for lipidic metabolism (reference range 140–160 mg/dL) [21]. Specific parameters which allowed the assessment of the hepatic function were also tested (i) aspartate aminotransferase (AST) (reference range 6-34 IU/L) [22], (ii) alanine transaminase (ALT) (reference range 20-60 IU/L) [22], and (iii) gamma-glutamyl transferase (GGT) (reference range 8-38 IU/L) [22]. The hepatic status was also evaluated through the use of ultrasound elastography imaging test (FibroScan 530, Echosens, Paris, France) for the degree of liver fibrosis in CHC patients. The scale used to assess the level of liver fibrosis was from zero (= absent) to four (= liver cirrhosis) [23].

2.5. Gingival Crevicular Fluid Sampling. After the supragingival plaque was removed with sterile cotton bullets, gingival crevicular fluid (GCF) was sampled from each of the 62 patients, by using absorbent paper strips (PerioPaper, Oraflow Inc., Smithtown, NY, USA). The two teeth with the deepest pocket depths were selected for sampling, using different paper strips for each tooth, inserted simultaneously at the two teeth. The paper strips were inserted within the periodontal pocket until mild resistance was felt and kept in place for 30 seconds. Upon removal, the strips were visually inspected for blood stains. In order to prevent the strips' contamination with saliva or blood, absorbent cotton roll isolation and air suction were used during the sampling procedure. The quantity of sampled GCF was standardized using the Periotron 8000 device (Oraflow Inc., Smithtown, NY, USA). Afterwards, the paper strips originating from both teeth were pooled together into a plastic microtube containing saline buffer solution (PBS). The sampling procedures were additionally repeated two times. Thus, three microtubes were obtained for each patient, corresponding to the three targeted mediators. The microtubes were preserved at -20 degrees Celsius, until analysis.

2.6. Immunological Assessment. After the GCF samples were collected from all 62 patients, they were transferred to the Immunology Laboratory of the University of Medicine and Pharmacy of Craiova for specific preparation and assessment. For the detection of the targeted inflammatory mediators (NLRP3 inflammasome, CASP-1, and IL-18) within the gingival fluid, the enzyme-linked immunosorbent assay (ELISA) method was used. Specifically designed commercial test kits were used for each of the mediators, (i) NLRP3—OKEH03368 (Aviva Systems Biology, San Diego, USA) (range 0.312-20 ng/mL), (ii) CASP1—OKEH01146 (Aviva Systems Biology, San Diego, USA) (range 15.6-1000 pg/mL), and (iii) IL-18—OKCD00106 (Aviva Systems Biology, San Diego, USA) (range: 15.6-1000 pg/mL), according to the manufacturer's indications and prescribed work method. The ELISA method was performed with a standard optical analyzer, at 450 nm wave length.

2.7. Statistical Analysis. All data was centralized and expressed as mean and standard deviation. Afterwards, it was subjected to statistical analysis (GraphPad Software, LLC, San Diego, CA, USA) in order to detect the differences between patients with CHC + P and CHC, CHC + P and P, and CHC and P groups, using Mann–Whitney *U* test (statistically significant at 5%, two-tailed). For categorical variables, the comparisons between the groups were evaluated using the Fisher's exact test. The existence of statistical correlations between the different types of data was assessed using Pearson coefficients. A power computation (G∗ Power 3, University of Dusseldorf, Germany) was completed and revealed that, for our samples, the effect size was large (1.468) and power equal to 0.979 (alpha equal to 0.05).

3. Results

3.1. Demographics, Glucose, and Liver Fibrosis Level. There was no statistical difference regarding the average age of the study's participants or the gender distribution among the study groups ($p > 0.05$) (Table 1). There was no significant difference concerning the level of serum glucose among the test groups. In the hepatitis C patients (groups CHC + P and CHC), there was a similar level of liver fibrosis (predominatly F1, 61.11% in the CHC + P group and 64.28% in the CHC group), with no significant difference ($p > 0.05$). The CHC + P group included significantly more patients with F2 stage liver fibrosis than the CHC group (Table 1).

3.2. Patients with CHC + P Expressed the Most Severely Modified Clinical Periodontal Status. The patients of the CHC + P group showed a significantly worsened periodontal status as compared to P patients, in terms of periodontal probing depth and clinical attachment loss (Table 2). A statistically significant difference was also found between the plaque index and gingival bleeding index of the CHC + P and P groups (Table 2).

Table 1: Study's demographic characteristics, serum glucose level, liver fibrosis, and statistical significance (p value) for differences between test groups.

Parameter	Groups				p CHC + P vs. CHC	p CHC + P vs. P	p CHC vs. P
	CHC + P	CHC	P	H			
Age (years)	62.61	57.76	55.86	50.13	> 0.05	> 0.05	> 0.05
Gender (male/female)	8/10	5/9	8/7	6/9	> 0.05	> 0.05	> 0.05
Glucose (mg/dl) ± SD	108.83 ± 27.22	103.14 ± 16.54	98.2 ± 13.43	95.53 ± 8.75	> 0.05	> 0.05	> 0.05
Liver fibrosis (%)			—	—		—	—
F1	61.11	64.28			> 0.05		
F2	22.22	7.14			< 0.05		
F3	11.11	14.28			> 0.05		
F4	5.55	14.28			> 0.05		

Table 2: Study's clinical results and statistical significance (p value) for differences between test groups.

Parameter	Groups				p CHC + P vs. CHC	p CHC + P vs. P	p CHC vs. P
	CHC + P	CHC	P	H			
Periodontal probing depth (mm) ± SD	3.85 ± 0.92	1.45 ± 0.2	3.76 ± 0.53	1.24 ± 0.2	< 0.05	< 0.05	< 0.05
Clinical attachment loss (mm) ± SD	5.42 ± 1.61	1.83 ± 0.42	3.98 ± 0.51	0.52 ± 0.19	< 0.05	< 0.05	< 0.05
Plaque index (%) ± SD	57.5 ± 17.27	25.85 ± 13.63	39.33 ± 12.61	13.86 ± 4.03	< 0.05	< 0.05	< 0.05
Gingival bleeding index (%) ± SD	50.53 ± 11.98	7.92 ± 5.31	43.6 ± 13.18	7.4 ± 2.35	< 0.05	< 0.05	< 0.05

3.3. Patients with CHC + P Exhibited Significantly Elevated Levels of Proinflammatory Mediators. The highest values of the assessed mediators were recorded for the CHC + P group, being significantly more elevated that those of the other groups (Table 3). Both groups of periodontitis patients (CHC + P and P) expressed significantly elevated levels for all of the three assessed proinflammatory mediators (NLRP3, CASP1, and IL-18) as compared to the nonperiodontitis groups (CHC and H) (Table 3). The proinflammatory mediators' levels in all three test groups were significantly more elevated than in controls (Table 3).

3.4. NLRP3, CASP1, and IL-18 GCF Levels Associated Positively with Certain Periodontal and Metabolic Parameters. The results delivered significant positive correlations between the GCF NLRP3 levels and the periodontal probing depth, clinical attachment loss, and gingival bleeding index ($r = 0.4$; $p < 0.05$) (Table 4). The NLRP3 GCF levels also positively correlated with certain metabolic parameters, including the glucose, aspartatetransferase (AST), and alaninetransferase (ALT) levels. Other significant positive correlations were found between the CASP1 GCF levels and the age of the patients, the clinical attachment loss, gingival bleeding index, and ALT level (Table 4). The GCF IL-18 levels significantly correlated with the age of patients, the periodontal probing depth, the clinical attachment loss, the gingival bleeding index, and triglyceride level (Table 4). No correlations were identified between the level of liver fibrosis and the assessed proinflammatory mediators.

4. Discussion

The development of the "inflammasome" concept has opened new perspectives for periodontal inflammation research. NLPR3 is a key element of this inflammatory reaction, being considered as its pioneer triggering factor, from the first initial contact with bacterial antigens, such as *Porphyromonas gingivalis*' lipopolysaccharides (LPS) [24–26]. While some authors state that LPS can stimulate NLRP3 synthesis by activation of the Toll-like 4 receptor [27], others have observed that the inflammasome decreases its activity when in contact with subgingival bacteria [28]. In our study, there was no correlation between the GCF NLRP3 levels and the level of bacterial plaque. However, certain studies reported that NLRP3 expression is more elevated in periodontitis patients than in healthy controls [29], both in specific cells [8], and within saliva [11, 30]. Our study delivered similar results, highlighting the significant differences of the GCF NLRP3 levels between the P and H groups and between the CHC + P and CHC ones, which suggest that periodontal pathological events trigger a considerable increase of NLRP3 expression. This hypothesis was also assessed and endorsed by a recent review on the subject, which concluded that periodontal diseases can be characterized by an upregulation of inflammasomes, alongside with a downregulation of their inhibitor proteins [29].

The statistical results of our study highlighted significant correlations between the GCF NLRP3 levels and serum glucose levels of the participating patients, in accordance with

Table 3: Study's immunological results and statistical significance (p value) for differences between test groups.

Parameter	Groups				p CHC + P vs. CHC	p CHC + P vs. P	p CHC vs. P
	CHC + P	CHC	P	H			
NLRP3 (ng/mL) ± SD	1.534 ± 0.32	0.989 ± 0.3	1.251 ± 0.36	0.583 ± 0.18	< 0.05	< 0.05	< 0.05
CASP1 (ng/mL) ± SD	0.369 ± 0.14	0.2 ± 0.07	0.283 ± 0.08	0.05 ± 0.04	< 0.05	< 0.05	< 0.05
IL − 18 (ng/mL) ± SD	0.287 ± 0.07	0.193 ± 0.05	0.2 ± 0.03	0.075 ± 0.07	< 0.05	< 0.05	< 0.05

Table 4: Synopsis of correlation statistical assessment (Pearson's test) for whole patient batch between NLRP3, CASP-1, and IL-18 levels and assessed parameters.

Parameter	Proinflammatory mediator		
	NRLP3	CASP1	IL-18
	r/p	r/p	r/p
Gender	0.05/0.8	0.08/0.71	0.14/0.52
Age	0.33/0.12	0.56/0.005*	0.43/0.04*
Periodontal probing depth	0.45/0.03*	0.39/0.06	0.47/0.02*
Clinical attachment loss	0.52/0.01*	0.46/0.02*	0.52/0.013*
Plaque index	0.27/0.21	0.36/0.09	0.4/0.06
Gingival bleeding index	0.45/0.03*	0.48/0.02*	0.42/0.04*
Glucose	0.43/0.04*	0.14/0.51	0.03/0.86
Cholesterol	0.02/0.9	0.26/0.22	0.39/0.06
Triglycerides	0.23/0.28	0.05/0.79	0.42/0.04*
Aspartate transaminase	0.45/0.04*	0.14/0.52	0.14/0.5
Alanine transaminase	0.51/0.02*	0.57/0.02*	0.08/0.69
Glutamyl transferase	0.2/0.35	0.18/0.4	0.05/081

r: Pearson's r; p: statistical significance. *Statistically significant value ($p < 0.05$).

other studies on the topic of NLRP3 inflammasome, periodontal disease, and diabetes association [9, 11, 31–34]. This correlation endorses the pathogenic links existing between periodontal diseases and cellular resistance to insulin [35]. Additional pathogenic connections also lay between chronic hepatitis C and insulin resistance [36]. These elements suggest that this pathologic mechanism could bridge the connection between chronic hepatitis and periodontal disease, through the significant impact and bi-directional consequences it inflicts on the inflammatory reaction.

The immunological analysis performed in our study generated comparable outputs on the average values of CASP1 within the GCF samples of periodontitis patients, which were significantly higher than those of healthy controls [37]. Histologically, CASP1 is predominantly expressed within gingival epithelial cells, as keratinocytes, and connective tissue cells, being almost absent when the periodontal tissues are not inflamed [37–39]. Moreover, important periodontal bacterial pathogens (*Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*) have been shown to trigger caspase expression within epithelial cells and macrophages [40, 41]. Within this finding, in our study also, a significant positive correlation was identified between the GCF CASP1 levels and the clinical periodontal parameters used for assessing the severity of periodontal damage (clinical attachment loss and gingival bleeding index).

Our current study's results show, as similar other ones, that the GCF IL-18 levels were significantly higher in the samples of periodontal patients as compared to healthy controls [42–45]. Moreover, the statistical analysis denoted significant correlations between the GCF IL-18 levels and the parameters of periodontal disease's severity (periodontal probing depth, clinical attachment loos, and gingival bleeding index), similar to those highlighted by other papers [29]. IL-18 has also been suggested as a possible indicator for periodontal structures' damage [45]. In accordance with this finding, our CHC + P patients expressed significantly elevated GCF IL-18, reflecting their unfavorable clinical periodontal status.

Regarding the hepatic pathology, NLRP3 has been observed to activate when stimulated by the hepatitis C virus, within white and red blood cells [46–48]. Moreover, in an *in vitro* setting, the presence of the virus determined cellular pyroptosis within infected hepatocytes, an event mainly controlled by NLRP3 and CASP1 activity [47]. NLRP3 is also involved in the onset of the chronic hepatic inflammatory reaction, consequent to HCV infection, together with IL-1β [48]. Thus, CHC patients are expected to exhibit elevated serum NLRP3 levels. This fact has also been shown by the results of our study that identified more elevated GCF NLRP3 levels in the samples of CHC patients, that those of the H group, given that GCF is a blood serum derivate. As shown by the delivered results, when CHC patients also suffer from P, their GCF NLRP3 levels increased significantly compared to those of patients suffering from CHC or P alone, suggesting that NLRP3 expression is upregulated when the two diseases occur in the same patient.

CASP1 is also involved in the pathological processes of CHC, as the HCV-infected cells are able to synthesis and release the NLRP3 inflammasome [49, 50]. Our study's results highlighted more elevated average GCF CASP1 levels in the samples of CHC + P patients, as compared to P patients. Significant differences were also identified between the average values of the CHC patients, with and without periodontal disease. These results suggest the significant impact that chronic inflammation, either hepatic or periodontal, could have on the GCF CASP1 levels.

Concerning the hepatic disease, IL-18 also has immunological and clinical implications on CHC, as affected patients often exhibit significantly elevated values of this mediator, compared to healthy controls [51–54]. In our study, significant differences of the GCF IL-18 levels were recorded

between the CHC + P group and the P group, endorsing the negative impact that HCV infection can have on the periodontal status, in similar periodontal pathological settings. This can amplify gingival pathogenic events and trigger a more intense and severe periodontal inflammatory reaction.

Our results show that CHC + P patients exhibited a more severely modified clinical periodontal status. In addition, these patients also exhibited the highest NLRP3 GCF levels. The negative character of their periodontal status could be in relation to the upregulation of this mediator. A study by Garcia-Hernandez et al. stated that the upregulated NLRP3 levels could increase the inflammatory response in uncontrolled type-2 diabetes periodontal patients [8]. This was also shown by a recent study by Isola et al., in samples of saliva and serum, originating from patients with periodontitis and diabetes [11]. Concerning CHC periodontal patients, future extended research is required to test this hypothesis.

Given the sample size limitations, all three assessed elements (NLRP3, CASP1, and IL-18) expressed significantly elevated GCF levels in CHC + P patients, as compared to the other groups, suggesting that the coexisting hepatic pathology might have an upregulating effect on these proinflammatory mediators. The decline of the clinical periodontal and immunological status of these patients should be taken into account when developing holistic therapeutical strategies.

5. Conclusions

Within the limitations of this study, we could conclude that chronic hepatitis C and periodontitis might have a joined effect on the upregulation of the NLRP3 inflammasome and its components in the GCF. This fact could justify the exacerbated manifestation of periodontal disease in chronic hepatitis C patients. This motivates future expanded research on the matter.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

Luminita Lazar, Cerasella Sincar, Mihail Boldeanu, Allma Pitru, and Cristina Florescu share equal contribution to that of the first author, thus can be considered as main authors.

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Review Article

Influence of Gestational Hormones on the Bacteria-Induced Cytokine Response in Periodontitis

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Periodontitis is an inflammatory disease that affects the supporting structures of teeth. The presence of a bacterial biofilm initiates a destructive inflammatory process orchestrated by various inflammatory mediators, most notably proinflammatory cytokines, which are upregulated in the gingival crevicular fluid, leading to the formation of periodontal pockets. This represents a well-characterized microbial change during the transition from periodontal health to periodontitis; interestingly, the gestational condition increases the risk and severity of periodontal disease. Although the influence of periodontitis on pregnancy has been extensively reviewed, the relationship between pregnancy and the development/evolution of periodontitis has been little studied compared to the effect of periodontitis on adverse pregnancy outcomes. This review is aimed at summarizing the findings on the pregnancy-proinflammatory cytokine relationship and discussing its possible involvement in the development of periodontitis. We address (1) an overview of periodontal disease, (2) the immune response and possible involvement of proinflammatory cytokines in the development of periodontitis, (3) how bone tissue remodelling takes place with an emphasis on the involvement of the inflammatory response and metalloproteinases during periodontitis, and (4) the influence of hormonal profile during pregnancy on the development of periodontitis. Finally, we believe this review may be helpful for designing immunotherapies based on the stage of pregnancy to control the severity and pathology of periodontal disease.

1. Introduction

Periodontal disease is an inflammatory condition of periodontal tissues with a heterogeneous aetiology and is one of the most common diseases in the world [1]. This disease affects the gum and supporting tissues of the teeth, alveolar bone, periodontal ligament, and root cementum. Approximately 60% of the total population has some degree of periodontal disease [2]. In Latin America, this number increases to up to 90% [3]. The early stage of development of this pathology is called gingivitis and affects only the soft tissues. The severe form, called periodontitis, severely affects periodontal tissues, mainly alveolar bone, with subsequent loss of insertion of dental organs.

The development of periodontal disease and its progression depend on different factors that modulate the host immune response against the biofilm, such as genetic and epigenetic predisposition including hereditary angyoderma [4], social factors, habits (such as tobacco and alcohol use and poor oral hygiene) [5], advanced age [6], and systemic conditions, such as obesity, malnutrition [7], infections (such as HIV/AIDS), osteoporosis and stress [8], type 1 and 2 diabetes [9], and scleroderma disease [10]. Notably, periodontitis affects 23% of women between 23 and 54 years of age and is present in 56% of pregnant women [11]. Furthermore, recent evidence suggests that hormonal treatment, the use of hormonal contraceptives, and pregnancy induce clinical, cytological, or microbiological changes in women

[12], which probably promote the development of this disease.

Inflammatory cytokines are upregulated during pregnancy and increased during ovulation, in early gestation, in term pregnancy, and during delivery [13]. However, it has not been established whether there is any relationship between pregnancy/proinflammatory cytokines and the development of periodontal disease. In this review, we summarize the current knowledge by providing a broad overview of periodontitis and then focusing specifically on recent findings related to the inflammatory response in pregnancy and its possible relationship to the development of periodontitis.

2. Periodontitis and the Oral Microbiome

The oral cavity has a dynamic environment that is formed by the oral microbiome with all of its interspecies interactions but also interactions with the oral cavity, creating a symbiotic relationship with the human host [14]. Periodontitis is initiated by polymicrobial synergy, and dysbiosis is modified by numerous risk factors. Competitive and cooperative interspecies interactions of microbial communities can shape the nature and function of the entire microbiome synergism [15]. The subgingival microbiome includes the red complex triad (*Treponema denticola*, *Tannarella Forsythia*, and *Porphyromonas gingivalis*) [16], orange complex triad (*Fusobacterium nucleatum*, *Prevotella intermedia*, and *Parvimonas micra*), *Actinobacillus actinomycetemcomitans*, *Campylobacter rectus*, *Eikenella corrodens*, *Bacteroides forsythus* [17], *Filifactor alocis* [18], *Peptoanaerobacter stomatis*, *Firmicutes phylum*, *Methanobrevibacter oralis* [19], *C. albicans* [20], and *human cytomegalovirus* and *Epstein-Barr virus* [21].

The periodontal microbiome is complex and constitutes the cornerstone in the development of periodontal disease. The characteristics of the bacteria themselves are essential in determining the course of the immune response. For example, *Porphyromonas gingivalis* can modulate the innate inflammatory response [22]. *Filifactor alocis* also induces oxidative stress and alters the recognition capacity of the inflammatory response by inactivating the complement pathways [23]. *Filifactor alocis* and other bacteria, such as *Porphyromonas gingivalis*, are highly invasive and promote dysbiosis of the microbiota [24]; therefore, a pathogenesis model has been proposed in which periodontal disease is initiated due to dysbiosis of the microbiota, called the PSD (polymicrobial synergy and dysbiosis) model [25].

3. Immune Response in Periodontitis

Immune cells interact with biofilms when their pattern recognition receptors (PRRs) detect pathogen-associated molecular patterns (PAMPs) present on bacteria. These receptors are expressed on innate immune cells, such as neutrophils, eosinophils, basophils, macrophages (Mφs), monocytes, dendritic cells (DCs), and natural killer (NK) cells, and adaptive immune cells, such as T and B lymphocytes, as well as on nonimmune cells, such as epithelial cells, endothelial cells, and fibroblasts [26]. This PAMP-PPR interaction activates the innate immune response characterized by neutrophil, eosinophil, and basophil recruitment, consequently activating the complement system [27].

This first recognition is characterized by acute inflammation; if biofilm dysbiosis persists, this response develops into chronic inflammation. In this phase, osteoclast activation is favoured. It results in bone resorption, with subsequent degradation of the bone matrix and periodontal ligament fibres by metalloproteinases (MMPs) and the formation of granulation tissue [28] (Figure 1). Thus, in both acute inflammation and chronic inflammation, cytokines, and inflammatory mediator's determinate disease progression factors.

Antigen-presenting cells (APCs), such as DCs, recognize pathogens expressing PAMPs, internalize these pathogens by phagocytosis, and degrade and process pathogen-derived antigens, transforming the antigens into small peptides that bind to major histocompatibility complex (HLA) molecules for display on the cell surface. This presentation is accompanied by the expression of the costimulatory molecules CD86 and CD40. DCs migrate to secondary lymphoid tissues (lymph nodes and lymphoid tissue) to present antigens and thus activate $CD4^+$ T cells to generate an antigen-specific immune response [29]. CD4+ T cells differentiate into regulatory and effector T cell subsets: Th1, Th2, Th17, follicular helper T (Tfh) cells, and regulatory T cells (Tregs) [30]. The differentiation of Th1 and Th2 cells is mutually antagonistic; Treg and Th17 cells share the same origin and have opposite effects, while Th17 cells cause autoimmunity and inflammation, and Treg cells inhibit these and maintain immune homeostasis.

The activation profile of $CD4^+$ T lymphocytes in periodontal disease varies depending on disease progression. In the initial phase, $CD4^+$ T lymphocytes exhibit a proinflammatory Th-1 profile characterized by the synthesis of macrophage inhibitory factor (MIF), interleukin- (IL-) 2, and interferon- (IFN-) γ, which promote cellular immunity and the activation of cytotoxic $CD8^+$ T lymphocytes (TCs) and Th-17 cells [31]. Other cytokines, such as IL-1α, IL-1β, IL-8, IL-6, and tumour necrosis factor- (TNF-) α produced by monocytes, Mφs, DCs, and neutrophils, are also produced under these conditions [32]. In addition, endothelial cells, fibroblasts, and osteoclasts produce prostaglandin E2 (PGE2) and granulocyte macrophage colony-stimulating factor (GM-CSF) [33]. Together, these conditions promote the expression of receptor activator of NF-κB ligand (RANKL), leading to osteoclastogenesis [34].

In the chronic phase of periodontitis, $CD4^+$ T lymphocytes differentiate towards an anti-inflammatory Th2 profile, characterized by the production of IL-4, IL-5, IL-6, and IL-10 [35]. This profile favours B lymphocyte activation and subsequent differentiation into IgG-type immunoglobulin-producing plasma cells [36]. In this way, when periodontitis becomes chronic, negative regulation of inflammation through the anti-inflammatory cytokines IL-4, IL-6, IL-10, IL-11, and IL-13 becomes predominant [37]. This immunoregulation is a complex phenomenon involving mediators such as RANKL-DCs, favouring the activation of $CD4^+$

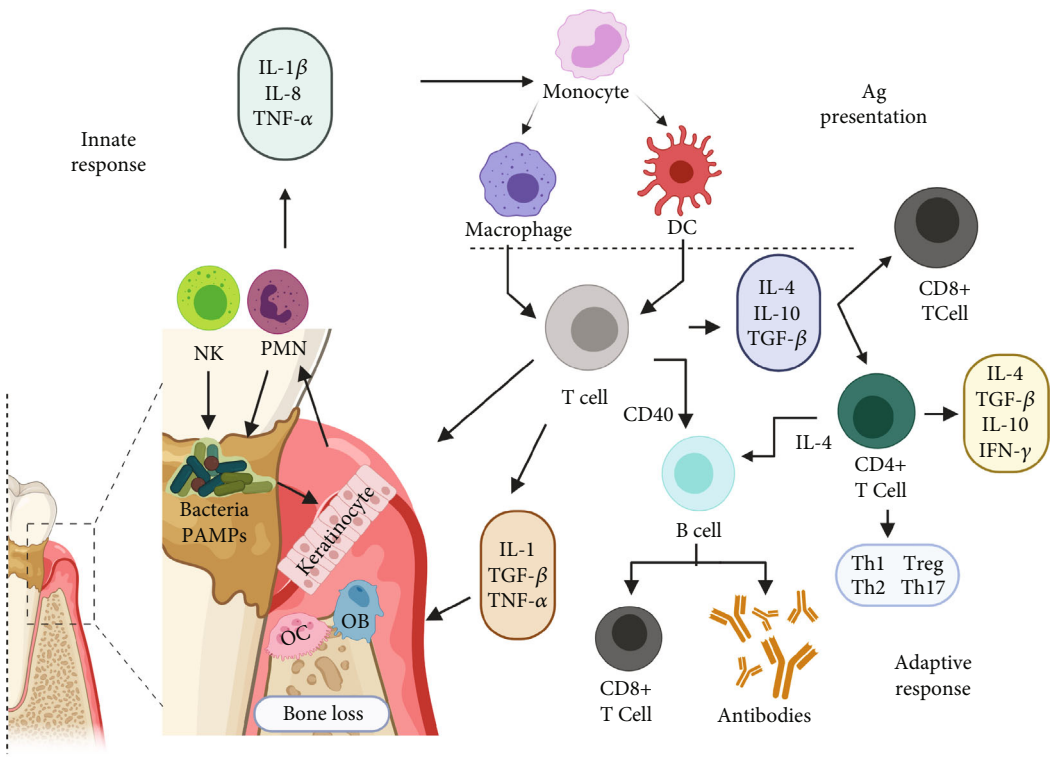

Figure 1: Innate immune cells, including keratinocytes, polymorphonuclear cells (PMNs), antigen-presenting cells (APCs), and natural killer (NK) cells, hold pathogen recognition receptors (PRRs) that detect pathogen-associated molecular patterns (PAMPs) present in biofilm bacteria, and these interactions promote the acute inflammatory response. Antigen-presenting cells (APCs) phagocytose pathogens, process antigens, and present the antigens in the form of peptides displayed by major histocompatibility complex (HLA) molecules. Costimulatory molecules stabilize this interaction. APCs migrate to secondary lymphoid tissues where they activate adaptive immune cells, including $CD4^+$ T cells, such as Th1, Th2, and Th17 cells; cytotoxic $CD8^+$ T cells; and B cells that will mature into antibody-producing plasma cells. Created with BioRender.com (https://biorender.com/).

$Foxp3^+$ T (Treg) cells [38], which also regulate the inflammatory Th1 response, thus, preventing the destruction of periodontal tissues [39].

Th17 cells are characterized by the production of the cytokine IL-17, although they also produce other cytokines, such as IL-17F, IL-21, IL-22, and GM-CSF [40]. Th17 cells are induced in the presence of TGF-β/IL-1β, IL-6, and IL-23 and express chemokine receptor- (CCR-) 6, which allows their migration to barrier and mucosal sites, such as gingival tissues, suggesting a protective role in the oral barrier. The inflammatory functions of Th17 cells depend on the different combinations of cytokines expressed in the local environment [33].

IL-17 signalling on epithelial cells is essential for the physiological regulation of mucosal immunity and barrier defences, promotes the production of antimicrobial factors, regulates the recruitment and generation of neutrophils through the induction of chemokines CXCL-1, 2, and 5, and induces the secretion of granulopoietic factors such as G-CSF and GM-CSF. Moreover, IL-17 induces the production of antimicrobial mediators such as β-defensins (HBD), regenerative proteins (ReG), S100 proteins, cathelicidins, lipocalins, and lactoferrins [40].

In periodontal lesions, an increase in Th17-related cytokines, such as IL-23 and IL-21, and other proinflammatory and osteoclastogenic mediators, such as IL-6 and RANKL, has been found [41]. IL-17 can enhance RANKL expression on osteoblasts by promoting the secretion of MMP-1, MMP-3, IL-6, and IL-8 from gingival fibroblasts and TNF release from macrophages in periodontal tissues [42] and activate RANK signalling on osteoclasts, promoting osteoclastogenesis [43]. In addition, IL-17 enhances inflammation through excessive neutrophil recruitment, enhances proinflammatory cytokine production, and activates osteoclasts, contributing to immunopathology and bone destruction.

In addition, the presence of Treg cells inhibits osteoclast formation and monocyte/Mφ differentiation through the secretion of transforming growth factor- (TGF-) β, IL-4, and IL-10 and the interaction of CTLA-4 (cytotoxic T-lymphocyte antigen) with the monocyte precursor/Mφ receptors CD80/CD86 [44].

Keratinocytes express several families of pattern recognition receptors, including TLR2, TLR4, NOD1, and NOD2, which are activated by both extracellular and intracellular bacterial molecular structures.

The mechanisms of tolerance include not only DCs but also Tregs. The function of oral Langerhans cells (LCs) under physiological conditions is to maintain a state of immune tolerance [45]. DCs also participate in peripheral tolerance in chronic periodontitis. These cells are capable of phagocytosing pathogens, but due to the anti-inflammatory cytokines IL-10 and TGF-β, their ability to

present antigens decreases; this decrease is associated with a deficiency in the costimulatory molecules CD80 and CD86, so they cannot activate T cells properly [29]. Natural killer cells, either through direct cell-to-cell contact or indirectly through cytokines, interact with dendritic cells to mediate T cell immune responses [46].

In summary, the severity of the pathology of periodontal disease, as well as its chronicity, depends on the balance and interaction between the Th1/Th17 inflammatory response and the Th2/Treg anti-inflammatory regulatory response [47]. The Treg/Th17 balance is shifted in favour of Th17 cells in the presence of proinflammatory cytokines.

4. Remodelling of Bone Tissue in Periodontitis

Bone tissue is one of the most affected tissues in periodontitis; under normal conditions, it is constantly remodelled, which requires cells that degrade the bone matrix (osteoclasts) and cells that synthesize the bone matrix (osteoblasts) [48]. Briefly, the bone matrix produces the growth factors TGF-β and insulin-like growth factor- (IGF-) 1. Both molecules favour the recruitment of preosteoblasts and promote their maturation; subsequently, some osteoblasts differentiate into osteocytes. This mechanism is regulated by paracrine and endocrine factors, such as epinephrine B2, IL-6, and parathyroid hormone (PTH) [49].

On the other hand, osteoclasts differentiate from a myeloid precursor under the influence of Mφ-colony-stimulating growth factor (M-CSF) and RANKL. Osteoprotegerin (OPG), produced by osteoblasts, modulates the osteoclast differentiation process [50]. Osteoclasts produce the proteolytic enzymes cathepsin K and metalloproteinases (MMPs), which degrade the bone matrix. In addition, H^+ proton transporters and ATPase generate an acidic environment that, together with chloride channels, hydrolyses, and solubilizes both organic matter and inorganic matter. All this happens in Howship's lacunae, and the osteoclasts seal them with their podosomes [51].

In periodontitis, lymphocyte infiltrates and mononuclear cells influence and alter the homeostatic balance of the bone. Although modulation of the bone immune system is complex, the balance of proinflammatory Th1 and anti-inflammatory Th2 immune responses is critical [52]. The cytokines that promote bone resorption include IL-1β, TNF-α, IL-6, IL-15, and IL-17, and the cytokines that inhibit bone resorption include IL-4, IL-10, IL-13, IL-18, GM-CSF, and IFN-γ [50]. The best example is TNF-α, which activates osteoclasts and inhibits osteoblast differentiation with a consequent decrease in bone formation [53]. Specifically, TRAIL (TNF-related apoptosis-inducing ligand) participates in osteoblast apoptosis and low bone quality in periodontitis [54].

The other important factor in bone resorption is a member of the TNF family, RANKL, which promotes osteoclast differentiation and modifies the relationship between osteoblasts and osteoclasts [55]. RANKL is overexpressed in proinflammatory systems, and the major source is B lymphocytes, followed by T lymphocytes and finally monocytes, although osteoblasts also produce RANKL after activation through TLRs [56].

The RANKL-RANK-OPG system is involved in bone regulation via regulation of the immune system to control other systems and several pathologies. These interactions have been described mainly in rheumatoid arthritis, which involves bone loss and bone remodelling [57, 58]. The regulation of the RANKL-RANK-OPG system and its mechanisms should be clarified in periodontitis since modulation of these mechanisms may favour treatment and prevent disease sequelae.

4.1. Involvement of Metalloproteinases in Bone Remodelling. Periodontal tissues are composed of connective tissue; the extracellular matrix (ECM) is mainly formed by collagen types I, III, IV, V, and VI and noncollagenous proteins, including elastin, fibronectin, laminins, and proteoglycans. In periodontitis, significant degradation of all of these constituent elements of periodontal tissues occurs. Overexpression of MMPs, a family of zinc-dependent endopeptidases, is associated with the development and severity of periodontal structure loss in this pathology. These enzymes are capable of degrading most of the components of the ECM [59]. In addition, MMPs favour processes involved in inflammation, such as inflammatory cell migration, chemokine recruitment and processing, cleavage and neutralization of complement components, phagocytosis, and cell lysis [60].

Different MMPs have the specificity to act in the degradation of specific types of tissues, e.g., MMP-2 in its proenzyme form (~72 kDa) and in its active form (~59–62 kDa) and the MMP-9 proenzyme (~92 kDa) and active forms (~88 kDa) degrade fibronectin, elastin, and collagen types IV, V, VII, X, XI, and XII; in acidic medium, they can degrade collagen type I [61]. MMP-13, both in its proenzyme form (~60 kDa) and in its active form (~45–50 kDa), degrades collagen I, collagen II, collagen III, collagen IV basal membrane, proteoglycans, fibronectin, fibrin, and tenasin [62].

The mechanisms that regulate homeostasis, such as the overexpression of MMPs in periodontitis, are complex processes. In homeostasis, one of the regulatory pathways involves tissue inhibitors of MMPs (TIMPS) and α2-macroglobulins that bind covalently and irreversibly to the active site of MMPs with high affinity. The levels of these endogenous mediators are elevated in healthy tissue and various fluids, such as serum, amniotic fluid, and saliva, and these mediators are synthesized by fibroblasts, monocytes, Mφs, endothelial cells, and osteoblasts [63]. Another mechanism of negative regulation of MMP expression is the presence of the oestrogen 17β-oestradiol, which negatively regulates the flow of calcium into cells [64] and consequently reduces the expression of MMPs, particularly MMP-1 [65].

On the other hand, the overexpression of MMPs can be triggered by different factors, such as the presence of PGE_2 [66], *in vitro* and *in vivo* are influenced by mechanical load as orthodontic movement [67, 68], interactions with periodontopathogenic bacteria, such as *Eikenella corrodens* [69], *Porphyromonas gingivalis*, and *Prevotella intermedia* [70], or polysaccharides and cytokines, such as IL-1β and TNF-

Table 1: Influence of sex hormones on some cells and cytokines of the immune response.

Hormone	Regulation	References
17β-estradiol	↑TCD8+ from spleen and in vitro.	[75]
	↑ maturation and activation of B lymphocytes, ↓Ig2a in peripheral blood mononuclear cells and spleen cells.	[76, 77]
	↓TNF-α, ↑IFN-γ e ↑IL-10 in peripheral blood mononuclear cells, spleen, and in vitro.	[78, 79]
	Peritoneal Mφ ↑, TLR4 in vitro.	[80]
	↑DCs ↑IL-12 in bone marrow, in vivo.	[81]
	Inhibits apoptosis by TNF-α via PI3k/Akt in neural progenitor cells.	[82]
	↓IL-1β and TNF-α in bone marrow.	[83]
	[↓E2] ↑Th1, [↑E2] ↑Th2 in peripheral blood mononuclear cells in vitro.	[84]
Progesterone	↓Mφ, DCs, and NKs in peripheral blood mononuclear cells	[85]
	↓NFκB transduction.	[86]
	↑Th2, ↑IL-4 e ↑IL-5, ↑Tregs, and ↓TH17 in peripheral blood mononuclear cells.	[87, 88]
Testosterona	↓LB, ↑apoptosis in bone marrow and lymph nodes.	[89]
	↑TCD8+ in peripheral blood mononuclear cells.	[90]
	↑Mφ, ↑TNF-α, ↑CCR2, ↑[IL-10], and ↓IFN-γ in skin and spleen.	[91, 92]
	Mφ, ↑IL-12 e ↑IL-1β in vitro.	[93]
	DCs ↓TNF-α, nitric oxide, TLR-4.	[94, 95]
	↓IgG e IgM, peripheral blood mononuclear cells.	[96]
	↑TGF-β e ↑IGFs ↑bone apposition, ↓ IL-6 osteoclastogenesis.	[97, 98]

α. In any case, these factors act on monocytes and Mφs by favouring the production of mediators that function as activators or modulators of MMPs [71]. For example, MMP-13 upregulates RANKL/OPG levels by activating MMP-9, increases TGF-β signalling in metastatic bone lesions [72], and influences osteoclastic activity [73].

Undoubtedly, the participation of MMPs in the development and severity of periodontitis is known. Establishing whether their expression is affected by hormonal conditions, such as gestation, is important because regulating their expression could be considered a component of the therapeutic treatment of gestational periodontitis.

5. Sex Hormones and Periodontitis

Sex hormones modulate immune functions, such as thymocyte maturation and selection, cell migration, MHC-II expression, cell proliferation, and cytokine production (Table 1) [74].

The regulatory effects exerted by hormones on the immune response depend on interactions with their receptors. For example, B cells have high expression of the genes encoding the two oestrogen receptors, ER1 and ER2. There is a moderate expression of these receptors on $CD4^+$ T, $CD8^+$ T, NK, and plasmacytoid DCs, while monocytes express reduced levels of ER1. Estradiol and ERs bind to transcription factors, such as NFκB, SP1, AP-1, and C/EBPβ, that are involved in the regulation of different cellular functions [99].

Progesterone receptors (PRs) are present on epithelial cells, mast cells, eosinophils, NK cells, Mφs, plasmacytoid DCs, and $CD4^+$ and $CD8^+$ T lymphocytes. Interestingly, the expression of PRs is higher in DCs from female rats than in those from male rats [100], which makes it clear that the expression of these receptors and consequently the response that is generated when their ligand binds are higher in females. Different PRs include two intracellular receptors (iPRs) and three membrane receptors (mPRs), with two isoforms each. iPRs were initially described in the lymphocytes of pregnant women, while mPRs were described in T lymphocytes and are overexpressed during the luteal phase in $CD8^+$ T lymphocytes. Differential expression of PRs may partially explain the differential activation of immune cells and differences in susceptibility to various infectious and noninfectious diseases between men and women [101].

In the same context, the sex hormone profile also has an impact on subgingival microbiology. It has been demonstrated that this profile promotes the development of periodontopathogenic bacteria, such as *Porphyromonas gingivalis* [102], subgingival anaerobic-aerobic bacteria, *Prevotella melaninogenica*, and *Prevotella intermedia* [103]. It is widely recognized that hormones related to gestation alter the immune response, modifying the pathogenesis of some diseases; for example, in multiple sclerosis and autoimmune encephalomyelitis, where an exacerbated inflammatory response is associated with the severity of the pathology, the disease severity decreases during gestation. Diseases such as malaria and influenza, which require acute inflammatory responses for their control, are exacerbated during pregnancy [80]. This phenomenon could be associated with estriol concentrations, which increase significantly during gestation. Estriol is produced in high concentrations by the fetoplacental unit during pregnancy; it accounts for 90% of the oestrogen produced during pregnancy, while the other 10% corresponds to oestradiol [104]. Although the immunological functions of estriol are similar to those of oestradiol because they share receptors, estriol seems to differentially influence the immune response; in experimental models of autoimmune pathologies, when estriol was administered, decreases in the proinflammatory cytokines TNF-α and IFN-γ have been observed, in addition to decreases in $CD4^+$ and $CD8^+$ cells [105]. This immune response modified

by the presence of estriol, together with other hormones present during pregnancy, could also influence the development of periodontitis. This observation is corroborated in pregnant women, who, due to their condition, have a modified hormonal profile that consequently favours the accumulation of *Bacteroides*, which is increased in abundance up to 55 times in pregnant women compared to nonpregnant women [106].

The involvement of hormones other than estriol in the development of periodontal diseases has been widely documented. Progesterone increases vascular permeability and favours oedema, erythema, and gingival bleeding, which are all associated with increased populations of *Porphyromonas gingivalis*, *Prevotella intermedia* [107], *Actinobacillus actinomycetemcomitans* [108], and *Prevotella melaninogenica* [109].

Oestrogens, particularly oestradiol, favour angiogenesis and fibroblast proliferation and promote osteoblast differentiation and maturation, osteoprotegerin (OPG) and RANKL expression in osteoblasts, and osteoclast apoptosis by inhibiting osteoclast activity [83]. Periodontal ligament (PLD) cells synthesize RANKL and OPG. *In vitro* cultures of oestrogen-treated PLD cells increase OPG expression and decrease RANKL expression through ER2 [110]; these observations demonstrate that oestrogens can modulate the activity of periodontal tissues and promote homeostasis.

Androgens participate in bone growth; they are anabolic agents that increase bone mass, mainly in males, although different androgens, including testosterone, are also present in females. Androgen receptor mRNA is expressed more in cortical osteoblasts than in trabecular bone and is more closely related to cortical osteoblasts, which generate a thicker cortical bone layer in males, while in osteoblasts, androgen receptor mRNA is expressed similarly between the sexes. Androgens promote osteoblast differentiation and decrease osteoclast apoptosis; specifically, dihydrotestosterone reduces OPG levels. The functions of androgens in women have not been clearly defined; however, they are involved in the maintenance of bone density [100].

Sex hormones are involved in bone regulation and immune system maturation and modulate the function of nonsexual tissues; therefore, these hormones may play a central role in the development of periodontitis in different stages of life.

5.1. Pregnancy and Periodontitis. Gestation is a condition that involves physiological changes in the mother, and these changes should allow "immune tolerance" towards the foetus to develop, as well as the appearance of new cells, such as trophoblasts [111].

Recently, the relevance of the model of a foetus as a semiallograft capable of inducing the absence of a specific immune response to prevent its destruction has been debated. It is not a simple absence of the immune response but a state of immunoregulation that allows the implantation of a foetus, which is also able to respond to injury or aggression from the environment with an immune response endowed with specificity and memory [112]. This immune tolerance, in order to not reject the foetus and at the same time allow protection of pregnant woman against pathogens, requires the transient modification of immunity, which favours a Th2 environment over a Th1 environment [113]. Different mechanisms have been described to explain immune tolerance to paternal antigens, including tolerance induction in T lymphocytes, including Treg and Th17 cells [114]. In a healthy pregnancy, the Th17/Treg ratio shifts in favour of Treg cells, while a decrease in Treg cells or an increase in Th17 cells is detrimental to normal pregnancy [115]. Tolerance is promoted by Treg and Th2 cells by repressing Th1 and Th17 cells, while Th17 cells protect trophoblasts from pathogens [116].

During gestation, the maternal-foetal interaction and the development of the placenta favour increased hormone concentrations. In particular, the placenta synthesizes and releases oestrogen and progesterone into circulation. This initiates events that stimulate "suppressive" immune responses, mainly at the level of lymphocytes. Suppression of $CD4^+$ and $CD8^+$ T lymphocytes decreases the secretion of IL-2, IFN-γ, TNF-β, TNF-α, IL-1β, and IL-6 [117]. The levels of oestradiol in human serum are ~0.1 μM, and those in blood from the intervillous space are ~0.25 μM, which is ~25 times higher than the concentration found in nonpregnant women at the midovarian cycle stage [118]. Oestradiol at a concentration of 0.04 ng/mL or higher and progesterone at 0.1 ng/mL both inhibit lipopolysaccharide- (LPS-) induced IL-1 and TNF-α secretion in monocyte cultures. In addition, the switch from the Th1 profile to the Th2 profile and the suppression of the cytolytic function of NK cells are processes regulated by progesterone-induced blocking factor (PIBF), which is secreted by $CD8^+\gamma\delta$ T cells [119]. High concentrations of PIBF favour the differentiation of $CD4^+$ T cells into Th2 lymphocytes, which increases IL-4, IL-5, and IL-10 concentrations and promotes the prevalence of an anti-inflammatory profile (Figure 2) [120].

In the gestational stage, polymorphonuclear cells (PMNs) show decreased chemotaxis and adhesion beginning in the second trimester and continuing throughout gestation [121]. This altered neutrophil activation and depressed leukocyte function during pregnancy may explain susceptibility to certain infections [122]. For example, gingival inflammation has been associated with increased serum levels of oestrogen and progesterone, even though no changes in TNF-α or IL-1β levels have been detected [123].

In animal models, levels of the cytokines IL-1β, IL-6, IL-8, IL-17, and TNF-α increase under different conditions. For example, in maternal infections with periodontal pathogens or *in vitro* models of placental cells and tissues, exposure to periodontal bacteria or products induces the secretion of COX-2, IL-8, IFN-γ, and TNF-α in addition to causing apoptosis [124], and PGE_2 causes uterine contractions [125].

On the other hand, during pregnancy, periodontal alterations increase, and the ratio of anaerobic to aerobic bacteria is modified in the second trimester of pregnancy, mainly through increases in *Prevotella melaninogenica* and *Prevotella intermedia* related to the plasma concentrations of estrogens and progesterone [126]. In animal models of periodontitis established with *Porphyromonas gingivalis* in pregnant mice, an increased immune response with decreased expression of anti-inflammatory cytokines and increased

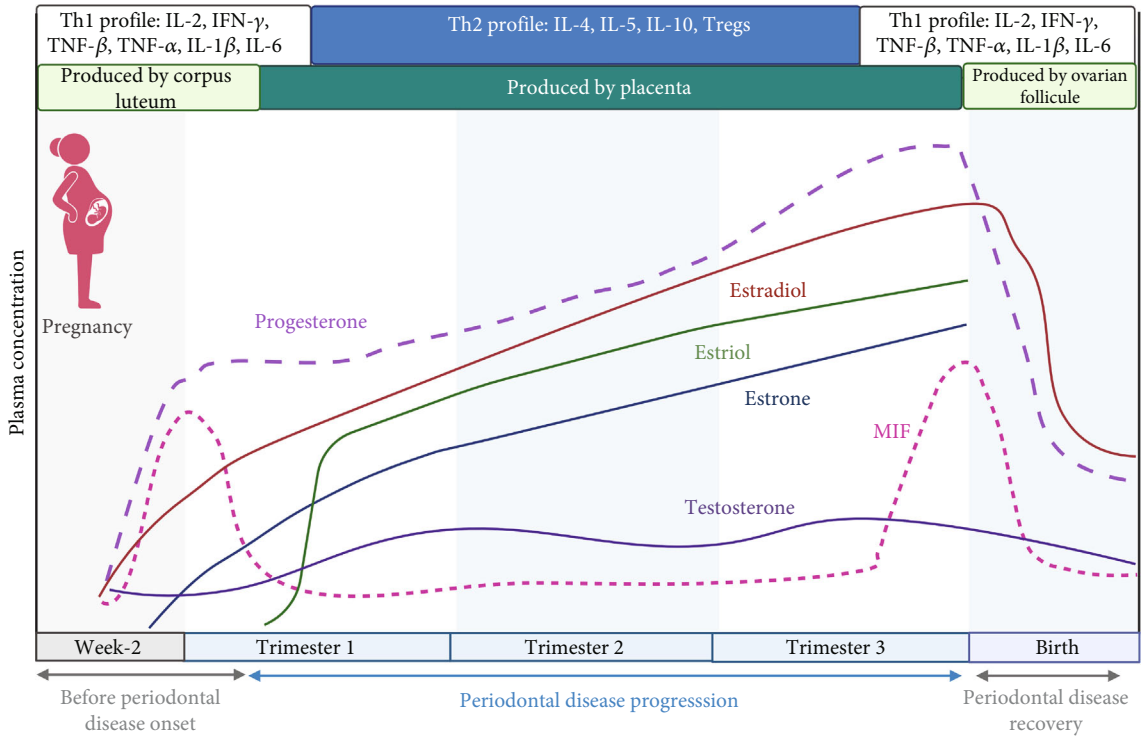

Figure 2: Time course of pregnancy hormones, periodontal disease, and the proinflammatory cytokine MIF. During early gestation, hormones are produced in the corpus luteum, and later until the end of gestation, they are produced in the placenta. Estriol is also synthesized in the placenta, and its production ceases when the pregnancy reaches term. Therefore, embryo implantation and early gestation require a Th1 profile with the expression of proinflammatory cytokines. During the second half of gestation, the expression of these cytokines decreases, and the profile is a Th2 and Treg cell profile, which is maintained until delivery, when the profile returns to a Th1 profile. The expression of MIF, which is a proinflammatory cytokine, maintains this trend during pregnancy. Created with BioRender.com (https://biorender.com/).

destruction of periodontal tissues is observed [102], and an imbalance in the Th17/Treg cell ratio with aggravation of periodontitis during pregnancy occurs [127].

Periodontitis causes gynecological problems, ranging from difficulty in embryo implantation to preterm delivery and low birth weight. Two possible causes have been proposed: first, periodontal bacteria cause infections in the placenta and foetus; second, inflammation can provoke responses at the maternal-foetal interface [128]. There are several contrasting and inconclusive reports on patients with recurrent miscarriages and multiple implantation failures during *in vitro* fertilization cycles which have a prevalent Th1 profile in their peripheral blood lymphocytes [129].

Offenbacher et al. noted that primary infections in distant systems can guide a pregnancy to an abnormal term [130]. Periodontal disease is an infectious process in periodontal tissues characterized by an increase in proinflammatory cytokines, and during pregnancy, the concentration of prostaglandins increases [35]. Therefore, there may be a relationship between both factors; periodontitis influences pregnancy, and that gestation influences the severity of periodontitis. One of the possible causes is the spread of bacteria or inflammatory mediators of periodontal origin by different routes, including (1) bacterial blood spread (bacteremia), (2) blood dissemination of inflammatory mediators, and (3) transmission of oral pathogens and colonization of the vaginal microbiome [131]. González-Jaranay et al. reported that in pregnant women with some degree of periodontitis, symptoms progress and worsen throughout gestation. However, in the postpartum period, clinical data improve [132]. Other authors have noted that maternal periodontal disease is not a risk factor if infectious processes are controlled [133].

Regarding the interaction of gestation with periodontitis, some studies did not find strong evidence of this interaction; however, they proposed routine periodontal therapy in pregnant women as a safe treatment for mothers and foetuses, in addition to improving the clinical signs of maternal periodontal disease [134]. In contrast, evidence of strong links between periodontitis and pregnancy disorders such as preeclampsia, preterm delivery, and low birth weight, attributable to periodontal disease, has recently been reported [135], as have associations of periodontitis with metabolic disorders such as obesity and diabetes [136].

6. Conclusions

This review shows that sex hormones modulate the immune response and participate in processes such as the maturation and selection of immune cells, cell trafficking, expression of histocompatibility molecules, cell proliferation, and cytokine production. Although pregnancy is a condition that modifies

the hormonal profile, little is known about its effects on the development of periodontitis. Here, we collect important evidence that gestational hormones, such as 17β oestradiol, estriol, and progesterone, influence the development of periodontitis. Importantly, the interaction between the concentration of gestational hormones and periodontal disease appears to be bidirectional: on the one hand, the hormonal profile during pregnancy seems to be decisive for the development and severity of periodontal disease, but on the other hand, the infectious process associated with periodontitis during pregnancy generates a proinflammatory immune profile that can produce alterations such as preeclampsia, preterm delivery, and low birth weight.

However, future studies are needed to understand the immune mechanisms underlying the interaction of pregnancy and periodontal diseases. The information gathered here has the potential to contribute to an understanding of the role of hormones in the development of periodontitis, allowing dental teams that care for pregnant and childbearing women to develop preventive and therapeutic strategies.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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