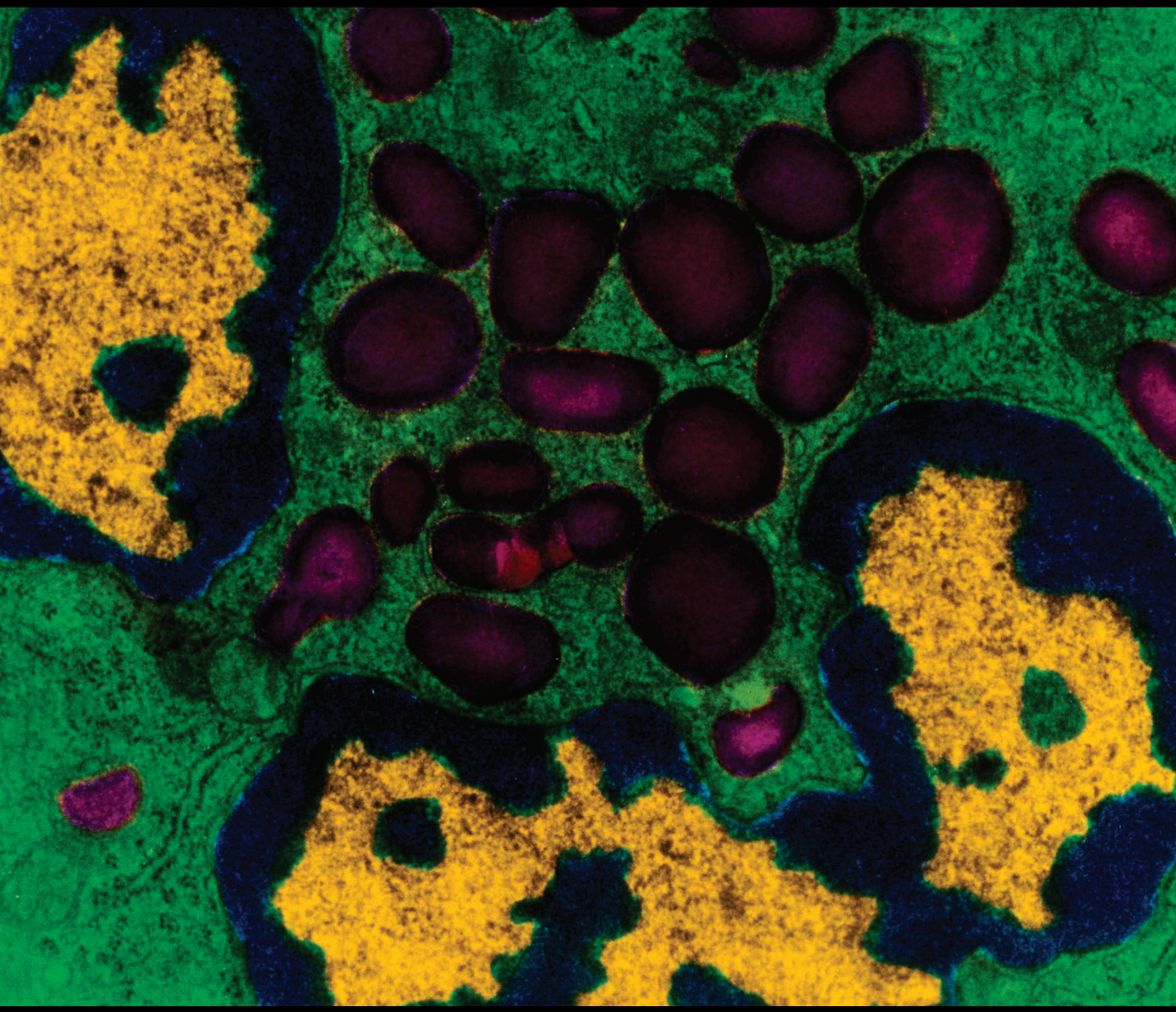
Mediators of Inflammation

Periodontal Disease and Its Systemic Associated Diseases

Guest Editors: Javier Fernandez-Solari, Paula Barrionuevo, and Claudio A. Mastronardi



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Editorial Board

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Editorial

Periodontal Disease and Its Systemic Associated Diseases

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Inflammatory processes can underlie the etiology of several pathological conditions ranging from metabolic to infectious diseases. Periodontitis, a chronic oral infectious disease, appears to occur as a result of a dysregulated host immune response elicited by subgingival microorganisms occurring in the dental biofilm. Whereas the activity of periodontal pathogens is required, their presence is not sufficient to account for the initiation and progression of periodontal disease. Thus, the combination of the bacterial-elicited insults and the poorly regulated host immune response causes deleterious effects on dentition supporting structures including the periodontal ligament, alveolar bone, and gingival tissues. Furthermore, emerging evidence suggests that periodontal disease can impact on host susceptibility for acquiring other diseases.

The relationship between periodontitis and other pathological conditions could be established by the immunogenic potential of host and/or bacterial products that reach the bloodstream and target distant organs and systems. For instance, lipopolysaccharide, a key component of the outer membrane of Gram-negative bacteria, stimulates host cells to produce a number of potent proinflammatory cytokines such as tumor necrosis factor alpha, interleukin-1, and interleukin-6. It also stimulates the production of other inflammatory mediators, such as prostaglandin E2 and nitric oxide. This inflammatory cascade promotes metalloproteinase matrix release from host tissues and causes deleterious effects in the extracellular matrix and alveolar bone. Thus, periodontitis could start as a local infection, but the triggering of a chronic inflammatory cascade could cause that oral bacteria, LPS, and/or several potent bacterial-induced proinflammatory molecules enter the bloodstream to increase the susceptibility of acquiring other infectious diseases and/or severe pathological conditions such as cardiovascular disease, cerebrovascular diseases, peripheral arterial disease, respiratory diseases, mental disorders (e.g., depression), diabetes, obesity, rheumatoid arthritis, osteoporosis, and complications of pregnancy. Additionally, chronic environmental insults such as exposure to stress, cafeteria diet, smoking, alcoholism, and drugs of abuse could also predispose the host to acquire and/or exacerbate the deleterious effects of periodontal disease and other related conditions. Some of these insults may in turn exacerbate the severity and incidence of periodontal disease by increasing the susceptibility of an impaired host immune response to oral bacteria and their by-products. Indeed, emerging evidence supports the fact that a bidirectional relationship between periodontitis and systemic diseases exists.

Since the understanding of the possible relationship between periodontitis and other systemic diseases still remains to be understood, we decided to edit this special issue on "Periodontal Disease and Its Systemic Associated Diseases" to bring attention on the deleterious impact that this chronic condition could impose on the host. The contributions to this special issue reported on some pathogenic and diagnostic aspects of periodontal disease, and its putative systemic associated conditions.

In the paper entitled "Inflammatory Mediators of Leprosy Reactional Episodes and Dental Infections: A Systematic Review" by D. Cortela et al., the authors conducted

a systematic review of primary literature published between 1996 and 2013, to determine the main shared inflammatory mediators in the immunopathological process of dental infections and leprosy reactions, concluding that these biomarkers have predictive and prognostic value to help in the early identification of patients at high risk of periodontal or leprosy diseases.

C-reactive protein (CRP), a well-known marker of inflammation, has been previously reported to predict risk of cardiovascular and cerebrovascular diseases. Interestingly, blood CRP levels also appear to reflect the severity of periodontitis. In the paper "C-Reactive Protein in Peripheral Blood of Patients with Chronic and Aggressive Periodontitis, Gingivitis, and Gingival Recessions" by S. Podzimek et al., the authors compare and evaluate the systemic levels of C-reactive protein (CRP) in peripheral blood samples of patients with chronic and aggressive periodontitis, gingivitis, and gingival recessions and compare them with periodontal clinical parameters, concluding that CRP levels increase subsequently with the severity of the periodontal disease.

The possible bidirectional relationship between periodontitis and systemic diseases was evaluated by R. Nagpal et al. In their paper "The Two-Way Association of Periodontal Infection and Systemic Disorders: An Overview", the authors revised the literature aiming at explaining a possible bidirectional link between the mechanism of periodontal diseases and systemic/metabolic diseases where both conditions could aggravate each other.

The putative relationship between periodontitis and rheumatoid arthritis was reviewed by V. Araujo et al. In their paper "Relationship between Periodontitis and Rheumatoid Arthritis: Review of Literature", the authors considered the link between both diseases considering epidemiological aspects, mechanical periodontal treatment, inflammatory mediators, oral microbiota, and antibodies. They concluded that there is a correlation between periodontal disease and rheumatoid arthritis, which could occur as a result of a similar shared imbalance in the immunoinflammatory response.

In the paper entitled "The Influence of Interleukin (IL) 17A and IL17F Polymorphisms on Chronic Periodontitis Disease in Brazilian Patients" by J. Zacarias et al., the authors investigated a possible role of *IL17A* and *IL17F* polymorphisms in the immunopathogenic mechanism of chronic periodontitis in a Southern Brazilian population. Their results demonstrate, through the analysis of genotypes by PCR-RFLP method, that the *IL17A* G197A rs2275913 polymorphism, *IL17A* AA genotype, and A allele were associated with an increased susceptibility to chronic periodontitis but show no evidence for risk or protection associations for *IL17F* T7488C rs763780.

The potential association between type 1 diabetes, a metabolic disease of autoimmune origin, and oral health has been reviewed by M. Novotna et al. In their paper "Periodontal Diseases and Dental Caries in Children with Type 1 Diabetes Mellitus", the authors considered the literature linking these two conditions. They have thoroughly discussed evidence supporting the fact that type 1 diabetes increases the risk of periodontitis. Moreover, there is compelling evidence showing an association between poorly controlled type 1 diabetes (higher HbA1c levels) and periodontitis. Thus, this review highlights the relevance of controlling oral health in patients undergoing type 1 diabetes.

In the paper entitled "HLA Haplotypes and Genotypes Frequencies in Brazilian Chronic Periodontitis Patients" by E. Â. Sippert et al., the authors studied the associations of HLA with chronic periodontitis (CP) in the Brazilian population to clarify the genetic predisposition to CP. Their results provide evidence that classes I and II HLA polymorphisms are associated with CP. HLA-A∗02/B∗40 haplotype seems to represent susceptibility factors, and HLA-B∗15/DRB1∗11 haplotypes were potential protective factors against the disease.

We hope that this special issue will not only be useful in providing insight into new and important aspects related to periodontitis and its systemic associated diseases, but also stimulate the development of novel research ideas and therapeutic strategies in this particular field.

Acknowledgment

We would like to thank both the authors for their excellent contributions and the reviewers for providing great efforts in improving these papers, which allowed the publication of this special issue.

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Paula Barrionuevo
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Research Article

C-Reactive Protein in Peripheral Blood of Patients with Chronic and Aggressive Periodontitis, Gingivitis, and Gingival Recessions

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CRP is a plasma protein that reflects a measure of the acute phase response to inflammation and is one of the markers of choice in monitoring this response. CRP can be used for the prediction and early detection of periodontal disease. The aim of this study was to compare and evaluate the systemic levels of CRP in the peripheral blood samples of patients with chronic and aggressive periodontitis, gingivitis, and gingival recessions and compare them with periodontal clinical parameters. All patients ($N = 158$) were examined prior to the initiation of periodontal treatment. Patients were divided into four groups. Group A consisted of 26 patients with aggressive periodontitis, Group B consisted of 111 patients with chronic periodontitis, Group C consisted of 13 patients with gingivitis, and Group D consisted of 8 patients with gingival recessions. Our study results indicate that CRP levels increase subsequently with the severity of the periodontal disease and that the bleeding on probing index showed much better positive correlation with the CRP levels compared to the pocket depth index in both periodontitis patients groups, especially in aggressive periodontitis patients.

1. Introduction

C-reactive protein (CRP) was discovered in 1930 during a study of patients with *Streptococcus pneumonia* infection [1]. CRP is a plasma protein that reflects a measure of the acute phase response to inflammation and is one of the markers of choice in monitoring this response [2]. CRP participates in the systemic response to inflammation. It is a pattern recognition molecule that binds to specific molecules that are produced during cell death or found on the surfaces of diverse bacterial pathogens. Rapid increase in CRP synthesis within hours after infection suggests its contribution to host defense as part of the innate immune response [3].

CRP is produced in response to many forms of injury other than periodontitis, such as other infections, trauma and hypoxia, and it is regulated by diverse cytokines. CRP levels have an association with smoking, obesity, triglycerides, diabetes, and periodontal disease [4]. Changes in peripheral blood cellular and molecular components can be found in patients with periodontitis due to inflammatory changes of the periodontal tissues [5].

Plasma levels of CRP rise rapidly and markedly as much as 100-fold or more within 72 hours following tissue injury and may increase as much as 1000-fold or more within a longer period after an acute inflammatory stimulus; therefore, CRP is a sensitive marker to evaluate the inflammatory status [3, 6]. Positive correlation between CRP and periodontal disease severity was proved by many studies [7–11], and levels of CRP decrease after nonsurgical periodontal therapy [12–16].

A study by Jayaprakash et al. revealed that the periodontitis group had a higher mean CRP level (2.49 ± 0.47 ng/mL) compared to the gingivitis group (1.40 ± 0.32 ng/mL) and healthy group (0.56 ± 0.20 ng/mL) [10]. The study conducted by Shojaee et al. demonstrated the difference between CRP in healthy subjects and patients with periodontal disease, gingivitis, chronic periodontitis and healthy control. The CRP levels were 5332.62 ± 5051.63 pg/mL in periodontitis

patients, 3545.41 ± 3061.38 pg/mL in the gingivitis group, and 3108.51 ± 3574.47 pg/mL in healthy subjects. The statistic analysis showed a significant difference in CRP concentrations between the periodontitis patients and healthy subjects ($p = 0.045$) [17]. The patients with gingivitis and healthy gingiva had lower levels of CRP than the patients with chronic periodontitis. Furthermore, with increasing inflammation, the high-sensitivity CRP levels increased proportionately [18]. It is possible to use CRP in prediction and early detection of periodontal disease [17].

Periodontal disease is an inflammatory disease that affects the soft and hard structures that support the teeth. In its early stage, called gingivitis, the gums become swollen and red due to inflammation, which is the body's natural response to the presence of harmful bacteria. In the more serious form of periodontal disease called periodontitis, the gums pull away from the teeth, and the supporting gum tissues are destroyed. Bone can be lost, and the teeth may loosen or eventually fall out [19].

Most studies have focused on CRP levels in chronic periodontitis, and very few are conducted on patients with aggressive periodontitis [7].

Some types of gingival recessions occur in the absence of periodontal disease. Such gingival recessions are considered mucogingival deformities and are included in the category of developmental or acquired deformities and conditions, according to Armitage's 1999 classification [20]. Gingival recessions can be localized or generalized, and one or more surfaces may be involved [21].

Positive correlation between CRP and periodontal disease severity with particular concern in younger individuals could be a possible underlying pathway in the association between periodontal diseases, and the observed higher risk of cardiovascular disease in periodontitis patients is mentioned in the study by Goyal et al. [7]. The inflammatory mediators, serum elastase, and CRP are all associated with an increased risk of coronary heart disease. Wohlfeil et al. compared these systemic inflammatory mediators in periodontally healthy controls, patients with untreated aggressive and chronic periodontitis. Serum elastase and CRP were significantly elevated in patients with untreated aggressive periodontitis compared to healthy controls as well as systemic inflammatory burden [22]. Patients with aggressive periodontitis have statistically significant elevations in serum CRP levels compared to subjects without periodontitis [23]. Periodontitis is a local inflammatory process mediating the destruction of periodontium triggered by bacterial insult, leading to systemic inflammation in the host. Epidemiologically, it has been modestly associated with cardiovascular diseases with elevated acute phase reactants. The increased serum CRP levels and neutrophils counts in chronic periodontitis subjects suggest an addition to the inflammatory burden of the individual, potentially striking towards an increasing risk of cardiovascular events [24].

Thus, the aim of this study was to compare and evaluate the systemic levels of CRP in the peripheral blood samples of patients with chronic and aggressive periodontitis, gingivitis, and gingival recessions and compare them with periodontal clinical parameters.

2. Materials and Methods

2.1. Study Population. All patients ($N = 158$) were recruited from the patient pool of the Periodontology Department, School of Dental Medicine, First Faculty of Medicine and General University Hospital, Charles University, Prague, Czech Republic, from 2014 to 2015 and all patients were examined prior to the initiation of periodontal treatment. Inclusion criteria were good general health, no medication, diagnosis of chronic periodontitis or aggressive periodontitis, gingivitis and gingival recession according to the ADA AAP Classification [25], and patient's agreement with CRP level determination from peripheral blood. Exclusion criteria included history of any systemic disease or any other disease manifested locally in oral cavity, current pregnancy or lactation, high blood pressure, sleep disturbances, depression, excessive alcohol use, and smoking recently or in past 10 years. All patients were of Caucasian origin.

Patients were divided into four groups. Group A consisted of 26 patients with aggressive periodontitis, Group B consisted of 111 patients with chronic periodontitis, Group C consisted of 13 patients with gingivitis, and Group D consisted of 8 patients with gingival recessions.

Diagnosis of aggressive and chronic periodontitis, gingivitis, and gingival recessions was based on a detailed clinical examination, medical and dental history, tooth mobility, and radiographic assessment of intraoral X-ray status performed in each patient.

Gingival recession is a noninflammatory periodontal disease; therefore, we used patients with this affection as a control group.

The study was performed with the approval of the Ethics Committees from the First Faculty of Medicine and General University Hospital, Charles University, Prague, Czech Republic. Written informed consent was obtained from all participants in line with the Helsinki declaration before inclusion in the study. This study was performed as a cross-sectional study.

2.2. Periodontal Evaluation. Patients with aggressive periodontitis, Group A, had to have at least one tooth with positive bleeding on probing (BOP) and a pocket depth (PD) of >5 mm in all quadrants (excluding the third molars). Patients with chronic periodontitis had to have at least one tooth with positive bleeding on probing (BOP) and pocket depth (PD) of >2 mm in all quadrants (excluding third molars). Pocket depth was assessed by WHO periodontal probe with a cut-off of 11.5 mm from six sites on every tooth present. Bleeding on probing is a sign of inflammation and indicates some sort of destruction and erosion to the lining of the sulcus [26] or the ulceration of sulcular epithelium. The blood comes from the lamina propria after the ulceration of the lining.

In patients with gingivitis, Group C, we used two types of evaluation indices: Papilla Bleeding Index (PBI) and clinical

Table 1: Groups of patients: characteristics.

	N	Females (F)/males (M)	Mean age ± SD	
Group A-aggressive periodontitis	26	F = 13	36.6 ± 7.6	37.5 ± 7.4
		M = 13	38.4 ± 7.4	
Group B-chronic periodontitis	111	F = 65	57 ± 11.3	55.1 ± 11.4
		M = 46	52.4 ± 11	
Group C-gingivitis	13	F = 10	41 ± 13.1	39.2 ± 12
		M = 3	33 ± 4.4	
Group D-gingival recessions	8	F = 3	53 ± 8.9	44.3 ± 10.3
		M = 5	39 ± 7.4	

attachment loss (CAL). PBI is a periodontal index that evaluates the gingival status, and bleeding is an indicator of this condition. This index is used for monitoring during treatment of gingivitis [27]. CAL is a sign of destructive (physiologically irreversible) periodontal disease. In gingivitis, inflammation localized to the supracrestal region of the periodontium leads to ulceration of the junctional epithelium. Although this is technically a loss of clinical attachment (because, in health, the epithelium attaches to the surface of the tooth), CAL is used almost exclusively to refer to connective tissue attachment loss. Sites with periodontitis exhibit clinical signs of gingival inflammation and loss of connective tissue attachment. Connective tissue attachment loss refers to the pathological detachment of collagen fibers from the cemental surface with the concomitant apical migration of the junctional or pocket epithelium onto the root surface [28]. The workshop of Australian dental association categorized a general guide for severity on the basis of clinical loss of attachment as follows: slight = 1-2 mm CAL; moderate = 3 to 4 mm CAL; and severe = 5 mm CAL [29]. In our study we measured CAL vestibulary for each tooth with a calibrated Williams probe with a cut-off of 11.5 mm. For the statistical evaluation we used the highest value from the upper and lower jaw.

In patients with gingival recessions, Group D, we used two types of evaluation indices: BOP and CAL. Gingival recessions are noninflammatory affections of periodontium, and this group can serve as control group for Groups A, B, and C, which represent inflammatory diseases of periodontium.

All determined evaluation indices were assessed according to WHO oral health surveys [30].

2.3. CRP Determination. CRP levels (mg/L) were measured in capillary blood using device QuikRead go CRP + Hb (Orion Diagnostica Oy, Finland), which works on the principle of photometry and turbidimetry. Capillary blood from the middle finger was collected from the patients before the clinical periodontal examination using a thin glass capillary. The samples were immediately processed and the established values were recorded. All patients were informed in detail about and consented to this marker determination.

2.4. Statistical Analysis. For calculations descriptive statistics were used: mean, standard deviation, frequencies, and standard error. This method was used to describe different groups in terms of age, CRP, and other indices present in groups.

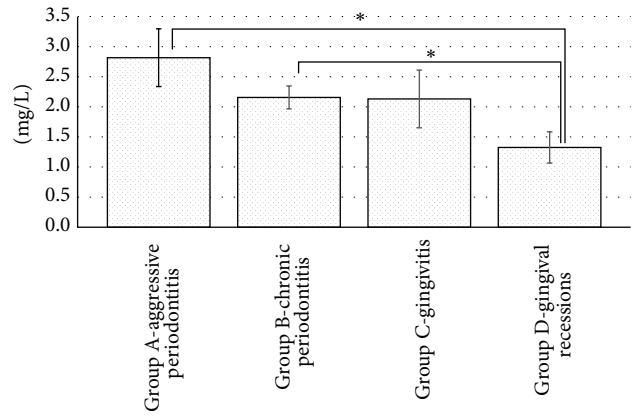

Figure 1: Levels of CRP.

For evaluation of the differences between groups, Student's t-test was used.

To test linear dependence between the characters, correlation coefficient was calculated and coefficient of determination was used in Figures 2 and 3.

Significance level of 0.05 was used in all tests.

MS Excel 2013 and Data analysis toolpack add-in statistical software were used.

3. Results

Characteristics of tested groups are shown in Table 1.

In Group A, mean BOP index was 23.5 ± 27.8% (mean ± SD), and mean PD was 5.7±2.7 mm (mean ± SD). In Group B, mean BOP index was 31.8±30.3% (mean ± SD), and mean PD was 5.2 ± 2.3 mm (mean ± SD). In Group C, mean PBI index was 1.2 ± 1.3 (mean ± SD), and mean CAL was 1.9 ± 1.8 mm (mean ± SD). In Group D, mean BOP index was 0.5 ± 1.1% (mean ± SD), and mean CAL was 4.0 ± 1.5 mm (mean ± SD).

The levels of CRP in Group A patients (2.82 ± 0.48 mg/L, mean ± SE), Group B patients (2.16±0.19, mean ± SE), Group C patients (2.13 ± 0.48, mean ± SE), and Group D patients (1.33 ± 0.26, mean ± SE) are shown in Figure 1. Statistically significant differences were found between Group A and Group D ($p = 0.01$) and between Group B and Group D ($p = 0.02$).

Table 2: Levels of CRP and clinical parameters in all tested groups.

Group	Number	CRP (mg/l)	BOP (%)	PD (mm)	PBI (%)	CAL (mm)
A	26	2.8 (±2.4)	23.5 (±27.8)	5.7 (±2.7)		
B	111	2.2 (±2.0)	31.8 (±30.2)	5.2 (±2.3)		
C	13	2.1 (±1.7)			1.2 (±1.3)	1.9 (±1.8)
D	8	1.3 (±0.7)	0.5 (±1.1)			4.0 (±1.5)

Table 3: Statistical comparisons of clinical parameters and CRP levels in all tested groups (R^2: coefficient of determination).

	R^2	Pearson	p value
A-PD, CRP	0.0108	0.1039	0.6132
A-BOP, CRP	**0.2611**	**0.5110**	**0.0076**
B-PD, CRP	0.0175	0.1323	0.1686
B-BOP, CRP	**0.1392**	**0.3731**	**0.0001**
C-PBI, CRP	**0.3831**	**0.6190**	**0.0241**
C-CAL, CRP	**0.3212**	**0.5667**	**0.0434**
D-CAL, CRP	0.0079	0.0889	0.8346
D-BOP, CRP	0.2516	0.5016	0.2053

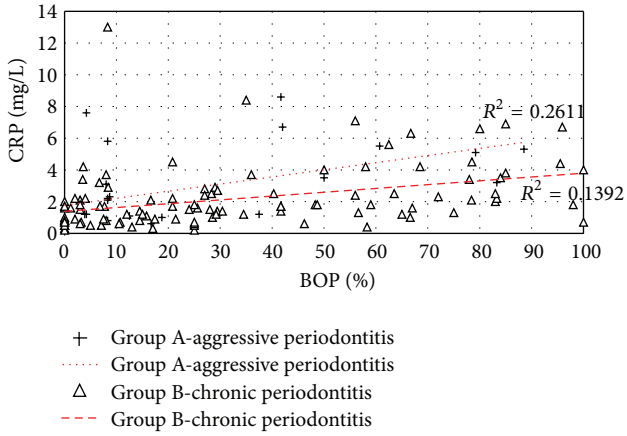

Figure 2: Statistical correlations between BOP and CRP levels in Group A and Group B (R^2: coefficient of determination).

All results are summarized in Table 2, and statistical comparisons of clinical parameters and CRP levels are shown in Table 3.

Statistical correlations between measured BOP indices and determined CRP levels in Group A and Group B are shown in Figure 2. Linear dependence in Group A (coefficient of determination 0.2611) was stronger than in Group B (coefficient of determination 0.1392).

Statistical correlations between measured PD indexes and determined CRP levels in Group A and Group B are shown in Figure 3. Linear dependence was low in Group A (coefficient of determination 0.0108) as well as in Group B (coefficient of determination 0.0175).

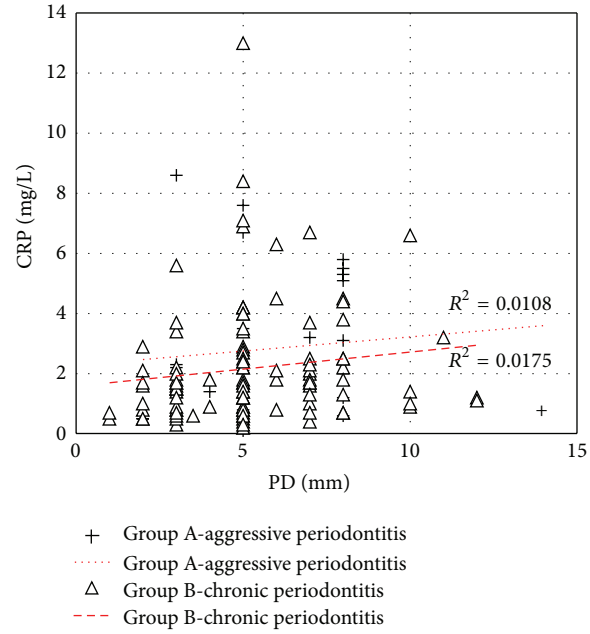

Figure 3: Statistical correlations between PD and CRP levels in Group A and Group B. (R^2: coefficient of determination).

4. Discussion

CRP represents a reliable marker of the acute phase response to infectious burdens and/or inflammation [2]. Due to its kinetics, CRP best describes the inflammatory status of human organism [31]. Recent evidence has indicated that patients with severe periodontitis have increased serum levels of CRP compared to unaffected control population [4].

We compared and evaluated the systemic levels of CRP in the peripheral blood samples of patients with chronic and aggressive periodontitis, gingivitis, and gingival recessions. CRP levels may fluctuate with various factors such as high blood pressure, alcohol use, smoking, chronic fatigue, diabetes, sleep disturbances, depression, many other systemic diseases, and pregnancy or lactation [32]. Therefore, we established strong exclusion criteria (determined in Section 2.1) for the patients to be included in this study.

Determined CRP levels were in correlation with the severity of periodontal affection. The highest mean value was found in patients with aggressive periodontitis (2.82 mg/L), and the lowest in patients with gingival recessions (1.33 mg/L). CRP, elastase, lipopolysaccharide binding protein, and IL-6 levels were elevated in patients

with untreated aggressive periodontitis compared to healthy control group. Serum elastase and CRP are significantly elevated in patients with untreated aggressive periodontitis. Aggressive periodontitis patients exhibit a stronger systemic inflammatory burden than control patients [22].

Ethnicity has been found to affect the levels of CRP [33] and data in diverse populations are not comparable. A study from the USA recorded CRP levels of 2.05 mg/L in aggressive periodontitis patients with generalized form and CRP levels of 1.1 mg/L in patients with localized form [23]. Another study from the USA showed CRP levels of 4.06 mg/L in subjects with high levels of clinical attachment loss mean [34]. A Swedish study showed median CRP of 2 mg/L in periodontitis patients [35]. In Netherlands, a study reported the highest CRP levels (1.45 mg/L) in patients with generalized form of periodontitis and CRP levels of 1.30 mg/L in patients with localized form [36]. Another study from India showed CRP levels of 7.49 mg/L in aggressive periodontitis patients and CRP levels of 4.88 mg/L in chronic periodontitis patients [37]. Therefore, in our study, only patients of Caucasian origin are included.

Statistical correlations between measured BOP and PD indices and determined CRP levels in periodontitis patient groups, Groups A and B, were established. BOP showed the best positive correlation with the levels of CRP in the aggressive periodontitis group compared to the chronic periodontitis group. In both periodontitis patient groups, BOP showed much better positive correlation with the levels of CRP compared to PD.

Significantly low levels of CRP were observed in the gingival recessions group compared to the aggressive and chronic periodontitis groups. This observation was in accordance with the previous study, where the levels of CRP were significantly lower in gingivitis patients compared to periodontitis patients [38].

A similar phenomenon to what was found in this study regarding CRP levels in patients with aggressive and chronic periodontitis and gingivitis was also published by other researchers. Study of Goyal et al. showed the highest CRP levels in patients with aggressive periodontitis and the lowest values in the group of healthy patients [7]. Other studies showed increased CRP levels in patients with chronic periodontitis compared to patients with gingivitis [10, 17]. Thus, CRP increases with disabilities of periodontium. The comparison of CRP and BOP index is a very important issue. BOP is one of the most important parameters for evaluating the periodontal status of patients with periodontitis. BOP is also the only periodontal parameter which shows the significant relationship with systemic parameters such as CRP and fibrinogen levels, and the white blood cell count, as confirmed in a recent study by Bokhari et al. [39].

Observed association between periodontal conditions and systemic CRP showed that periodontal affections may be one of the factors contributing to systemic inflammation. In the study by Beck et al. it was demonstrated that while attachment loss, pocket depth (PD), and bleeding on probing (BOP) are individually associated with serum soluble intercellular adhesion molecule and CRP, only BOP remains significant for serum soluble intercellular adhesion molecule when all 3 are in the model, and for CRP, only PD remains significant. Both of these clinical parameters were more robust in estimating the degree of systemic inflammation than traditional classifications of mild, moderate, and severe periodontitis or other measures of disease severity such as attachment loss [40].

Novelty of obtained results was based on the comparison of four types of periodontal diseases, as usually two or three types are compared, and on comparison of diverse periodontal indices with subsequently established CRP levels in patient's peripheral blood.

5. Conclusion

Our study results show that CRP levels increase subsequently with the severity of the periodontal disease. The lowest CRP levels were found in patients with gingival recessions, increasing in patients with gingivitis and patients with chronic periodontitis, with the highest levels found in aggressive periodontitis patients.

The bleeding on probing index showed much better positive correlation with the CRP levels compared to the pocket depth index in both periodontitis patient groups, especially in aggressive periodontitis patients.

Further studies are needed to clarify this association and the associated confounding factors. In further research, other systemic markers that might be more specific to periodontal disease such as fibrinogen, leptin, white blood cell count, and interleukin-6 should be considered. Changes in their values during the treatment of periodontal disease could lead to improved monitoring of periodontal tissue status during therapy. Elevated levels of such markers of systemic inflammation are connected to both systemic diseases and periodontal diseases. Studying these markers would certainly be beneficial for monitoring periodontal disease therapy.

Conflict of Interests

The authors declare that they have no conflict of interests associated with this work.

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Review Article

Periodontal Diseases and Dental Caries in Children with Type 1 Diabetes Mellitus

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Type 1 diabetes mellitus is a chronic metabolic disease of an autoimmune origin with early manifestation predominantly in the childhood. Its incidence has been rising in most European countries. Diabetes has been intensively studied by all branches of medicine. There were a number of studies investigating oral consequences of diabetes; however, unambiguous conclusions were drawn only for the relationship between diabetes and periodontal impairment. Many studies confirmed higher plaque levels and higher incidence of chronic gingivitis both in adults and in children with diabetes. Juvenile periodontitis is rare both in healthy subjects and in those with type 1 diabetes. Yet certain findings from well-conducted studies, for example, differences in oral microflora or the impact of metabolic control of diabetes on periodontal health, indicate a higher risk of periodontitis in children with type 1 diabetes. As for the association of diabetes and dental caries, the results of the studies are inconsistent. However, it was found that some risk factors for dental caries are either more or less prevalent in the diabetic population. Despite an extensive research in this area we have to acknowledge that many questions have remained unanswered. There is a need for continued, thorough research in this area.

1. Introduction

1.1. Diabetes: Definition and Current Classification. Diabetes mellitus is a common term for a group of chronic metabolic diseases, the basic feature of which is hyperglycemia.

Currently, classification of diabetes by ADA (American Diabetes Association) is being used, which emphasizes etiology of the disease (Table 1) [1].

Previously used classification by WHO (World Health Organization) from 1985 was based on the treatment of the disease and distinguished diabetes mellitus (DM), impaired glucose tolerance (IGT), and gestational diabetes mellitus (GDM). Type 1 diabetes belongs to the subgroup insulin-dependent diabetes mellitus according to this classification [2].

In ICD-10 (International Statistical Classification of Diseases and Related Health Problems, 10th revision), diabetes is classified under codes E10–E14, where type 1 diabetes mellitus is the title of E10 code [3].

This review focuses on the relationship between type 1 diabetes and oral health.

1.2. Type 1 Diabetes Mellitus: Etiology. Diabetes mellitus is a serious and relatively common chronic childhood disease. The basis of the disease is an autoimmune insulitis leading to a destruction of the beta cells of the pancreatic islets of Langerhans producing hormone insulin. This results in the clinical manifestation of the disease. The disease manifests itself in genetically predisposed individuals (polygenic genetic predisposition) after interaction of genetic and environmental factors [4]. It is estimated that, in the pathogenesis of the disease, the genetic and nongenetic factors are involved approximately to the same extent. Major environmental factors presumably associated with an increased risk of autoimmune insulitis include enterovirus infections (coxsackievirus type B) [5, 6], some nutritional factors (e.g., early exposure to cow's milk proteins, high intake of cow's milk products in the childhood, duration of breastfeeding,

Table 1: Classification of diabetes by ADA [1].

I	Type 1 diabetes	Immune mediated Idiopathic
II	Type 2 diabetes	
III	Other specific types	
IV	Gestational diabetes mellitus	

the effect of nitrates and nitrites, and vitamin D deficiency) [7], and perinatal and early childhood factors (e.g., higher age of the mother, lower birth order, and limited contact with other children) [8]. It is probably environmental factors, that is, changes in the exposure to certain nongenetic factors, that are responsible for a dramatic increase in an incidence of type 1 diabetes over the recent decades, because such an increase in the proportion of risk genotypes for type 1 diabetes in the population is not likely. As the pathogenesis of diabetes is still not fully explained, there are no effective preventive measures [9].

The incidence of the disease is predominantly nonfamilial; only 5–10% of the patients are siblings of diabetic children or children of diabetic parents [4]. In monozygotic twins, a simultaneous occurrence of type 1 diabetes was demonstrated in 23–53% of them [9].

The first clinical signs of diabetes in children include polyuria and polydipsia caused by an exceeded renal threshold for glucose excretion, followed by a gradual metabolic breakdown with typical symptoms.

Laboratory findings include hyperglycemia, glycosuria, and ketonuria. In order to evaluate the metabolic control of diabetes and treatment success rate, the blood levels of glycated hemoglobin (HbA1c) are monitored, as they reflect the blood glucose fluctuations over the last six weeks.

The treatment of type 1 diabetes is currently based on an intensified insulin therapy, which should mimic the normal insulin secretion, on regular self-monitoring of specific metabolic biomarkers in the patient, and on an education of the patient and his/her family [4].

1.3. Type 1 Diabetes Mellitus: Prevalence in Europe and in the Czech Republic. The incidence of type 1 diabetes differs significantly among diverse ethnic groups and nations. This is due to genetic differences and probably also due to still-not-fully-explained nongenetic factors.

In Europe, there is a north-south gradient in the disease incidence. The highest incidence is observed in Finland (40.2/100000/year), while the lowest incidence rates are reported by Balkan countries, particularly by Macedonia (3.2/100000/year), with the exception of the island of Sardinia (Figure 1) [4, 10–12].

When compared to other European countries, the Czech Republic has an intermediate but steadily rising incidence of diabetes. Over the recent years, type 1 diabetes mellitus was diagnosed in approximately three hundred Czech children per year [9].

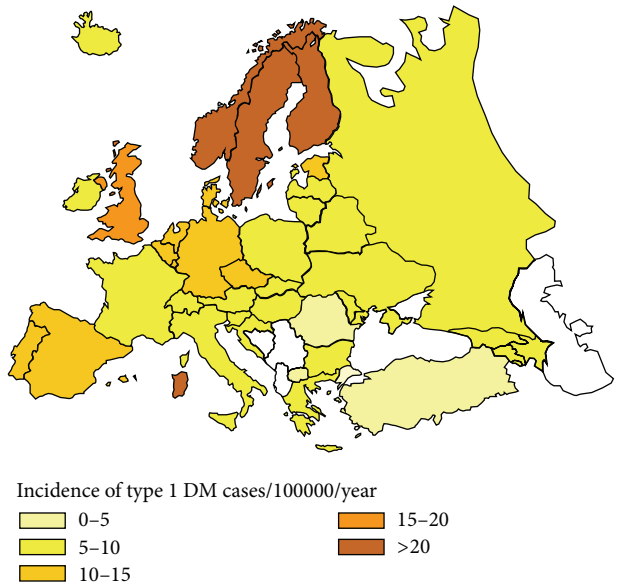

Figure 1: Incidence of type 1 diabetes in children up to 14 years of age/100000/year over the years 1990–1994 [12].

2. Oral Health in Children with Type 1 Diabetes

The relationship of type 1 diabetes as an underlying disease and different aspects of oral health has been investigated by a number of studies worldwide. Even though some associations have been confirmed, some others are still being discussed and the results of individual studies are often controversial not only due to methodology differences but also due to multifactorial etiology of most oral pathologies. After carrying out a thorough search of PubMed database, the authors can state the following points. Only a limited number of studies have been performed in children with type 1 diabetes probably due to a relative rareness of the disease. Nevertheless, the majority of the results of the research performed in adults with type 1 diabetes could be applied also to the child population. Case studies and reviews of the literature focusing on diabetic adults regardless of diabetes type and treatment are most frequent; however, the applicability of their findings to children with type 1 diabetes is debatable, given significant differences between the two types of diabetes and with respect to a higher proportion of type 2 diabetics in the studies.

2.1. Gingivitis, Dental Plaque, Calculus, and Periodontitis. Association between the terms mentioned above could be explained in a simplified way. It is now well accepted that bacteria in dental plaque are the major villains of periodontal diseases, which are infections of the structures around the teeth (the gums, the cementum, the periodontal ligament, and the alveolar bone). Calculus is a mineralized form of dental plaque which facilitates plaque deposition and irritates the gums. In the earliest stage, only the gums are affected (gingivitis). Without treatment, the infection spreads after a period of time and in the end all of the supporting tissues

are involved (periodontitis). After bone resorption, the teeth become loose and finally fall out [13].

A vast majority of studies have concluded that the incidence of chronic gingivitis in patients with type 1 diabetes is significantly higher than that in the healthy population and it increases with age. In a group of diabetic children aged 5–9 years, the mean value of gingival inflammation index (score of 0–3) was 1.54 ± 0.5, while in the control group it was 1.14 ± 0.5. In a group of diabetic children aged 10–14 years the mean value of gingival inflammation index versus the control group was 1.98 ± 0.6 and 1.17 ± 0.5, respectively [14]. In a Swiss clinical trial on experimental gingivitis induced by refraining from oral hygiene for three weeks, there were no differences in the plaque index scores or in the composition of bacterial plaque between the type 1 diabetics and healthy controls, but the diabetics responded to plaque irritation by an earlier developed and more severe gingival inflammation, which corresponded to a significantly higher levels of some inflammatory biomarkers in crevicular fluid [15, 16]. Another research performed in a large group of Brazilian child diabetics with a mean age of 13 ± 3.5 years observed gingivitis and periodontitis in 21% and 6% of the study subjects, respectively [17]. In a group of Lithuanian children with diabetes aged 10–15 years, the prevalence of gingivitis versus the control group was 27% and 13%, respectively [18]. Similarly, there are reports of a higher incidence of dental plaque [19] and earlier and heavier formation of calculus in diabetic children. The significant differences between the diabetic and healthy individuals appear in adolescence [14, 18].

Although periodontitis does not belong to clinical manifestations of any type of diabetes mellitus, it is still being labeled as "the sixth chronic complication of diabetes." It has been confirmed that, in individuals with diabetes, there is about a three times higher risk of periodontitis. Thus, diabetes is considered to be a predisposing factor for periodontitis [20]. In the diabetic patients, the periodontal disease develops at a younger age than in the healthy population [21, 22]. In children with diabetes, the periodontal impairment usually manifests in the adolescence [19, 23] and sometimes even earlier [24]. It was confirmed that there is an association between poorly controlled diabetes (higher HbA1c levels) and a development of periodontitis, even in children with type 1 diabetes [17, 20, 22, 25, 26]. Some studies show relationship between the duration of diabetes and severity of periodontitis [17, 21]. On the other hand, it was confirmed that there is a negative effect of periodontitis on blood glucose levels. This is due to an increased insulin resistance of tissues in reaction to systemic inflammatory mediators [20]. Recently, a presumption that treatment of periodontitis results in an improved metabolic control of diabetes has been confirmed [20], although some earlier studies did not support this hypothesis [25, 27, 28]. According to the recent clinical trials, a successful treatment of periodontitis decreased the HbA1c levels (reflecting a long-term diabetes control) of 0.4% [20], but this was observed mainly in patients with type 2 diabetes [29].

The onset and progression of periodontitis in diabetic patients are probably induced by diabetic microangiopathy, impaired immune response and a lower resistance to infections, different oral microflora, and disorders in collagen metabolism [23]. *In vitro* a direct negative effect of hyperglycemia and hypoglycemia on periodontal cells has been demonstrated. Hyperglycemia has also an indirect adverse effect, stimulating immune system cells to release inflammatory cytokines [30]. Recently, a number of studies have been published dealing with biochemical principles of periodontal damage in diabetes.

2.2. Impaired Immune Response in Diabetes and Periodontitis. Hyperglycemia caused by diabetes mellitus can alter immune system in many ways. First of all, it increases salivary concentration of glucose as well as its concentration in gingival crevicular fluid. This increased availability of glucose in the environment of oral cavity increases proliferation of periodontopathic and cariogenic bacteria and increases oral inflammation. Presence of elevated levels of proinflammatory mediators in the gingival crevicular fluid of periodontal pockets of poorly controlled diabetics, compared to nondiabetics or well-controlled diabetics, resulting in significant periodontal destruction with an equivalent bacterial challenge has been shown [31–33].

Hyperglycemia caused by diabetes mellitus can lead also to microangiopathy. Endothelial cells lining blood vessels use more glucose than usually and form more glycoproteins on their surface and basement membrane grows thicker and weaker. The vessel walls become thick and weak and vessels bleed easily and leak proteins. These vascular changes in periodontium decrease polymorphonuclear cells functions such as chemotaxis, adherence, phagocytosis and migration, oxygen utilization, and antigens elimination leading to progression of periodontitis.

Hyperglycemia also increases the formation of advanced glycation end-products. The overexposure of proteins (such as collagen) or lipids to aldose sugars induces nonenzymatic glycation and oxidation. These glycosylated products can create complex molecules, reducing collagen solubility and increasing levels of proinflammatory mediators responsible for the degradation of connective tissues. Changes to collagen metabolism result in accelerated degradation of both nonmineralized connective tissue and mineralized bone [32, 34]. The interaction of advanced glycation end-products with target cells, such as macrophages, via cell-surface polypeptide receptors stimulates the production of cytokines and matrix metalloproteinases, including collagenases and other connective tissue-degrading enzymes [31]. Monocytes from diabetics have shown a hyperresponsive phenotype with overexpression of proinflammatory mediators such as interleukin-1β, tumor necrosis factor-α, and prostaglandin E_2 [35, 36]. This exacerbation of the proinflammatory response in diabetics can lead to impaired wound healing and amplify connective tissues damage. This proinflammatory response may be further increased by the chemotactic properties of advanced glycation end-products for human monocytes which differentiate into the chronic inflammatory macrophages [32]. Degradation of newly synthesized collagen in connective tissues and alterations in the immune response can both contribute to progression of periodontal disease and impaired wound healing.

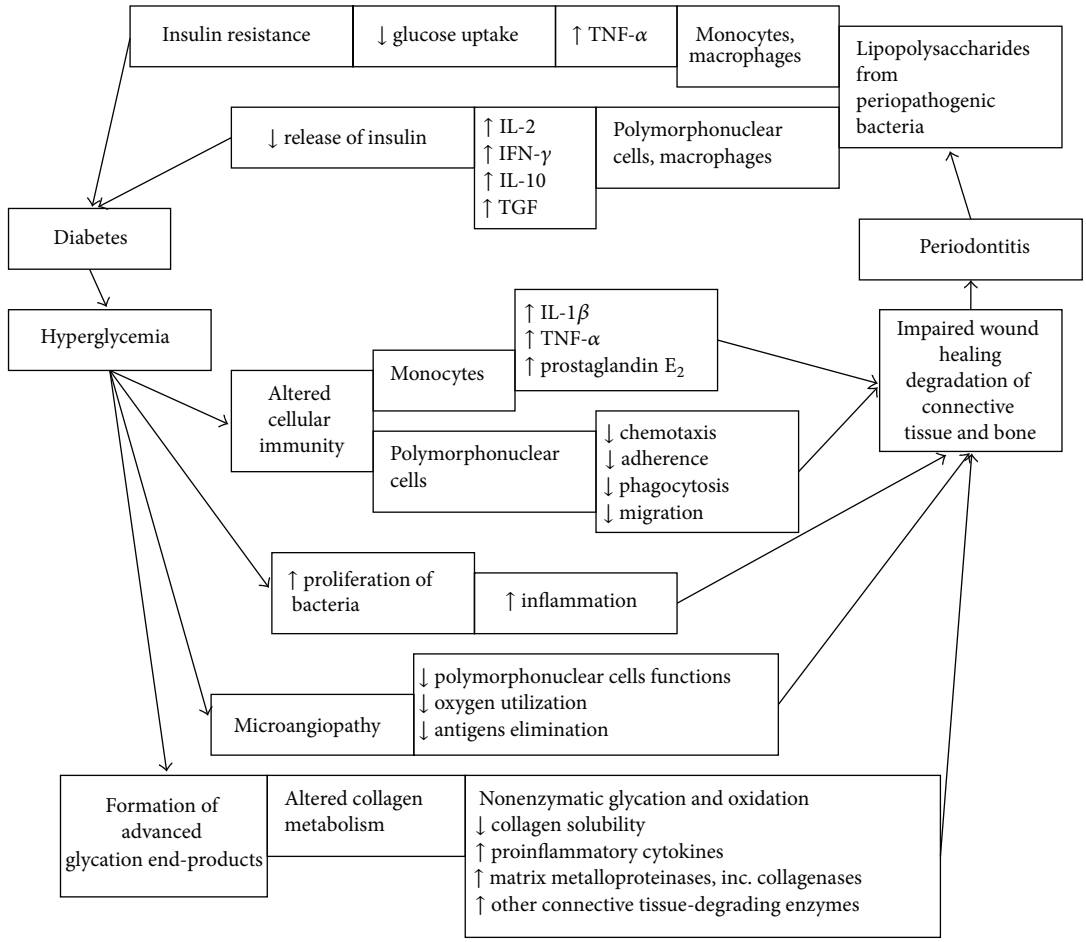

Figure 2: Associations between diabetes and periodontitis.

On the other hand, inflammation caused by inflammatory connective tissue disease such as periodontitis can trigger insulin resistance. Lipopolysaccharides from periopathogenic Gram-negative bacteria are able to induce tumor necrosis factor-alpha production by monocytes and macrophages. This cytokine can interfere with lipid metabolism, reduce glucose uptake by cells, and cause insulin resistance. An inflamed periodontium is highly vascular and may serve as a gate to the systemic circulation for bacterial products and produced local inflammatory mediators [37].

Periodontitis as an inflammatory condition leads to changes in cellular and humoral immunity. Polymorphonuclear cells and macrophages functions are affected; IL-2 and interferon gamma as well as cytokines important for humoral response such as IL-10 and transforming growth factor are produced. These changes in immune responses affect release of insulin and glycemic control [38, 39].

Periodontitis can thus obstruct glycemic control and obstructed glycemic control can further stimulate periodontal disease; a cycle worsening both conditions may be created (Figure 2).

Therefore, prevention and control of oral inflammatory diseases are essential for appropriate prevention and optimal management of diabetic complications [40].

2.3. Dental Caries. Regarding dental caries, the results of the studies are inconsistent. Dental caries is a multifactorial disease, and while some factors increase the risk of caries disease in type 1 diabetes, others reduce it.

Dental caries risk factors include oral cariogenic bacteria, intake of fermentable carbohydrates as a substrate for cariogenic bacteria, and sufficient time allowed for caries formation. The protective factors against caries include the saliva, oral hygiene, and fluorides [26]. The research has shown that the levels of cariogenic bacteria, particularly of *Streptococcus mutans*, are higher in diabetic patients and the proportion of individuals with high levels of cariogenic bacteria, particularly *Streptococcus mutans*, is higher in the diabetic population [26]. According to the nutritional recommendations, the diet of diabetic children should ideally be low in simple sugars, especially the so-called extrinsic sugars artificially added to food, while the so-called intrinsic sugars, contained mainly in fruits and vegetables, do not need to be

restricted. The frequency of meals should be higher compared to healthy individuals; however, patient compliance is critical [41, 42]. The saliva of diabetics shows both quantitative and qualitative changes [43–48]. The oral hygiene habits and education of diabetic patients seem to be similar or slightly better compared to healthy individuals [18, 49].

There are studies that show a lower incidence of dental caries in diabetic children compared to the healthy peers, such as the one by Orbak et al., who also reported a higher prevalence of dental caries in permanent teeth of children with poorly controlled diabetes [14]. The extensive study by Lal et al. showed a lower prevalence of dental caries in deciduous teeth of diabetic children compared to the controls [50]. In the study by Siudikiene et al. performed in healthy and diabetic children aged 10–15 years, over a two-year follow-up, there were no significant differences in the incidence of dental caries between the two study groups [45]. The same pattern (i.e., nonsignificant differences in the dental caries incidence between healthy and diabetic children) was observed in a large trial by Lalla et al. [19] and in several recent studies from Brazil, Egypt, and Belgium, where the study groups consisted of approximately 50 diabetic children [51–53]. Contrary to the above findings, higher caries experience in diabetic children was observed by investigators in Kuwait, India, and Puerto Rico [54–56].

The importance of adequate metabolic control of diabetes was partially confirmed in the study by Siudikiene et al., which compared prevalence of dental caries and levels of mutant streptococci in pediatric diabetic patients with adequate and poor metabolic control of diabetes based on HbA1c levels [57]. A relationship between caries risk and metabolic control was found in Twetman et al.'s study as well [58].

Studies that assess caries experience using dmft/DMFT (decayed, missing, filled temporary/permanent teeth) index often find nonsignificant differences between healthy and diabetic subjects; however, individual components of the index reveal a higher proportion of dental caries (component d/D, decayed) in the control subjects and higher proportion of fillings (component f/F, filled) and teeth extracted due to caries (component m/M, missing) in the diabetic subjects [14, 59].

The results of the studies performed in adults with type 1 diabetes are also inconsistent regarding the prevalence of dental caries. Some studies report a higher incidence of cervical, interproximal, or root caries in diabetics. These findings are considered to be due to a higher content of glucose in the saliva and crevicular fluid in the diabetics [60].

2.4. Saliva. Compared to the healthy subjects, there are both quantitative and qualitative changes in the saliva of diabetic patients. Diabetics are considered to have a reduced salivary flow [26], especially that of unstimulated saliva. The study by Moreira observed that the unstimulated salivary secretion in children with diabetes versus the control group was 0.15 ± 0.1 mL/min and 0.36 ± 0.2 mL/min, respectively. As for the stimulated saliva, there were no significant differences observed [43]. Similar results were observed in the study by López et al., where the unstimulated salivary secretion in children with diabetes versus the control group was 0.15 ± 0.1 mL/min and 0.25 ± 0.1 mL/min, respectively [44]. The reduced unstimulated and stimulated salivary secretion in children with diabetes were demonstrated by Siudikiene et al. [45]. As for the qualitative parameters, the typical findings are lower buffering capacity and pH of the saliva in diabetics (salivary pH in pediatric patients with diabetes versus the control group was 6.0 ± 0.8 and 7.0 ± 0.6, resp.) [43], higher viscosity of the saliva, higher levels of carbohydrates, glucose, and total protein in the saliva [44, 45], and higher levels of IgA and IgG antibodies [45–47] but lower levels of antimicrobial proteins, for example, lactoferrin and lysozyme [48]. As for the calcium levels in the saliva of diabetic children, the results of the studies are inconsistent: there are reports of both higher calcium levels [43] (which results in an enhanced tartar build-up) and lower calcium levels [44].

Siudikiene et al. found an association between the incidence of dental caries and glucose levels in the saliva of diabetic children [45].

2.5. Oral Microflora. Differences in oral microflora of diabetics and healthy individuals may significantly influence the incidence of diseases caused by bacteria, such as periodontal impairment and dental caries.

It was observed that in diabetic individuals including adolescents there are differences in the type and amount of periodontal pathogens, their habitat, and patients' age of their occurrence compared to the healthy population [21, 22].

As for the levels of cariogenic species, the results of the studies are inconsistent. There are reports of both insignificant differences in the levels of *Streptococcus mutans* and lactobacilli in healthy and diabetic individuals [21, 45, 52] and higher proportion of diabetics with high levels of *Streptococcus mutans* in the saliva compared to the control group [26]. As for the levels of *Candida* species in the oral cavity, there are reports of not only no differences between the diabetic and healthy children [45, 52] but also higher levels in the diabetics versus the healthy population [26, 57].

With respect to inconsistent findings, more microbiological studies are needed to clarify potential differences between healthy and diabetic children.

2.6. Diet. A recommended diet for children with type 1 diabetes corresponds to traditional rules of rational nutrition. The intake of fat and in some cases also of proteins should be restricted, but according to the recent nutritional recommendations the intake of carbohydrates should be up to 50–60% of the daily caloric intake. Dietary carbohydrates should primarily come from the complex carbohydrates, starch and fiber, whereas foods and beverages high in simple carbohydrates, which result in a significantly increased postprandial glycemia, should be excluded [4].

The frequency of daily meals and snacks partially depends on whether the patient uses the insulin pen or pump and on the type of insulin regimen used. Nevertheless, the frequency of food intake in diabetics is generally higher compared to the healthy population, and a standard recommendation is six meals per day [4].

The nutritional compliance particularly in adolescent patients is debatable, and about half of the patients in this age group are considered to be noncompliant [4].

2.7. Metabolic Control of Diabetes. A long-term metabolic control of diabetes is usually verified based on not only glycated hemoglobin HbA1c levels but also the total daily dose of insulin and blood glucose fluctuations.

The metabolic control of diabetes is associated with the risk of both acute and chronic complications. The main chronic complications of type 1 diabetes include neuropathy and microangiopathy. In poorly controlled patients, there is a decreased leukocyte phagocytic activity but also antibody and cell-mediated immunity [4], which results in an increased risk of bacterial infections [4, 26].

The effect of proper metabolic control and occurrence of chronic complications of diabetes on the development of periodontal diseases has been confirmed by a number of studies [17, 22, 25], in contrast to dental caries. The study by Dusková and Broukal demonstrated a positive correlation between the levels of glycated hemoglobin (HbA1c) and those of *Streptococcus mutans* and *Candida* species in the oral cavity [22].

2.8. Behavioral Aspects. A positive correlation between the metabolic control of diabetes and the occurrence of oral pathologies may be related, besides the biological factors, to certain psychological features of the patients. These features influence the patient compliance and self-care related to both diabetes and oral health problems. This association was observed in a study performed in 149 insulin-dependent diabetics, which included a questionnaire survey, clinical assessment of oral health, and analysis of diabetic patients' medical records. The study showed that 82% of diabetic patients without gingivitis had a good metabolic control and lower mean levels of HbA1c versus the diabetics with gingivitis [61]. The patients with a higher self-reported frequency of tooth brushing and lower plaque index score had lower mean levels of HbA1c [62]. On the other hand, a positive correlation between an inadequate interdental plaque removal and noncompliance in diabetes self-care was found [61]. Although the abovementioned studies were performed in the adult populations, it can be assumed that the above findings can be applied also to the child and adolescent populations with diabetes.

3. Conclusion

The authors have used both latest and older studies to bring a comprehensive overview of the relationship between type 1 diabetes and oral health in children.

There have been a number of studies on this topic published in dentistry; however, unambiguous conclusions have been reached only concerning the relationship between diabetes and periodontal diseases. A number of studies have shown higher amounts of dental plaque and increased incidence of chronic gingivitis in both adults and children with type 1 diabetes. Periodontitis in children is rare both in healthy subjects and in children with type 1 diabetes. Yet some of the findings of well-performed studies indicate a higher risk of periodontitis in children with type 1 diabetes. Regarding the impact of diabetes on dental caries development, the results of clinical trials are inconsistent. However, it has been confirmed that some of minor caries risk factors are more or less prevalent in a diabetic population compared to a nondiabetic control group. Quantitative and qualitative salivary changes in diabetics have also been confirmed, even though particular detailed results of individual studies vary. Oral health studies focusing on behavioral features of diabetic patients yield even more interesting insights.

The latest studies tend to focus on the research of possible direct and indirect influence of type 1 diabetes on oral health and vice versa, but their results are not crystal clear in most aspects. On that account, relationships between diverse oral diseases and their causal factors and type 1 diabetes should become a subject of intensive research in the future.

Conflict of Interests

The authors declare that they have no conflict of interests associated with this work.

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Review Article

Relationship between Periodontitis and Rheumatoid Arthritis: Review of the Literature

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Periodontitis (PD) and rheumatoid arthritis (RA) are immunoinflammatory diseases where leukocyte infiltration and inflammatory mediators induce alveolar bone loss, synovitis, and joint destruction, respectively. Thus, we reviewed the relationship between both diseases considering epidemiological aspects, mechanical periodontal treatment, inflammatory mediators, oral microbiota, and antibodies, using the keywords "periodontitis" and "rheumatoid arthritis" in PubMed database between January 2012 and March 2015, resulting in 162 articles. After critical reading based on titles and abstracts and following the inclusion and exclusion criteria, 26 articles were included. In the articles, women over 40 years old, smokers and nonsmokers, mainly constituted the analyzed groups. Eight studies broached the epidemiological relationship with PD and RA. Four trials demonstrated that the periodontal treatment influenced the severity of RA and periodontal clinical parameters. Nine studies were related with bacteria influence in the pathogenesis of RA and the presence of citrullinated proteins, autoantibodies, or rheumatoid factor in patients with PD and RA. Five studies investigated the presence of mediators of inflammation in PD and RA. In summary, the majority of the articles have confirmed that there is a correlation between PD and RA, since both disorders have characteristics in common and result from an imbalance in the immunoinflammatory response.

1. Introduction

Periodontitis (PD) is a chronic inflammatory disease where resident cells and preformed mediators induce leukocyte infiltration and progressive destruction of the tooth supporting tissues as a result of interaction between bacterial products, cell populations, and mediators in disease-susceptible individuals [1, 2]. This is also influenced by genetic and environmental risk factors and is characterized as a complex disease with multifactorial etiology [3, 4]. In this context, environmental factors, including oral hygiene/bacterial plaque, smoking, and stress, play an important role in the expression of PD [3]. Furthermore, it has been evidenced by some authors that there is a joint influence of polymorphisms in multiple genes [5], such as the genes of IL-10 [6] and IL-6 [7].

Polymorphonuclear neutrophils (PMNs) represent the first line of defense to protect the host from periodontal pathogens in the gingival sulcus and junctional epithelium. Data on the role of the pathogenesis of periodontitis are mixed. PMNs are a critical arm of defense against periodontitis, but bacterial evasion of the neutrophil microbicidal machinery coupled with delayed neutrophil apoptosis may transform the neutrophil from defender to perpetrator [8]. Actually, these cells can release a variety of factors, such as reactive oxygen species, collagenases, and other proteases, [1, 9], such as stimulation from a wide range of cytokines. In this scenario, macrophages can act as antigen-presenting cells, promoting the activation of lymphocytes [1]. Therefore, the cellular concentration of neutrophils in the inflammatory infiltrates decreases during the transition between gingivitis and periodontitis, in which there is a predominance of lymphocytes [9].

It has been described that proinflammatory cytokines, prostaglandin E_2, matrix metalloproteinase (MMP), nitric

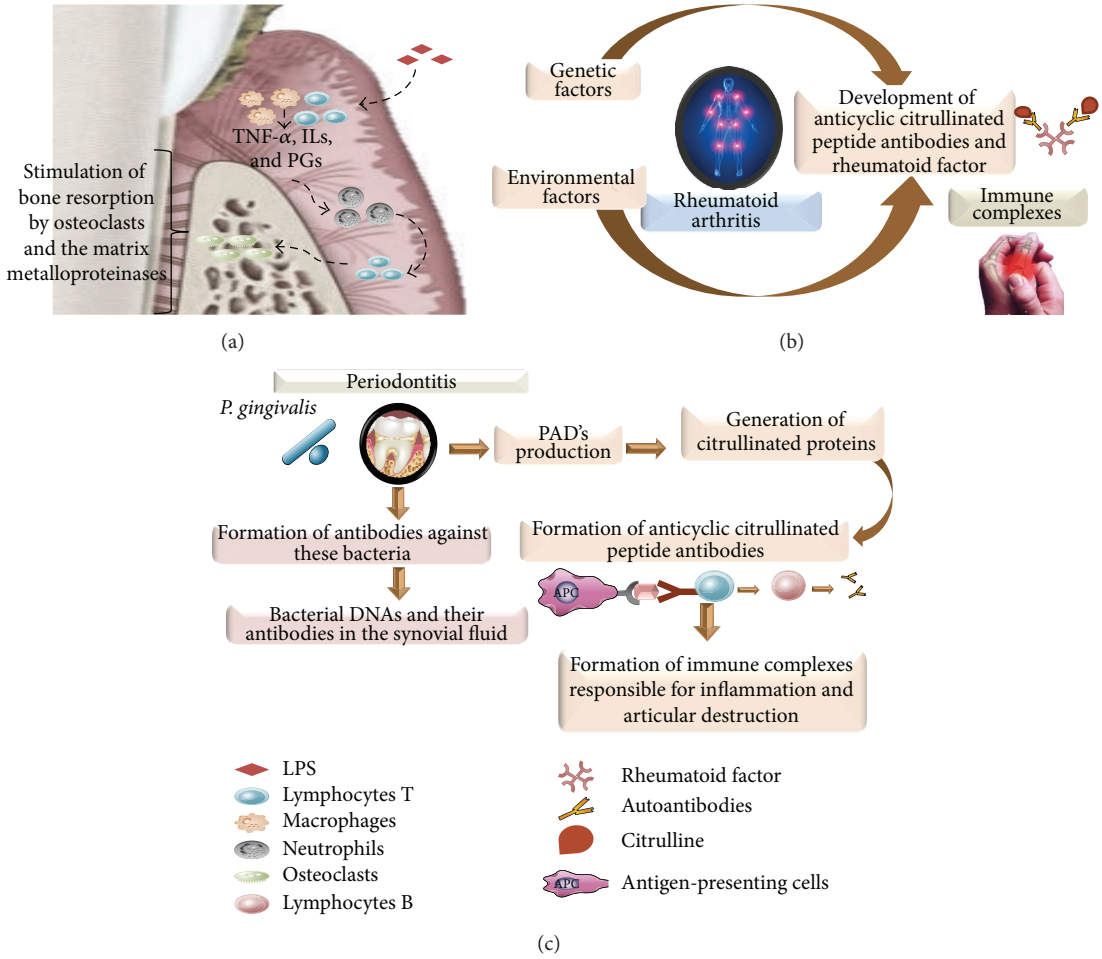

Figure 1: Scheme on the relationship between periodontitis and rheumatoid arthritis. (a) Pathogenesis of periodontitis and the effects promoted by lipopolysaccharides present in periodontopathogens. (b) The involvement of genetic and environmental factors in the development of rheumatoid arthritis. (c) Possible mechanisms that explain the relationship between rheumatoid arthritis and periodontitis.

oxide (NO), and other inflammatory mediators play a crucial role in the pathogenesis of PD [10–12]. Moreover, an increase of TNF-α, IL-1β, IL-6, IL-11, and IL-17 can induce osteoclastogenesis by increasing the expression of Receptor Activator of NF-κB Ligand (RANKL) and by reducing the osteoprotegerin (OPG) production in osteoblasts and stromal cells [13]. In fact, it was demonstrated that IL-17 and RANKL were overregulated and IL-10, an anti-inflammatory cytokine, and TGF-β1 were downregulated in active periodontal lesions compared with inactive lesions [14, 15] (Figure 1(a)).

Considering that an imbalance between bone formation and resorption is also linked to various diseases, studies suggest that PD may be a risk factor for other diseases [16], such as rheumatoid arthritis (RA) [17], but without consensus. Although pathogenesis of RA is not completely understood, it is recognized that the activation of the complement system is important in disease development [18], the abnormal response of circulating lymphocytes from patients, and an alteration in the structure of these cells, which contribute to the autoimmunity, immunosuppression, and the genesis of the disease [19]. Studies report there is a correlation between both PD and RA since the mechanisms for the development of RA have consonance with the pathogenesis of chronic PD. In fact, RA is defined as an inflammatory and autoimmune disease characterized by accumulation of leukocyte inflammatory infiltrate in the synovial membrane, as well as mediators such as PGE_2, TNF-α, IL-1β, IL-6, IL-12, IL-17, IL-18, IL-33, granulocyte macrophage colony-stimulating factor (GM-CSF), Monocyte Colony-Stimulating Factor (M-CSF), RANKL, MMPs, and NO, all being found in the synovial fluid [20–24], and leading to synovitis and joint architecture destruction.

Some studies have suggested that the susceptibility of RA may be associated with genetic or environmental factors [25]. One of the most important genetic factors is the human leukocyte antigen (HLA) class II. Certain alleles of this antigen are often associated with the development of rheumatoid arthritis (HLA-DRB1*0101, HLA-DRB1*0102, HLA-DRB1*0401, HLA-DRB1*0404, HLA-DRB1*0405, HLA-DRB1*0408, HLA-DRB1*0410, HLA-DRB1*1001, and HLA-DRB1*1402) [14]. Other factors include the allele of 620W of PTPN22 (protein tyrosine phosphatase nonreceptor type

22), a gene encoding tyrosine phosphatase that is involved in controlling the intracellular signaling triggered through T and B receptors [26]; C5-TRAF1, which can interfere with disease susceptibility and severity of the alteration in the structure, function, and levels of complement component c5/factor 1 associated with the TNF receptor [27]; gene encoding the CTLA4 (cytotoxic T lymphocyte antigen-4), the protein responsible for the regulation of T lymphocyte activation [28]; peptidylarginine deiminase (PAD2), the enzyme responsible for the generation of citrullinated proteins, which are related to the formation of anticyclic citrullinated peptide autoantibodies [29] (Figure 1(b)). With regard to environmental factors, smoking is a risk factor that duplicates the risk of developing RA, but its effect is limited to those with antibodies to citrullinated peptides [30, 31]. Other factors refer to the excessive consumption of coffee (more than 10 cups daily) which can be related to the development of the disease [32] and bacterial microbiota, including oral bacterial species which can participate in the etiopathogenesis of RA [33]. On the other hand, the intake of alcohol may exert a protective effect in rheumatoid arthritis in a dose-dependent manner [32]. The literature shows that the basic difference between both diseases is that RA is an inflammatory autoimmune disease, while PD is an immunoinflammatory disease of bacterial origin [9]. However, it is noteworthy that many epidemiological studies seem to dilute the subtle differences expressed by some parameters, though clinically important. Indeed, analyses of inflammatory mediators and other molecular markers are examples where the differences found in a trial with few participants could disappear in a large and diverse sample. In this sense, this review is a critical appraisal of studies that address potential associations of periodontitis with RA and with an overall comprehensive approach.

2. Methods

For this review, the US National Library of Medicine National Institutes of Health PubMed was searched by two independent researchers who agreed with the search criteria of studies with patients with both PD and RA and checked by a third researcher separately. The keywords *periodontitis* and *rheumatoid arthritis* were used and 367 articles published in English were found. The time period was limited from January 2012 to March 2015, and 162 references were found. Then, a critical reading based on titles and abstracts was made and 136 papers were excluded, such as reviews, assays *in vitro* and animal studies, articles that were not in English, studies not related to both PD and RA, case study, workshop, or unavailable and incomplete articles. Then, 26 articles were finally included for this review, which related to PD and RA, considering epidemiological aspects, mechanical periodontal treatment, mediators of inflammation, oral microbiota, and antibodies as seen in Figure 2.

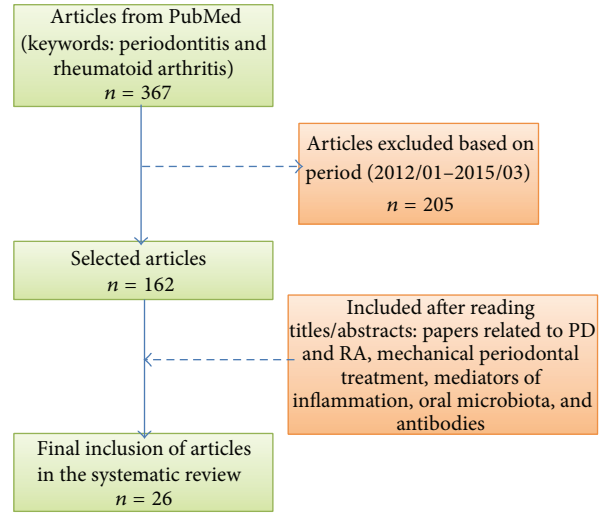

Figure 2: Search flow-chart and selection of articles for the review of the literature considering a bit more than the last three years. PD = periodontitis; RA = rheumatoid arthritis.

3. Results

Table 1 shows demographic data, such as gender, age and habits, comorbidities and medications, and the relationship between both diseases investigated through clinical and epidemiological associations, presence of oral bacterial DNA in patients with RA, proinflammatory mediators, antibodies against bacteria, and autoantibodies, as well as the effects of mechanical periodontal treatment, related to the 26 selected articles.

In most articles (92.3%), the analyzed groups were mainly composed of women. Regarding age, most patients were 40 years old, except for the study of Dev et al. (2013) [45] and Ranade and Doiphode (2012) [37], whose patients were above 20 and 30 years old, respectively. Among the 26 articles, 57.7% [34–36, 38, 39, 42, 50–57, 59] used samples with smoker patients, while 30.8% established smoking as criteria for excluding [37, 41, 43, 45–49]. 11.5% did not mention smoking status of patients [40, 44, 58]. Comorbidities such as diabetes, Sjögren's syndrome, hypertension, cardiovascular disease, hyperlipidemia, renal disease, and osteoporosis/osteopenia have only been reported in studies of Mikuls et al. (2012) [35], Khantisopon et al. (2014) [54], and Gonzales et al. (2015) [59]. Regarding the medication used by patients, 50% [35–37, 40, 41, 44, 45, 49, 50, 52, 53, 55, 58] of the articles did not specify the pharmacological treatment. In the remainder of the studies, the most frequently reported treatment for rheumatoid arthritis included disease-modifying antirheumatic drugs (methotrexate, sulfasalazine, and leflunomide) [38, 39, 42, 43, 46, 51, 54, 56, 57, 59], biologic therapy (anti-TNF-α) [34, 38, 39, 42, 59], corticosteroids (prednisolone) [38, 42, 43, 46, 51, 54, 56, 59], and/or nonsteroidal anti-inflammatory drugs [43, 46–48, 51, 54, 57].

Among the selected trials, eight studies broached the epidemiological and clinical relationship of patients with PD and RA [38, 41, 44, 45, 49, 54, 56, 59], indicating a higher prevalence of PD in patients with RA, which have worse periodontal parameters. The effect of mechanical removal of foci of infection in the oral cavity on the severity of RA and periodontal clinical parameters were shown by four

Table 1: Summary of papers relating to the relationship between periodontitis and rheumatoid arthritis.

Authors	Study population	Demographic characteristics	Exclusion criteria	Periodontal disease evaluation	RA treatment	Results	Association of PD × RA
Ishida et al., 2012 [34]	30 RA patients; 30 patients with PD; 30 healthy volunteers	RA (27 females and 3 males; 66 ± 2 years; 3 smokers); PD (20 females and 10 males; 62.3 ± 1.5 years; 1 smoker and 2 former smokers); healthy (20 females and 10 males; 53.4 ± 2.7 years; nonsmokers)	Periodontal therapy 6 months prior to examination Less than 15 teeth Diabetes mellitus and pregnancy	CAL, PPD, and missing teeth	Anti-TNF-α	The hypomethylated status, a single region of the IL-6, may contribute to elevated serum levels of this cytokine, implying a role in the pathogenesis of PD and RA	$P < 0.0001$
Mikuls et al., 2012 [35]	Patients: 171 autoantibody negative; 75 autoantibody positive; 38 high risk based on the presence of a positive ACPA or positivity to 2 or more RF assays	Negative antibody (69% female; 44 ± 14 years; 37% smokers; 5% with DM); positive antibody (73% female; 48 ± 15 years; 31% smokers; 4% with DM); high risk (76% female; 51 ± 16 years; 29% smokers; 5% with DM)	Age < 18 years	Not analyzed	Not informed	Anti-*P. gingivalis* concentrations were higher in high-risk, with autoantibody (anti-CCP and RF), positive group than in the autoantibody negative group	$P = 0.01$
Nesse et al., 2012 [36]	15 patients with PD; 6 healthy volunteers; 4 RA patients	PD and Ab5612+ (63% female; 48.9 ± 11.4 years; 50% smokers); PD and Ab5612− (71% female; 53.6 ± 17.9 years; 29% smokers); PD and F95+ (50% female; 55 ± 12.4 years; 40% smokers); PD and F95− (100% female; 43.2 ± 16.3 years; 40% smokers); PD either+ (58% female; 52.3 ± 14 years; 42% smokers); PD both− (100% female; 46.2 ± 18.5 years; 33% smokers); controls either+ (100% female; age: 28.5 ± 5.5 years; 50% smokers); controls either− (50% female; 28 ± 8.6 years; 25% smokers)	Other systemic conditions (excluding RA)	PPD	Not informed	Formation of citrullinated proteins in periodontal tissues was shown, which appear to be a variety similar to those observed in RA synovial tissue affected by RA	—

Table 1: Continued.

Authors	Study population	Demographic characteristics	Exclusion criteria	Periodontal disease evaluation	RA treatment	Results	Association of PD × RA
Ranade and Doiphode, 2012 [37]	40 RA patients; 40 healthy volunteers	80% female 20–70 years	Systemic diseases; medications that affect the periodontium; tobacco habit Dental treatment (a month before)	ABL, CAL, PPD, GI, and PI	Not informed	High prevalence of mild to moderate PD in patients with RA presenting significantly higher GI, PI, PPD, and CAL, when compared to healthy volunteers	$P < 0.001$
Scher et al., 2012 [38]	31 patients with new-onset RA; 34 chronic RA patients; 18 healthy volunteers	New-onset RA (68% female; 42.2 years; 16% smokers, 16% former smokers, and 68% nonsmokers); chronic RA (79% female; 47.7 years; 6% smokers; 24 former smokers; 70% nonsmokers); healthy (65% female; age: 42.2 years; 6% smokers; 16% former smokers; 78% nonsmokers)	Recent use of any antibiotic therapy; current extreme diet; inflammatory bowel disease Malignancy; consumption of probiotics; tract surgery leaving permanent residua; liver, renal, or peptic ulcer diseases	CAL, PPD, and BOP	Corticoids, DMARDs, and biologic therapy	New-onset RA patients exhibit a high prevalence of PD at disease onset; the colonization with *P. gingivalis* correlates with PD severity; overall exposure was similar among groups	$P < 0.01$
Smit et al., 2012 [39]	95 RA patients; 44 non-RA controls; 36 healthy volunteers	RA (68% female; 56 ± 11 years; 23% current smokers; 40% former smokers); non-RA controls (57% female; 54 ± 9.7 years; 27% current smokers; 43% former smokers); healthy (56% female; 34 ± 15 years; 14% current smokers)	Age < 18 years; edentulism; diabetes mellitus; active thyroid disease; nonoral infections Malignancy; myocardial infarction or stroke; pregnancy Antibiotic use	BOP, PPD, and CAL	DMARDs and anti-TNF-α	Association between PD and RA and the increased prevalence of PD in patients with RA Anti-*P. gingivalis* titers were higher in RA patients with severe PD compared with non-RA patients	$P < 0.001$ $P < 0.05$
Témoin et al., 2012 [40]	11 RA patients; 25 patients with OA	RA (100% female; 45–70 years); OA (9 males and 16 females; 50–80 years)	Antibiotic use Edentulism	Not analyzed	Not informed	Bacterial DNA was detected in 13.9% of RA patients; *F. nucleatum* consisted in the pathogen most prevalent	—
Torkzaban et al., 2012 [41]	53 RA patients; 53 healthy volunteers	RA (41.5 years); healthy (43.5 years); 58 females and 48 males	<7 teeth; systemic diseases such as diabetes or Sjögren's disease; antibiotics use; treatment for PD; immunosuppressive drugs; smokers	PI, BOP, and CAL	Not informed	Patients with RA had a higher percentage of sites presenting plaque, BOP, and CAL	$P < 0.001$

Table 1: Continued.

Authors	Study population	Demographic characteristics	Exclusion criteria	Periodontal disease evaluation	RA treatment	Results	Association of PD × RA
Bıyıkoğlu et al., 2013 [42]	10 patients with PD and RA; 15 patients with PD	PD and RA (9 females; 46.6 ± 8 years; 8 smokers); PD (6 females; 46.73 ± 7 years; 9 smokers)	Systemic disease or infection other than RA; history of antibiotic therapy Periodontal treatment <10 teeth	PPD, CAL, BOP, and PI	MTX, leflunomide, prednisolone, chloroquine, sulfasalazine, anti-CD20, and anti-TNF-α	The nonsurgical periodontal treatment reduced the clinical periodontal parameters and promoted an improvement in the scores of RA	$P < 0.001$
Cetinkaya et al., 2013 [43]	17 RA patients; 16 patients with PD; 16 healthy volunteers	RA (14 females and 3 males; 47.82 years) PD (6 females and 10 males; 44 years); healthy (8 females and 8 males; 28 years)	Conservative or prosthetic restorations; caries at the anterior region; systemic or local disease with an influence on the immune system (cancer and cardiovascular and respiratory diseases); history of hepatitis or HIV infection; immunosuppressive chemotherapy; current pregnancy or lactation; antibiotic prophylaxis; history of antibiotic therapy Periodontal treatment <18 years; smokers	PI, GI, PPD, and CAL	MTX, sulfasalazine, leflunomide, NSAIDs, and corticoids	No significant differences in the levels of pro- and anticytokine between PD and RA were observed	$P > 0.05$
Chen et al., 2013 [44]	13779 RA patients; 137790 non-RA patients	RA (77.4% female; 52.6 ± 14.4 years); controls (77.4% female; 52.4 ± 15.4 years); comorbidities: diabetes mellitus and Sjögren's syndrome	Age < 16 years	Periodontal surgery, number of PD-related visits	Not informed	PD severity was related to a history of periodontal surgery, more PD-related visits, and higher costs of medical care; an association between periodontitis and incident RA was demonstrated	$P < 0.001$
Dev et al., 2013 [45]	852 patients with PD; 668 healthy volunteers	52.8% female and 47.2% male 30–70 years	Smokers; diabetes mellitus; periodontal therapy (3 months before); antibiotic use (3 months before); systemic disease and osteoporosis; antibiotic prophylaxis; pregnancy; lactation	PPD, BOP, and CAL	Not informed	Moderate to severe periodontitis is an independent risk factor for RA	—

Table 1: Continued.

Authors	Study population	Demographic characteristics	Exclusion criteria	Periodontal disease evaluation	RA treatment	Results	Association of PD × RA
Erciyas et al., 2013 [46]	30 RA patients with moderate to high disease activity and chronic PD (LDA); 30 RA patients with low disease activity and chronic PD (MHDA)	LDA (25 females; 42.6 ± 10.05 years); MHDA (22 females; 43.83 ± 10.97 years)	Periodontal therapy (6 months); presence of any other systemic diseases; smokers; <18 teeth Antibiotic therapy	PI, PPD, CAL, and BOP	DMARDs Corticoids NSAIDs or anti-TNF-α	SRP might prove beneficial in reducing RA severity as measured by ESR, CRP, TNF-α levels in serum, and DAS28 in RA patients with chronic periodontitis	$P < 0.001$ $P < 0.05$ related to TNF-α
Gümüş et al., 2013 [47]	17 RA patients; 19 patients with OPR; 13 healthy volunteers	RA (17 females; 44 years) OPR (19 females; 58 years) Healthy (13 females; 54 years)	Systemic disease; antibiotic use (6 months); corticosteroids; β-blockers use; diabetes mellitus; periodontal therapy (6 months) <10 teeth; smokers	PPD, CAL, and BOP	NSAIDs	Concentrations in serum and GCF of RANKL and OPG were significantly higher and lower, respectively, in patients with RA when compared to individuals with OPR and healthy volunteers; the total counts of the IL-17 and IL-17F were significantly higher in patients with RA compared to the control group	$P < 0.05$
Gümüş et al., 2013 [48]	17 RA patients; 19 patients with OPR; 13 healthy volunteers	RA (17 females; 44 years) OPR (19 females; 58 years) Healthy (13 females; 54 years)	Systemic disease Antibiotic use (6 months); corticosteroids; β-blocker use; diabetes mellitus; periodontal therapy (6 months); <10 teeth; smokers	PPD, CAL, BOP, and PI	NSAIDs	Despite the long-term use of various anti-inflammatory drugs in RA and osteoporosis, patients involved in this study showed an increase in gingival crevicular and serum levels of TNF-α	$P < 0.05$
Joseph et al., 2013 [49]	100 RA patients; 112 healthy volunteers	RA (76 females and 24 males; 46.54 ± 8.5 years) Healthy (86 females and 26 males; 45.91 ± 9.76 years)	Systemic diseases; smokers; conditions that may alter the serum CRP and blood ESR levels; antibiotic use; periodontal therapy	GI, PPD, CAL, missing teeth, and OHI-S	Not informed	Patients with RA, compared to healthy volunteers, showed a significant difference in PPD and CAL, and 58% of patients with RA had moderate to severe PD	$P < 0.05$
Lappin et al., 2013 [50]	38 RA patients; 36 healthy volunteers	RA (17 females and 21 males; 31–70 years; 24 nonsmokers and 16 smokers); healthy (16 females and 20 males; 30–65 years; 20 nonsmokers and 16 smokers)	Systemic disease; previous antibiotic use (3 months)	PPD, CAL, BOP, and missing teeth	Not informed	Although smokers have shown lower antibody titers, individuals with periodontitis showed higher levels of anti-CCP antibodies	$P < 0.001$

Table 1: Continued.

Authors	Study population	Demographic characteristics	Exclusion criteria	Periodontal disease evaluation	RA treatment	Results	Association of PD × RA
Okada et al., 2013 [51]	55 RA patients	Treatment group (84.6% female; 60.7 years; 9 former smokers and 17 nonsmokers); control group (82.8% female; 62.7 years; 11 former smokers and 18 nonsmokers)	Diabetes mellitus; pregnancy; antibiotic use (3 months); periodontal therapy (3 months)	GI, PI, CAL, BOP, and PPD	Corticoids, DMARDs, and NSAIDs	SRP decreased RA parameters and serum levels of IgG to *P. gingivalis* Citrulline in patients with RA	$P < 0.01$ $P < 0.04$
Reichert et al., 2013 [52]	42 RA patients; 114 healthy volunteers	Healthy (40.4% female; 53.8 ± 16.7 years; 10.7% smokers, 14.3% former smokers, and 75% nonsmokers); RA (52.4% female; 56.1 ± 15.2 years; 14.3% smokers, 11.9% former smokers, and 73.8% nonsmokers)	Pregnancy; antibiotic use; periodontal therapy	BOP, CAL, and PI	Not informed	There was a significant amount of *P. gingivalis* DNA in synovial fluid and in subgingival plaque from patients with RA	$P < 0.05$
De Pablo et al., 2014 [53]	96 patients with PD; 98 without PD	PD (62% female; 46 ± 8.9 years; 24% smokers) Without PD (59% female; 29 ± 7.3 years; 22% smokers)	Pregnancy; lactation; antibiotic and NSAIDs use (3 months before); vitamin supplementation (3 months before); regular mouthwash; dietary requirements (celiac disease)	Not analyzed	Not informed	Serum antibodies were significantly higher in patients with PD compared with those without PD for antibodies against CEP-1, REP-1, vimentin, and fibrinogen Cit-vim	$P < 0.0001$ $P = 0.003$
Khantisopon et al., 2014 [54]	196 RA patients	87% female; 51.7 ± 9.7 years; 78% nonsmokers, 30.69% with hypertension; 34.16% with dyslipidemia; 2.97% with DM; 2.47% with chronic kidney disease; 58.97% with osteoporosis; and 23.08% with osteopenia.	Pregnancy; lactation; systemic conditions that could affect the progression of periodontal disease, such as uncontrolled diabetes mellitus, severe hypertension, severe renal insufficiency, or malignancies; antibiotic uses	GI, PI, CAL, PPD, and gingival recession	MTX, prednisolone, DMARDs, and diclofenac	RA Patients had a high prevalence of moderate or severe periodontitis Increasing age, the male sex, history of previous or current smoking, and high PI were associated with the severity of periodontal disease	— $P < 0.02$

Table 1: Continued.

Authors	Study population	Demographic characteristics	Exclusion criteria	Periodontal disease evaluation	RA treatment	Results	Association of PD × RA
Mikuls et al., 2014 [55]	287 RA patients; 330 healthy volunteers	RA (63% male; 59 years; 19% smokers, 43% former smokers; 38% nonsmokers); healthy (60% male; 59 years; 11% smokers, 35% former smokers, and 54% nonsmokers)	Tetracycline or antibiotic use (6 months); cyclosporine or dilantin; antibiotic prophylaxis prior to dental probing	PPD, BOP, PI, and gingival recession	Not informed	Periodontitis was more common in patients with RA positive for anticyclic citrullinated peptide; there was an association between periodontitis and the number of inflamed joints and RF Antibodies specific for anticyclic citrullinated peptide were higher in patients with *P. gingivalis* subgingival plaque	$P < 0.02$
Wolff et al., 2014 [56]	22 RA patients; 22 healthy volunteers	68% female; 51.7 ± 9.7; 14% smokers	Current therapy with biological DMARDs; poor oral hygiene or disabilities that interfere with adequate oral hygiene; periodontitis as a manifestation of systemic disease; periodontal therapy within the past 5 years; professional to antibiotics use; pregnancy or nursing during the past 6 months	PPD, BOP, GI, PI, and CAL	DMARDs and corticoids	PPD, BOP, and CAL were increased in RA patients when compared to healthy volunteers	$P < 0.001$
Seror et al., 2015 [57]	694 early-RA patients; 79 healthy controls; 61 patients with PD; 54 patients with sicca	RA (78.2% female; 48.5 ± 12.3 years; 48% ever smokers); healthy (84.6% female; 47.6 ± 11.9 years; 16.2% ever smokers); sicca (85.2% female; 48.9 ± 11.5 years; 37.3% ever smokers); PD (41% female; 50.7 ± 8.3 years; 65.6% ever smokers)	DMARDs (except within the 15 days before inclusion) or steroids use; inflammatory rheumatic disease other than RA	Not analyzed	NSAIDs and DMARDs	Anti-*P. gingivalis* antibody titres did not significantly differ between early-RA patients and healthy controls, sicca controls, or PD controls	$P = 0.66$ $P = 0.53$ $P = 0.17$

Table 1: Continued.

Authors	Study population	Demographic characteristics	Exclusion criteria	Periodontal disease evaluation	RA treatment	Results	Association of PD × RA
Silosi et al., 2015 [58]	21 healthy controls, 16 with active RA, 14 with PC, and 12 RA-CP association	Controls (7 males and 14 females; 35–58 years) RA (4 males and 12 females; 38–62 years); PC (6 males and 8 females; 39–68 years) RA-PC (3 males and 9 females; 38–62 years)	History of medication other than NSAIDs Drugs (6 months); periodontal treatment; pregnancy; hormonal or vitamin therapy	PI, BOP, and PPD	Not informed	Differences of serum MMP-9 between RA and CP groups and control Serum levels of MMP-9 were similar in RA and RA-CP Increased MMP-9 CGF levels in RA-CP subjects as compared to CP	$P < 0.01$ $P > 0.05$ $P < 0.05$
Gonzales et al., 2015 [59]	287 with RA and 330 controls with OA	RA (63% male; 59 ± 12 years; 38% never smokers; 43% former smokers; 19% current smokers; 18% with DM; 45% hypertension; 13% cardiovascular disease; 11% osteoporosis); OA (60% male; 59 ± 11 years; 54% never smokers; 35% former smokers; 11% current smokers; 25% with DM; 57% hypertension; 10% cardiovascular disease; 15% osteoporosis)	Tetracycline or related antibiotic use (6 months); antibiotic premedication Pregnancy or breastfeeding; prior use of cyclosporine or phenytoin; systemic inflammatory disease	ABL	MTX, prednisolone, and biologic therapy	ACPA-positive patients with RA had a statistically significantly higher mean percentage of sites with ABL >20% than patients with OA After multivariate adjustment, greater ABL was significantly associated with higher serum ACPA concentration, DAS28, health assessment questionnaire disability, tender joint count, and joint space narrowing scores among patients with RA	$P = 0.03$ $P = 0.004$ $P = 0.023$ $P = 0.05$ $P = 0.02$ $P = 0.05$

ABL, alveolar bone loss; anti-CCP, anticyclic citrullinated peptide; anti-TNF-α, tumor necrosis factor-alpha antagonists; BI, bleeding index; BOP, bleeding on probing; CAL, clinical attachment level; CI, calculus index; DAS28, disease activity score in 28 joints; DM, diabetes mellitus; DMARDs, disease-modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; GAP, generalized aggressive periodontitis; GBI, gingival bleeding index; GBTI, gingival bleeding time index; GCF, gingival crevicular fluid; GI, gingival index; HCQ, hydroxychloroquine; IL, interleukin; JIA, juvenile idiopathic arthritis; LAP, localized aggressive periodontitis; MMP, matrix metalloproteinase; MTX, methotrexate; NSAIDs, nonsteroidal anti-inflammatory drugs; OA, osteoarthritis; OHI-S, oral hygiene index-simplified; OPR, osteoporosis; PBI, papillary bleeding index; PD, periodontitis; PI, plaque index; PPD, probing pockets depths; PsA, psoriatic arthritis; PSI, periodontal screening index; RA, rheumatoid arthritis; RANK, Receptor Activator of Nuclear Factor κB; RANKL, Receptor Activator of Nuclear Factor κB Ligand; RF, rheumatoid factor; SRP, scaling and root planning; TNF-α, tumor necrosis factor alpha; VPI, visible plaque index. $P < 0.05$ was considered significant.

studies [37, 42, 46, 51], which demonstrated the beneficial effects of the mechanical treatment in the improvement of clinical parameters of RA. Two studies were related to the oral bacteria influence of the pathogenesis of RA [40, 52]. Seven trials highlighted the presence of citrullinated proteins and their antibodies, antibodies to *P. gingivalis* in patients with RA and periodontitis, and also the association between anti-*P. gingivalis* and periodontal parameters, and the titers of rheumatoid factor and antibodies anticyclic citrullinated peptide, which were also related to the severity of PD [35, 36, 39, 50, 53, 55, 57]. Regarding the inflammation in both diseases, five trials considered the mediators of inflammation to the PD and RA [34, 43, 47, 48, 58], such as MMP-9, TNF-α, IL-17, RANKL, and OPG. Considering the relationship between rheumatoid arthritis and periodontitis, only two articles showed no statistical significant association, while 24 studies have established this association, either by descriptive (3 studies) or statistical analysis (21 studies).

4. Discussion

In this review, demographic data and other aspects that can modify one or both diseases were presented, as well as the relationship between both diseases investigated through clinical and epidemiological associations, effects of mechanical periodontal treatment, presence of oral bacterial DNA in patients with RA, proinflammatory mediators, antibodies against bacteria, and autoantibodies.

The articles showed higher prevalence of female patients. This aspect was interesting, as a possible relationship between female sex hormones and susceptibility of rheumatoid arthritis had been reported in the literature, so that low levels of those hormones at menopause promote the risk of developing the disease early [60]. However, a protective role of oral contraceptives on the risk for rheumatoid arthritis in women is still controversial [61–63]. On the other hand, there is strong evidence that estrogen deficiency influences the severity of periodontitis, since worse periodontal parameters were observed as bleeding on probing, gingival recession, and clinical attachment loss in postmenopausal women with osteoporosis [64].

The smoking status was also recorded in the selected studies. Cigarette smoking is considered an important risk factor for the development of rheumatoid arthritis, since it was demonstrated that lifelong cigarette smoking was positively associated with the risk of RA even among smokers with a low lifelong exposure [65]. Moreover, it has been related that smoking interacts with HLA-DR SE genes and increases the risk of anti-CCP antibodies in patients with rheumatoid arthritis [66]. Regarding the periodontium, it was shown that smokers presented greater probing depths, when compared to the probing depths of patients who never smoked [67].

The literature shows that PD does not usually require pharmacological treatment, except for mechanical periodontal treatment as routine. In this review, this fact was also observed, while half of the studies had shown that rheumatoid arthritis involved some pharmacological approach. The use of disease-modifying antirheumatic drugs (DMARDs) aims to reverse the symptoms of the disease, reduce the progression of joint damage, and consequently improve the quality of life of patients [68]. The conventional synthetic DMARDs include methotrexate, sulfasalazine, and leflunomide; the available tumor necrosis factor inhibitors (adalimumab, etanercept, and infliximab), the T cell costimulation inhibitor (abatacept), the anti-B cell agent (rituximab), and the interleukin-6 receptor blocking monoclonal antibody are included in biological DMARDs [69]. These medications may be associated with glucocorticoids (GC) or nonsteroidal anti-inflammatory drugs (NSAIDs). The long-term, low-dose glucocorticoid and NSAIDs therapy were shown to reduce joint symptoms, pain, and other systemic manifestations [70, 71]. Although these benefits are present, the long-time treatment with GC and methotrexate decreased immune response and promoted oral changes, such as candidiasis, periodontitis, and oral ulceration besides impaired saliva secretion [72]. Indeed the literature demonstrated that patients on corticosteroids exhibited higher levels of candidiasis, clinical attachment loss, and probing pocket depth [73]. These aspects, at least in part, may contribute to the worse periodontal status of RA patients when compared to healthy patients. Moreover, the use of medications referred to in half of the articles could compromise the evaluation of this review. However, it is noteworthy that the other half of the articles did not use any medication [35–37, 40, 41, 44, 45, 49, 50, 52, 53, 55, 58].

Analysing the articles, it was observed that most patients with RA showed a significant increase in the incidence of PD as compared to healthy individuals, while only few articles concluded the opposite, probably due to the lack of standardization of parameters in evaluating the different types of periodontitis. Although epidemiological studies outlined by Dev et al. (2013) [45] have not observed a significant RA incidence in subjects with periodontitis where these authors suggested that periodontitis is an independent factor for RA, several other studies have shown that patients with RA were more susceptible to the development of periodontitis [38, 44], since these patients had worse periodontal parameters, such as clinical attachment level [37, 56], alveolar bone loss [56, 59], probing depth [37, 49], plaque index, and bleeding on probing [37, 41, 54]. Indeed, the mechanical periodontal treatment as scaling and root planning in the control of periodontal infection interfered not only with the severity of RA but also with the periodontal clinical parameters [74]. This result can be explained by a reduction in the foci of oral bacteria, and therefore the low levels of inflammation demonstrated a decrease of DAS28 (disease activity score in 28 joints) and serum levels of IL-1β, TNF-α, C-reactive protein, and erythrocyte rate sedimentation [37, 42, 46, 51]. In this sense, studies have defended the hypothesis that oral infections play an important role in the pathogenesis of RA, promoting the citrullination of proteins, which can be based on the detection of bacterial DNA using the techniques of DNA isolation (PCR and DNA-DNA hybridization) and high titers of antibodies against bacteria in synovial fluid and serum samples from patients with RA [40, 51, 52]. Most of the studies have shown the presence of oral bacteria in patients with RA, highlighting *P. gingivalis* and *F. nucleatum* [40, 52]. Markedly,

P. gingivalis is the most elucidated in the development of RA, and studies using animal models have demonstrated the potential of this proinflammatory bacterium promoting the development of experimental arthritis and increased serum levels of C-reactive protein, TNF-α, IL-1β, IL-17, MMP-13, and RANKL [75]. Furthermore, RA is an autoimmune disease characterized by autoantibodies specific for citrullinated peptide antigen (anticyclic citrullinated peptide), which are synthetized by peptidylarginine deiminase and characterized as the most specific markers for the diagnosis of the disease [76, 77]. Considering that the *P. gingivalis* is regarded as being capable of expressing this enzyme (PAD), it is suggested that infection with this microorganism could influence the pathogenesis of RA [78, 79]. These citrullinated proteins were also found in periodontal tissues, indicating a link between these peptides generated in the oral cavity and those observed in articular tissues [36, 80].

Additionally, the presence of antibodies to *P. gingivalis* was investigated. Although Seror et al. (2015) [57] have not detected this, antibody titres significantly differ between early rheumatoid arthritis and healthy controls. Other studies observed the antibodies to *P. gingivalis* in patients with RA and severe periodontitis [39] and were associated with probing depth and clinical attachment level and the titers of rheumatoid factor and anticyclic citrullinated peptide autoantibodies [35, 50], which may be found in patients with RA and related to the severity of periodontitis [55]. In summary, the studies suggested that *P. gingivalis* might play a role in the pathogenesis of RA. The response in periodontitis was related to uncitrullinated peptide, suggesting that these peptides break tolerance and can be involved in pathogenesis of RA [53] (Figure 1(c)).

Inflammatory conditions and mechanisms for bone destruction in PD and RA have many similarities. Most of the studies have found high levels of proinflammatory cytokines and other mediators of inflammation, such as MMP-9 [58], TNF-α [48], IL-17, RANKL, and OPG [47]. Moreover, it was demonstrated that the hypomethylated status, a single region of the IL-6, may contribute to elevated serum levels of this cytokine, implying a role in the pathogenesis of these diseases [34], while the anti-inflammatory cytokines in the GCF, such as IL-4 and IL-10, showed no consensus among studies regarding the differences observed among individuals with PD and RA [43]. In addition, hypotheses have been proposed to explain the relationship between periodontitis and systemic diseases, such as rheumatoid arthritis. In the literature, studies have suggested that chronic periodontitis generates local constant high levels of microparticles, which have been considered inflammatory biomarkers or mediators responsible for distant cell signalling and regulation [81]. Moreover, it has been reported that these microparticles play an important role in thrombosis and angiogenesis and mediate cellular communication by transferring mRNAs and microRNAs from the cell of origin to target cells [82]. Thus, the microparticle participation and its spread into the bloodstream could constitute the explanation to the increased risk for systemic disease in patients with periodontitis [83].

Despite these evidences showing a link between rheumatoid arthritis and periodontitis, the exact mechanisms involving this association have not been fully elucidated. Thus, well-designed longitudinal multicentre clinical trials and further studies with sufficient sample sizes are required to determine the biochemical processes and clinical relationships between these chronic inflammatory conditions. Moreover, these studies should consider other potential confound factors such as the drugs administered for the treatment for each disease or differences in oral hygiene or smoking habits in these patients.

5. Conclusion

The majority of the articles have confirmed that there is a correlation between PD and RA, since both disorders have characteristics in common and result from an imbalance in the immunoinflammatory response. Although it is necessary to highlight the importance of the mechanical treatment for periodontitis and pharmacological treatments mainly for RA patients, more research is needed to assess whether the coexistence of both diseases can affect the clinical signs of periodontitis and systemic markers of rheumatoid arthritis and strengthen the capacity of oral bacteria to stimulate an autoimmune response, thus establishing that cell constituents or mediators could share common pathophysiological pathways for both diseases and therefore define the best therapy.

Conflict of Interests

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

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Review Article

Inflammatory Mediators of Leprosy Reactional Episodes and Dental Infections: A Systematic Review

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Reactional episodes in leprosy are a result of complex interactions between the immune system, *Mycobacterium leprae*, and predisposing factors, including dental infections. To determine the main inflammatory mediators in the immunopathological process of dental infections and leprosy reactions, we conducted a systematic review of primary literature published between 1996 and 2013. A three-stage literature search was performed (Stage I, "leprosy reactions" and "inflammatory mediators"; Stage II, "dental infections" and "inflammatory mediators"; and Stage III, "leprosy reactions," "dental infections," and "inflammatory mediators"). Of the 911 eligible publications, 10 were selected in Stage I, 68 in Stage II, and 1 in Stage III. Of the 27 studied inflammatory mediators, the main proinflammatory mediators were IL-6, IFN-γ, TNF-α, IL-1β, and IL-17; the main anti-inflammatory mediators were IL-10 and IL-4. Serum IL-6 and TNF-α concentrations were significant during periodontal and reactional lesion evolution; IFN-γ and IL-1β were associated with types 1 and 2 reactions and chronic periodontal disease. The proinflammatory mediators in dental infections and leprosy reactions, especially IL-6 and TNF-α, were similar across studies, regardless of the laboratory technique and sample type. IFN-γ and IL-1β were significant for leprosy reactions and periodontal diseases. This pattern was maintained in serum.

1. Introduction

Leprosy reactions are sudden acute immune-inflammation episodes against *Mycobacterium leprae* superimposed on the chronic course of leprosy. They predominate in individuals classified as multibacillary and are responsible for irreversible nerve damage, increasing the disease burden and associated stigma [1].

Identified as type 1 reactions (T1Rs), type 2 reactions (T2Rs), or neurological reactions, leprosy reactions show distinct immunological characteristics and may occur before or during treatment as well as up to 5 years or more after the conclusion of polychemotherapy. A T1R is clinically characterized by the increase and exacerbation of preexisting lesions with no involvement of the individual's general condition. In a T2R, nerve involvement is less frequent; the individual presents with general malaise, fever, and systemic involvement, which is not restricted only to the skin. Isolated neuritis results in symptoms and neurological signs without the cutaneous manifestations of T1Rs and T2Rs; in the absence of pain, they are called silent neuritis [2–4]. Approximately 25–50% of sick individuals can develop reactions [5–8].

Among individuals with borderline leprosy, 30% show a risk of T1R; the incidence is significantly higher in borderline-borderline and borderline-lepromatous (BL) cases than in borderline tuberculoid cases. In contrast, T2R occurs more frequently in individuals with lepromatous leprosy (LL), affecting 20% of LL cases and 10% of BL cases [9, 10].

Studies published in the past 5 years have addressed the possible relationship between the occurrence of reactional episodes and dental infections [11–14]. The oral health conditions in individuals with leprosy are poor, that is, high rates of caries and periodontal disease (PD) [15–20], with little involvement of dentists to control these diseases [11, 21].

Leprosy reactions and dental infections have some common characteristics. They are both slowly evolving chronic infections, modulated by a number of inflammatory and immunopathological events resulting from the interaction between bacteria and their products and the host immune

response. Both the complications of leprosy and extent and severity of PD manifest as secondary damage, arising from an unsuccessful defense mechanism of the host [22–24]. Common and important mediators expressed in both conditions include IL-1, IL-1β, IL-4, IL-6, IL-8, IL-10, TNF-α, and IFN-γ [25–29].

The release of cytokines in response to oral bacteria is among the mechanisms underlying the systemic effects of periodontitis [30, 31]. Motta et al. [12–14] investigated the role of dental infections in the triggering, maintenance, or exacerbation of reactive episodes and emphasized the possible role of IL-6, IL-10, and IL-1 in these events. However, there is need for additional studies to understand this possible interaction.

The hypothesis of a close relationship between oral diseases and certain systemic conditions is not new. The scientific evidence in dentistry and medicine has corroborated the bidirectional relationship between an individual's general health and oral health as well as specific oral diseases, such as PDs [32–34].

Considering the scarcity of studies aimed at investigating the relationship between dental infections and leprosy reactions and the possibility of a systemic effect of cytokines in the immunopathological mechanisms of these diseases, this systematic review aimed at analyzing scientific publications reporting the inflammatory mediators involved in the immunopathological processes of dental infections and leprosy reactions.

2. Materials and Methods

2.1. Type of Study. This was an exploratory systematic review of the primary literature on inflammatory mediators involved in the immunopathological process of reactional episodes in leprosy and dental infections.

2.2. Data Sources and Time Period. A search of the literature was conducted between January and December 2013 in the following electronic databases: (i) national database (BBO Dental/Brazil, Spanish Bibliographic Index of Health Sciences/IBECS, and Scientific Electronic Library Online/SciELO); (ii) international database (Latin American and Caribbean Health Sciences/LILACS, US National Library of Medicine/PubMed, and U.S. National Library of Medicine's bibliographic database/MedLine); and (iii) the cochrane library.

2.3. Search Strategy and Selection of Articles. Considering that the term "periodontal medicine" was first used in dentistry in 1996 to designate the branch of periodontology addressed to the investigations of the bidirectional relationships between PDs and the general condition of the individual [33, 34], we limited the literature search to studies published between January 1, 1996, and December 31, 2013.

The search strategy was constructed with descriptors in English, Spanish, and Portuguese and considering their synonyms, according to the specificities of the databases.

We identified the descriptors in the health sciences by consulting the DeCS according to Keywords in Context and the Medical Subject Headings (MeSH).

The following MeSH terms and keywords were used:

(1) Pulpitis.

(2) Gingival diseases, gingivitis.

(3) Periodontitis; periodontal diseases, hierarchical term in MeSH: aggressive periodontitis; chronic periodontitis; periapical periodontitis; periodontal abscess; periodontal pocket.

(4) Cytokines; interleukin(s) (blood/skin).

(5) Inflammation mediators; biological markers; biomarkers.

(6) Type I reversal reaction; reversal reaction; erythema nodosum leprosum.

(7) Leprosy reaction; leprosy reactions; leprosy reactional.

(8) Biopsy.

(9) Skin.

The search was conducted in three stages. During Stage I, the bibliographic search was conducted for the terms "*leprosy reactions*" and "*cytokines.*" In Stage II, we used "*dental infections*" and "*cytokines,*" and, in Stage III, we used "*leprosy reactions,*" "*dental infections*," and "*cytokines*."

Studies were included that investigated the participation and involvement of cytokines in the inflammatory process of dental infections and/or during the occurrence of leprosy reactions.

We excluded all articles that had any of the following groups: pregnant women, syndromic individuals, smokers, experimental animals, or individuals with systemic diseases or conditions (diabetes, menopause, cardiovascular disease, and chronic renal failure). We also excluded studies involving cell cultures, influence of drugs, the production of cytokines and periodontopathic bacteria, genetic polymorphisms, mutations, case reports, systematic reviews, literature reviews, and meta-analyses.

After the article selection, we constructed a form with the following pieces of information: author and year, study population (sample size, age, type of dental infection, and/or reactional episode), laboratory techniques used in the studies, cytokines analyzed, and additional relevant results.

Exploratory analyses using tables, figures, and flowcharts were conducted.

3. Results

We identified 911 publications dated between January 1, 1996, and December 21, 2013, of which we excluded 795; a further 37 articles were duplicates. We selected the remaining 79 publications for analysis: 10 articles (12.7%) in Stage I, 68 articles (86.0%) in Stage II, and 1 article (1.3%) in Stage III. In these articles of dental infections and the occurrence of reactional episodes, the 27 researched inflammatory mediators, independent of the laboratory technique and type of sample,

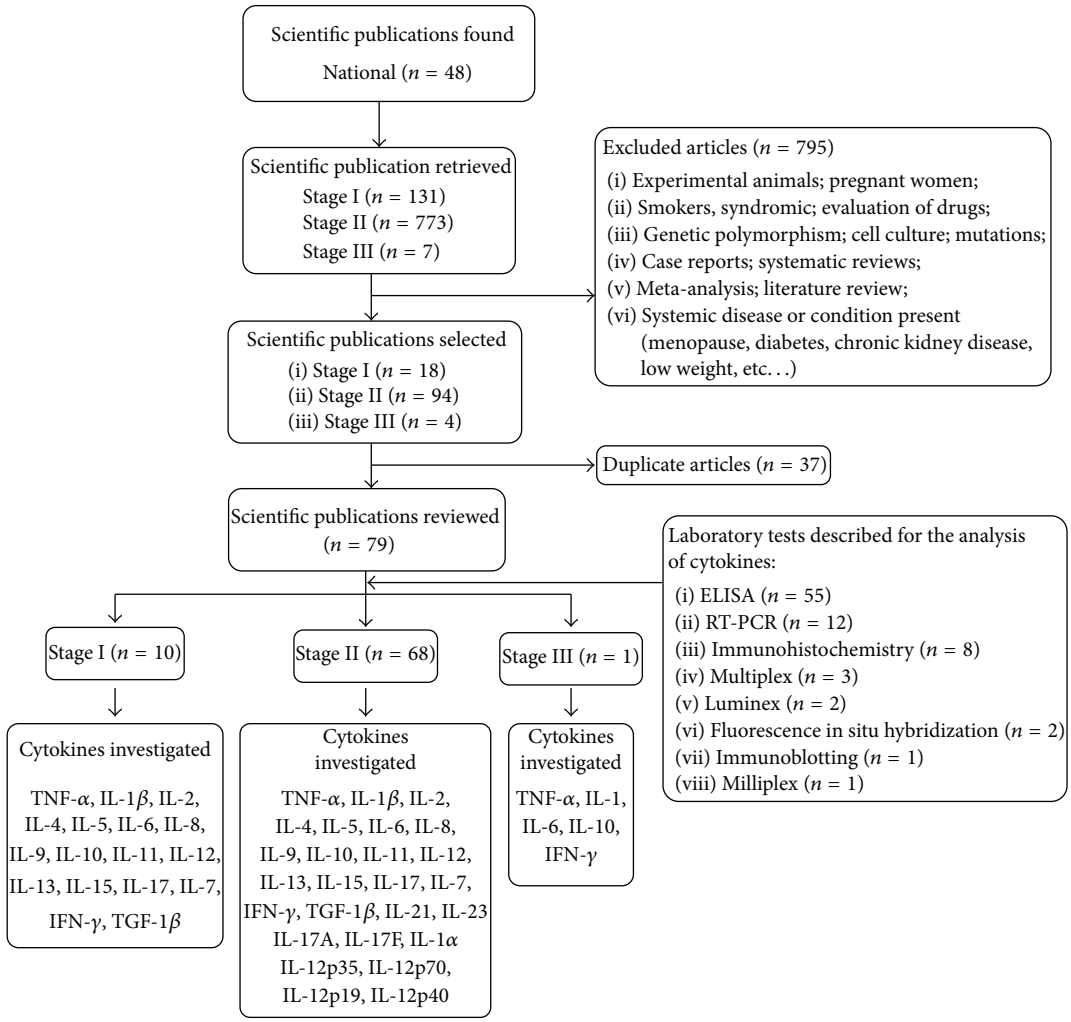

Figure 1: Flowchart of the selection of scientific articles published between January 1, 1996, and December 31, 2013, regarding inflammatory mediators involved in leprosy reactional episodes and dental infections. Stage I (bibliographic search for inflammatory mediators/leprosy reactions); Stage II (bibliographic search for inflammatory mediators/dental infections); Stage III (bibliographic search for inflammatory mediators/dental infections/leprosy reactions).

were TNF-α, IFN-γ, IL-1/IL-1β, IL-1α, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-12p35, IL-12p70, IL-12p40, IL-15, IL-17, IL-17A, IL-17F, IL-18, IL-21, IL-23, IL-23 p19, and TGF-1β (Figure 1).

The use of ELISA (69%) and RT-PCR (15%) to detect the inflammatory mediators was more frequently reported (Figure 1).

Among the studies on dental infection, the most common proinflammatory mediators were IL-1β (29 articles), TNF-α (25 articles), IL-6 (24 articles), and IFN-γ (17 articles), and the most common anti-inflammatory mediator was IL-4 (15 articles). For leprosy reactions, the most common proinflammatory mediators were TNF-α (7 articles), IFN-γ (5 articles), IL-6 (4 articles), and IL-17 (3 articles), and the most common anti-inflammatory mediators were IL-4 (4 articles) and IL-10 (4 articles) (Table 1).

Of the publications regarding the role of inflammatory mediators in the immunopathological process of dental infections, 10% were associated with periapical lesions (e.g., cyst, periapical granuloma, keratocyst, chronic periapical lesion, radicular cyst, and periapical lesion), 10% were associated with the presence of severe caries and/or pulpitis, and 79% referred to PD.

Of the 19 studies that analyzed the participation of inflammatory mediators in PD, defined as mild, moderate, or severe, or whose definition parameters included only measurements of the periodontal pocket depth, clinical attachment loss, and presence of gingival bleeding, inflammatory mediators were correlated with the clinical parameters of PD. The following cytokines were included in these articles: TNF-α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-11, IL-13, IL-17, IL-18, IL-23, IFN-γ, and IL-12p35 (Table 2).

Laboratory analyses of inflammatory mediators were conducted in serum (11 articles), gingival, pulp, or periapical tissue biopsy (28 articles), and gingival crevicular fluid (GCF) or saliva (29 articles). Only one article presented the analysis of inflammatory mediators in plasma (Table 2).

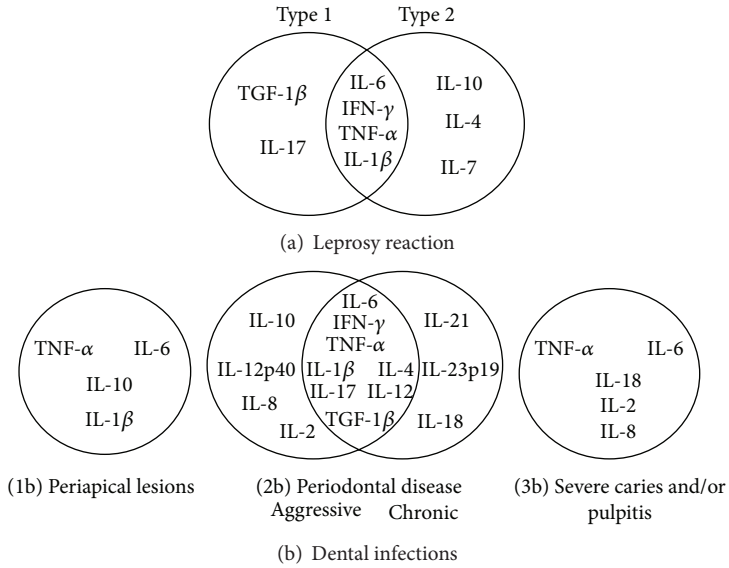

Figure 2: Main inflammatory meditators identified for leprosy reaction (a) and dental infections (b) in articles published between January 1, 1996, and December 1, 2013, and selected for the systematic review.

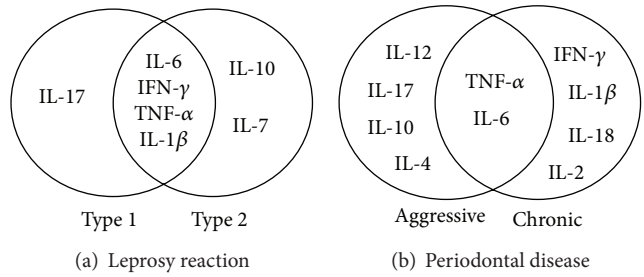

Figure 3: Main serum inflammatory mediators that were identified in leprosy reactions and periodontal diseases, by type, in the articles published between January 1, 1996, and December 31, 2013, that were selected for the systematic review.

Only one publication investigated the role of mediators during reactional episodes in individuals with dental infections. We identified higher serum IL-1, IL-6, and IL-10 levels in individuals with leprosy and dental infection compared with individuals with leprosy without dental infection (Table 3).

Among the articles of leprosy reactions, regardless of the type of sample, IFN-γ, TNF-α, IL-6, and IL-1β were involved in T1Rs and T2Rs. In addition, IL-17 and TGF-β1 were involved in T1Rs, and the anti-inflammatory mediators IL-10, IL-4, and IL-7 were involved in T2Rs (Figure 2(a)).

IL-6 and TNF-α were involved in the immunopathological process of dental infections (Figure 2(b)), which included periapical lesions (Figure 2(b)(1b)), PD (Figure 2(b)(2b)), and severe caries and/or pulpitis (Figure 2(b)(3b)). In studies of aggressive periodontitis (PDag) and chronic PD (CPD), 6 proinflammatory mediators (IL-1β, IL-6, IFN-γ, TNF-α, IL-17, and IL-12) and 2 common anti-inflammatory mediators (TGF-β and IL-4) were involved.

Among the common proinflammatory mediators identified in serum during the occurrence of leprosy reactions (a) and PD (b), IL-6 and TNF-α were predominant (Figure 3).

In the immune process of T2Rs, only the anti-inflammatory mediators IL-10 and IL-7 were present in serum. During the occurrence of T1Rs and T2Rs, the proinflammatory mediators IL-6, IFN-γ, TNF-α, and IL-1β were detected. IL-17 participated during the occurrence of T1Rs (Figure 3).

In CPD, we also identified IL-β1, IFN-γ, IL-18, and IL-2 as the proinflammatory mediators in serum. The anti-inflammatory mediators IL-4 and IL-10 were only identified in serum for PDag. For T2Rs and CPD, the common serum proinflammatory mediators included IL-6, IFN-γ, TNF-α, and IL-β1 (Figure 3).

4. Discussion

In this systematic review, we identified important pro- and anti-inflammatory mediators involved in the occurrence of dental infections and leprosy reactions, including IL-6, IFN-γ, TNF-α, IL-1β, IL-17, IL-10, and IL-4, which were independent of the laboratory technique and sample. In serum, significant concentrations of IL-6 and TNF-α were present during the evolution of periodontitis and reactional

Table 1: Frequency of articles published between January 1, 1996, and December 31, 2013, that were selected for the systematic review regarding dental infections, leprosy reactions, and the types of investigated inflammatory mediators.

Inflammatory mediators	Type of article			
	Dental infections		Leprosy reactions	
	$n = 68^*$	%	$n = 10^{**}$	%
IL-1β	29	42.7	3	30.0
TNF-α	25	36.8	7	70.0
IL-6	24	35.3	4	40.0
IFN-γ	17	25.0	5	50.0
IL-4	15	22.0	4	40.0
IL-10	13	19.1	4	40.0
IL-17[§]	13	19.1	3	30.0
IL-8	12	17.6	2	20.0
IL-2	11	16.2	1	10.0
IL-12	6	8.8	2	20.0
IL-1α	6	8.8		
IL-18	5	7.3		
IL-23	4	5.9		
IL-5	4	5.9	2	20.0
IL-11	4	5.9		
TGF-1β	4	5.9	1	10.0
IL-13	4	5.9	1	10.0
IL-15	3	4.4	1	10.0
IL-7	2	2.9	1	10.0
IL-12p40	2	2.9		
IL-12p70	2	2.9		
IL-23p19	2	2.9		
IL-9	1	1.5	1	11.1
IL-12p35	1	1.5		
IL-21	1	1.5		

[*]Number of articles about dental infections.
[**]Number of articles about leprosy reactions.
[§]Included in the cytokines IL-17A and IL-17F.

lesions, while IFN-γ and IL-1β were related with T1R, T2R, and CPD.

Such inflammatory mediators are produced by a wide variety of cells during the acute and chronic phases of inflammation, and they have important modulatory and regulatory functions in the inflammatory responses of the immune system. They function together to create a complex network with redundant, synergistic, or antagonistic properties. Furthermore, some molecules are pleiotropic and may have endocrine activity, such as IL-6, TNF-α, and IL-1β [35, 36].

4.1. IL-6. IL-6 is mainly synthesized in the presence of IL-1, TNF-α, and lipopolysaccharides that are present in the cell walls of gram-negative bacteria, including the periodontopathogens. It is multifunctional and is present in both the innate and adaptive immune responses, with a key role in the acute immune inflammatory response. It stimulates the T lymphocytes, contributes to the increase of B lymphocytes, and contributes to the production of antibodies in the Th2-cell-mediated immune response [10, 26, 37, 38].

IL-6 and TNF-α have been found in biopsy specimens of all individuals with T1R or T2R [39], who have also demonstrated increased levels of IL-6 in serum [26, 40]. Considering its proinflammatory potential and ability to stimulate the production of antibodies, some authors have suggested this cytokine as a valuable prognostic marker for leprosy reactions [7, 9, 36, 37, 40, 41].

In more recent studies, an association between increased plasma IL-6 levels and the occurrence of T1R and T2R has been reported. In T1R, this condition can be explained by the probable participation of cells related to the T1 type response, resulting from nongenetic and/or genetic determinants. In contrast, in T2R, the main determinant for the significant increase in IL-6 seems to be the presence of polymorphisms in the encoding gene of this cytokine [7, 41].

In the present review, regardless of the type of sample used, IL-6 in dental infections was associated with the presence of severe caries, symptomatic periapical lesions, and PD status. As osteoclast-activating factors, IL-6, TNF-α, and IL-1β are involved in bone resorption during the evolution of PDs [42–50]. IL-6 was correlated with the probing depth and sulcus impairment; it was identified in biopsy specimens, saliva, and gingival fluid, in addition to serum and plasma [42, 43, 46, 47, 49–52]. Individuals with CPD in advanced stages, severe periodontitis, or PDag had significant IL-6 levels [42, 49–51]. The presence of polymorphic variants in the IL-6 gene has indicated an association with the pathogenesis of CPD [53], as well as an increased risk for PDag [54]. Interestingly, this polymorphism seems to have a similar location as that of SNP rs1800795, which is associated with T2R [41].

According to Motta et al. [12], IL-6 is among the mediators possibly involved in the maintenance of reactional episodes, in addition to serum IL-1 and IL-10. Multibacillary individuals with oral infections showed a greater risk for reactions, especially of the erythema nodosum leprosum type, with a clinical improvement of reactional episodes after dental therapy [12, 13, 55]. Recently, it was also observed that dental infections in individuals with leprosy could increase the proinflammatory response mediated by IFN-γ, while the opposite effect occurred for the immunoregulatory activity of IL-4, resulting in exacerbation of the inflammatory reaction [14].

4.2. IFN-γ. Serum IFN-γ levels during the occurrence of T1R, T2R, and CPD favor the phagocytic activity in inflammation and amplify the response activity of T cells. It has also been observed in gingival tissue biopsies from lesions of patients with leprosy, saliva, and GCF [43, 45, 49, 50, 56–60]; it is secreted by CD4+ T cells, CD8+ lymphocytes, peripheral blood mononuclear cells, and natural killer (NK) cells, which are also related to periodontal bone loss [14, 26, 61, 62].

High serum IFN-γ levels during the reactivation or in excessive acute immune inflammatory responses during the occurrence of reactional episodes have been discussed in

Table 2: Articles selected for the systematic review on dental infections and the presence of inflammatory mediators in serum (a), biopsy specimens (b), and gingival crevicular fluid (GCF) (c) according to the publication year, author, type of sample, and obtained results.

Year	Authors	*N*	Significant results
(a) Dental infections and Presence of mediators in serum			
2011	Kinney et al.* [105]	83 (PD)	IL-1β, MMP-8, and MMP-9 were strongly correlated with PD status.
	Özçaka et al.* [106]	22 (CPD), 21 (C)	Individuals with CPD had lower IL-17 levels in saliva.
	Robati et al. [51]	25 (PDagG) 25 (C)	Low levels of IL-4 were associated with PDagG, and IL-6 levels were high compared with the control group.
	Sánchez-Hernández et al.** [35]	18 (CPD), 12 (PDag), 9 (C)	Individuals with PDag had higher IL-12 levels in gingival tissue and serum. Those with CPD had higher serum IL-18 concentrations than controls.
2010	Duarte et al. [70]	14 (PDagG) 14 (CPDg); 14 (C)	After periodontal treatment, the serum TNF-α concentration remained high in the PDagG group
	Schenkein et al. [73]	53 (PDagL), 49 (PDagG), 67 (C)	IL-17 was associated with the loss of clinical insertion. Individuals with PDagG or PDagL had higher serum IL-17 concentrations.
2008	Abdolsamadi et al. [107]	40 (LPC) 40 (C)	Production of IL-6 in LPC could be used as a marker of chronic apical periodontitis.
	de Queiroz et al. [108]	17 (CPD), 8 (C)	Serum levels of RANTES, MIG, and eotaxin differed between healthy individuals and those with periodontitis.
2005	Bretz et al.*** [42]	1131 (severe, moderate, or absent disease)	High levels of plasma TNF-α were associated with the extent of PD and number of teeth. IL-6 levels were higher in individuals with more extensive PD than in other individuals.
2003	Górska et al.** [56]	25 (CPD) 25 (C)	Serum and gingival tissue biopsy specimens of individuals with CPD had higher levels of IL-1β, TNF-α, IL-2, and IFN-γ than those of the control group.
2001	Murata et al. [52]	276 individuals	The severity of PD was not associated with the average serum IL-6 concentration. Further, 54% were positive for IL-6 in serum.
(b) Dental infections and presence of inflammatory mediators in biopsy specimens			
2012	Dutzan et al. [80]	10 (CPD), 8 (C)	Individuals with CPD showed increased expression of IL-21, IL-1β, IL-6, IL-17, and IL-23p19 and decreased expression of IL-10 and TGF-β1.
2011	Dutzan et al.† [109]	15 (CPD), 19 (C)	Individuals with CPD had higher IL-21 levels in gingival tissue and GCF than controls.
	Santos [57]	36 (DGC), 31 (CPD), 15 (C)	IFN-γ was present in the gingival tissue of all samples and was present at higher concentrations in more advanced stages.
2009	Dutzan et al.† [58]	106 (moderate or advanced CPD), 25 active sites; 25 inactive sites	The IFN-γ level in gingival fluid was higher than at the active site. Progressive periodontal lesions in individuals with CPD had higher expression of IFN-γ and had more frequent IFN-γ expression.
	Fukada et al. [110]	20 (GP), 10 (cysts), 8 (C)	Granulomatous tissue showed increased expression of IL-10, whereas periapical tissue with granuloma and cyst had similar expressions of IFN-γ and IL-4.
	Ohyama et al. [74]	15 (PD) 11 (C)	Individuals with PD had higher levels of IL-23 and IL-12 in periodontal lesions than the control group.
2008	Honda et al. [75]	24 (PD) 23 (G)	Expression of IL-17A mRNA was higher than that of IL-17F mRNA. The expression of IL-17A differed in gingivitis and periodontitis.
	Menezes et al. [85]	57 (GP) 38 (C)	Periapical granulomas showed higher TNF-α, IL-10, and RANKL mRNA expression than healthy periodontal tissues.
2007	Johnson and Serio [43]	59 (BP = 3 mm and SG) 73 (BP = 4–6 mm) 53 (BP > 6 mm) 58 (C)	Affected gingival tissue (3–6 mm) showed higher concentrations of IFN-γ, IL-2, IL-4, IL-6, IL-10, and IL-13 than controls. IL-6 showed a positive correlation with sulcular impairment.
	Jurisic et al. [86]	43 (CR), 15 (keratocysts)	A higher concentration of TNF-α was observed in radicular cysts.
	Kokkas et al. [44]	6 (reversible pulpitis) 6 (irreversible pulpitis), 6 (C)	The increase in TNF-α gene expression was associated with irreversible pulpitis compared with the control group. TNF-α was positively associated with the severity of clinical parameters.
	Brekalo Pršo et al. [87]	Group I: 15 (sensitive LP), Group II: 15 (insensitive LP), 15 (C)	Groups I and II had higher levels of TNF-α. Symptomatic periapical tissues had higher levels of IL-6 than asymptomatic periapical tissues and controls.

Table 2: Continued.

Year	Authors	N	Significant results
2006	Honda et al. [59]	25 (CPD) 23 (G)	Individuals with periodontitis had higher levels of IL-1β, IFN-γ, RANKL, HSP60, and TGF-β1. The levels of IL-4 were slightly higher in periodontitis than in gingivitis.
2005	Johnson and Serio [45]	36 (BP = 3 mm and SG) 39 (BP 4–6 mm) 15 (BP > 6 mm) 42 (C)	Concentrations of IL-2, IL-4, IL-6, IL-10, IL-18, and IFN-γ were higher in biopsy specimens from tissue adjacent to BP of 4–6 mm than in controls. Higher concentrations of IL-6 and IL-18 were noted adjacent to sites with a probing depth >6 mm than in healthy sites.
	Rodríguez and López [46]	13 (G), 9 (CPD) 13 (C)	Individuals with gingivitis and periodontitis had higher concentrations of IL-6 in gingival tissues than in healthy tissues.
2004	Johnson et al. [47]	19 (BP = 3 mm and SG) 24 (BP 4-5 mm) 11 (BP ≥ 6 mm) 31 (C)	IL-6 concentration increased with probing depth; the IL-11 concentration was higher around BP = 3 mm, and the IL-17 concentration was higher around BP of 4-5 mm compared with the other sites.
2003	Zehnder et al. [111]	11 (severe caries, symptomatic), 13 (C)	Teeth with severe caries showed a higher expression of IL-6, IL-8, and IL-18.
2002	Pezelj-Ribaric et al. [112]	20 (irreversible pulpitis) 20 (extensive caries restoration), 20 (C)	Teeth with irreversible pulpitis showed higher concentrations of TNF-α than controls.
2001	Lappin et al. [60]	10 (PIP) 10 (CPD)	IFN-γ and IL-2 were involved in disease progression, suggesting a modulator role in the inflammatory response.
2000	Danin et al. [113]	25 (LPC)	TGF-β per mg tissue was correlated with the diameter of the lesions.
1999	Barkhordar et al. [114]	6 (pulpitis), 6 (LP) 8 (C)	Samples of the periapical and inflamed pulp tissue showed medium levels of IL-6, which were higher compared with control levels.
	Huang et al. [115]	Teeth (irreversible pulpitis and C)	Teeth with irreversible pulpitis had higher levels of IL-8 than those with healthy pulp.
1998	McGee et al. [48]	$N = 8$ n_{I}: BP ≤3 mm n_{II}: BP with 4–6 mm; n_{III}: BP >6 mm	There was a higher concentration of IL-8 around BP ≤3 mm and a higher concentration of IL-6 and IL-1β around BP >6 mm.
	Shimauchi et al. [81]	29 teeth with pulp exudates (EP) (symptomatic and asymptomatic)	There was a positive correlation between IL-1ra and IL-1β, at relatively higher levels of IL-1ra when compared with IL-1β.
1997	Rauschenberger [116]	12 (irreversible pulpitis), 17 (C)	IL-2 concentrations differed significantly between inflamed pulp tissue and healthy pulp tissue.
	Roberts et al. [117]	17 (CPD)	TNF-α and IL-1ra mRNA expression were higher in CPD than in healthy gingival tissue.
	Roberts et al. [118]	34 (CPD) 5 (C)	TNF-α mRNA expression was higher in CPD than in controls. IL-1β, IL-1ra, and IL-1α were seen more often in healthy tissue.
	Tokoro et al. [89]	13 (moderate or advanced (PD), 5 (G)	Gingival tissue with periodontitis showed a predominant expression of IL-4 and IL-5. There was a predominance of IL-1α, IL-1β, and TNF-α in gingivitis.
(c) Dental infections and presence of inflammatory mediators in GCF and saliva			
2013	Ertugrul et al. [90]	21 (PDagG), 21 (CPD) 21 (G), 21 (C)	PDagG had higher total levels of IL-8 in GCF than CPD, G, and controls. Levels of IL-1β and TNF-α were higher for the group with PDagG.
	Rathnayake et al. [82]	441 (PD)	IL-1β can be used as a marker in PD. Individuals with severe periodontitis showed a higher concentration of IL-1β.
	Yue et al. [49]	40 (PDag) 40 (C)	In PDag, there were higher concentrations of IL-1β, IL-2, IL-6, IFN-γ, and TNF-α in saliva and GCF.
2012	Ay et al. [119]	20 (PDagG), 18 (C)	The frequency of IL-11 was lower in the group with PDagG, and the concentration of IL-17 was lower than in the control group.
2011	Chaudhari et al. [95]	30 (CPD) 30 (C)	IL-1β was positively correlated with the following clinical parameters: bleeding on probing, pocket depth, periodontal disease rating, and tooth mobility.
	Garrido Flores et al. [88]	14 (PAA), 14 (C)	Higher TNFα concentrations were noted in gingival sites of teeth with PAA than in the control group.
	Kaushik et al. [83]	28 (CPDg), (C)	Individuals with PD had a medium level of elevated IL-1β compared with the control group.
	Shaddox et al. [50]	34 (PDagL) 9 (C)	Patients with PDag had higher levels of TNFα, IFNγ, IL1β, IL2, IL6, IL10, and IL12p40 than healthy individuals.
	Stashenko et al.[††] [120]	103 (PIP_1), 42 (PIP_2) 45 (C)	Levels of IL-1β in GCF increased according to the severity of PD.

Table 2: Continued.

Year	Authors	*N*	Significant results
2010	Burgener et al. [121]	40 teeth (PA) 40 teeth (C)	Teeth with apical lesions had higher levels of IL-1β in the gingival fluid than the controls.
	Fitzsimmons et al. [122]	430 (moderate or severe PD), 509 (C)	PD was independently associated with higher levels of IL-1β and C-reactive protein.
	Perozini et al. [96]	12 (CPD), 12 (G) 12 (C)	IL-1β concentrations were higher in CPD than in the other groups. IL-1β levels were positively correlated with PD, the volume of gingival fluid, and pocket depth.
	Teles et al. [97]	20 (CPD) 20 (C)	Clinical parameters (PD, BOP, vol GCF, R, and Sup) were positively correlated with the levels of IL-1β and IL-8 in GCF.
	Teles et al. [98]	31 (PDagG) 25 (C)	PDagG had higher average levels of IL-1β. There was a tendency for levels of IL-2 and IL-13 to be higher in PDagG.
2009	Ay et al. [123]	40 (CPD): BP ≤4 mm; BP ≥5 mm 20 (C)	The total rate and concentration of IL-11 and IL-17 were lower in the group with BP ≥5 mm.
	Bastos et al. [91]	14 (PDag) 13 (C)	TNF-α concentrations were higher in PDag than in controls.
	Fitzsimmons et al. [124]	511 (moderate or advanced PD), 562 (C)	There were higher levels of IL-1β and PCR in individuals with PD. Clinical parameters were positively correlated with biomarker levels.
	Pradeep et al. [71]	20 (CPD), 20 (G), 20 (C), 3 individuals after treatment	IL-18 levels increased according to the severity of periodontal disease, decreasing after the treatment.
	Teles et al. [125]	74 (CPD) 44 (C)	Mean salivary levels of IL-8 were positively correlated with probing depth and the average percentage of sites with bleeding on probing.
2008	Frodge et al. [92]	35 (PD) 39 (C)	Individuals with PD had higher levels of TNF-α.
	Tóbon-Arroyave et al. [126]	30 (CPD) 18 (PDag) 18 (C)	The salivary level of IL-1β did not differ between groups with periodontitis, but it was higher than in the control group.
	Toker et al. [72]	15 (PDagG), 15 (C)	There were higher levels of IL-1β in sites with moderate or deep initial pocket than in the shallow pockets.
	Yücel et al. [84]	12 (CPD), 14 (G) 14 (C)	IL-1β and IL-12 concentrations in GCF were higher in CPD than in the control group.
2007	Tsai et al. [69]	17 (CPD)	Nonsurgical periodontal treatment resulted in a decrease in IFN-γ and an increase in IL-4.
2006	Gürkan et al. [127]	30 (PDagG), 32 (CPD), 15 (G) 16 (C)	The rate of TGF-β1 expression was higher in groups of individuals with PDagG and CPD than in the control group.
2003	Nicolau et al. [99]	20 (CPD) 20 (C)	Individuals with CPD showed higher concentrations of IL-1β in the gingival fluid compared with the controls.
2000	Guo et al. [128]	Chronic pulpitis Acute pulpitis Control	There were higher IL-8 concentrations in teeth with acute pulpitis than in those with chronic pulpitis.
1997	Ishihara et al. [94]	7 (PD) 2 (C)	IL-1β and IL-1α were associated with the severity of periodontal disease.
1996	Mathur et al. [93]	20 (PD) 20 (C)	The average rate and concentration of IL-1α in GCF were higher in individuals with PD than in controls. The site status is the major determinant of the cytokine levels in unhealthy sites.

Note 1. * Analysis in serum and saliva; ** analysis in serum and biopsy specimens; *** analysis in plasma; † analysis in GCF and biopsy specimens; †† analysis in GCF and serum. PD, periodontal disease; MMP, matrix metalloproteinases; GCF, gingival crevicular fluid; *Note 2.* CPD, chronic periodontitis; C, control; PDagG, generalized aggressive periodontitis; PDag, aggressive periodontitis; CPDg, generalized chronic periodontitis; PDagL, localized aggressive periodontitis; LPC, chronic apical periodontitis; RANTES, regulated on activation, normal T cell expressed and secreted; MIG, monokine induced by gamma interferon; GP, periapical granuloma; G, gingivitis; RANKL, receptor activator of nuclear factor kappa-B ligand; BP, periodontal pocket; SG, gingival bleeding; CR, radicular cyst; LP, periapical lesion; PIP, early onset periodontitis; EP, pulp exudates; BOP, bleeding on probing; R, recession; Sup, suppuration; PAA, asymptomatic apical periodontitis; PA, periapical periodontitis.

the literature; however, its immunoregulatory mechanisms remain unclear. Verhagen et al. [63], when assessing the change of T-cell subsets in the occurrence of T1R and the profile of secreted cytokines, observed a significant amount of Th0 cells with production of both IFN-γ and IL-4. However, individuals with T1R recurrence also showed a bias for Th1 with production of IFN-γ. In both T1R and, mainly, T2R, there is evidence of the involvement of IFN-γ in cellular processes [10, 26, 62, 64–66].

The balance between Th1 and Th2 cells and the change in the serum mediator and skin expression profiles (i.e., IFN-γ, TNF-α, IL-1β, IL-6, and IL-4), in the occurrence of both T1R

Table 3: Articles selected for the systematic review on leprosy reactions and presence of mediators in skin biopsy and/or serum (a) and leprosy reaction, dental infection, and presence of cytokines (b) according to the publication year, authors, type of sample, and obtained results.

Year	Authors	*N*	Results
(a) Leprosy reaction and presence of inflammatory mediators in skin biopsy specimens and/or serum			
2013	Abdallah et al. [67]	31 (L), 6 (T1R), 6 (T2R), 43 (C)	Increased production of IL-4 in multibacillary forms can be responsible for the development of erythema nodosum leprosum. IL-17 was lower in cases than in controls.
2012	Chaitanya et al. [77]	80 (T1R), 21 (T2R), 90 (L), 94 (NL)	Serum IL-17 level increased during reactional states. There was higher elevation during T1R than during T2R and nonreactional states.
2011	Lockwood et al. [29]	299 (tissue)	TNF-α and TGF-1β were detected in 78% and 94% of the samples, respectively, and were associated with T1R.
	Madan et al. [129]	51 (L), 10 (R)	Levels of IFN-γ, IL-1β, and IL-10 were higher in T2R, whereas the TNF-α level was higher in T1R.
2009	Stefani et al. [40]	20 (R), 19 (L)	Potential biomarkers for T1R (CXCL10 and IL-6) and T2R (IL-6, IL-7, and PDGF-BB) were identified.
2007	Belgaumkar et al. [26]	94 (L), 5 (T1R), 1 (T2R)	Levels of IFN-γ were higher in T1R, whereas the T2R individuals showed higher levels of IL-6 compared to the nonreactional states.
	Iyer et al. [130]	49 (R), 82 (L), 112 (NL)	IFN-γ showed a greater association with the reactional states, mainly for T2R.
2004	Faber et al.* [131]	7 (L)	It was not possible to establish a relationship between the serum profile of cytokines and T1R.
2002	Teles et al. [39]	9 (T1R), 16 (T2R)	TNF-α and IL-6 were detected in all individuals in a reactional state.
1998	Moubasher et al. [64]	55 (L), 35 (R)	Individuals with T1R and T2R had higher serum levels of IFN-γ, TNF-α, and IL-1β than those in a nonreactional state. Higher levels of IFN-γ and IL-6 were noted in T1R and T2R, respectively.
(b) Leprosy reaction, dental infection, and presence of inflammatory mediators in serum			
2010	Motta et al. [12]	19 (L and OI), 19 (L without OI), 10 (C: NL and OI)	It was observed that 78.8% of individuals with leprosy and OI presented erythema nodosum and 15.8% presented with a reverse reaction. Seven days after dental treatment, the serum levels of IL-1, IL-6, and IL-10 were significantly different between the groups. The IL-6 and IL-10 levels in Group C were higher than those in the group with L and OI. Clinical improvement of the reactional episode was noted after dental treatment in 68.4% (13/19) of individuals.

Note. *Nonsignificant result; L, leprosy; T1R, type 1 reaction; T2R, type 2 reaction; C, control; NL, nonleprosy; R, reaction; OI, oral infection; PDGF-BB, platelet-derived growth factor two B (-BB) chain.

and T2R, seem to be closely related with the clinical spectrum of the disease. Studies show that, in borderline tuberculoid individuals with T1R, the infiltration of LT CD4+ observed in skin and nerve lesions favored by the synergism between IFN-γ and TNF-α may possibly contribute to the exacerbated cell-mediated response, resulting in the elimination of mycobacterial antigens and the development of tissue damage. On the other hand, the immunosuppressive activity of IL-4 and IL-10, the increase in IFN-γ and TNF-α, and also the increase in IL-β, and IL-6 observed in BL and LL individuals support the evidence of a systemic inflammatory response in the evolution of T2R [26, 61, 62, 64, 65, 67].

With regard to PDs, there is no consensus on the immunological patterns involved in its pathophysiology. In early/stable periodontal lesions (gingivitis), migration of neutrophils of the junctional epithelium to the gingival sulcus and activation of macrophages and T cells are observed, with a predominance of TNF-α, IL-12, and IFN-γ, suggesting a cellular response against the pathogens with a Th1 profile and infection control. In advanced/progressive lesions (periodontitis), there are similar proportions of cells with a Th1 profile expressing IFN-γ and IL-2 and cells with a Th2 profile expressing IL-4 and IL-6 as reported by Berglundh et al. [68], with combined functioning of these cells in chronic periodontitis. The recruitment of B lymphocytes and the production of immunoglobulins strengthen the Th2 profile signaling [22, 36, 60].

The IFN-γ produced by Th1 cells in the initial lesion may contribute to infection control by increasing the phagocytic activity of neutrophils and macrophages. When the pathogen or its antigens persist in the dental biofilm, the lesion is not contained.

On reviewing studies that included biopsies, we found that IFN-γ was associated with generalized aggressive periodontitis (PDagG) [50] as well as with advanced stages and periodontal pockets up to 6 mm [43, 45, 57]. Recently, Yue et al. [49] identified a positive correlation between the presence of IFN-γ in saliva and gingival crevicular fluid and the clinical parameters of individuals with PDag. According to Lappin et al. [60], this mediator is involved in the progression of PD, with a decrease in its gingival crevicular fluid levels after nonsurgical therapy [69].

Although the literature review revealed distinct methodologies and values for clinical measures that characterize PDs (e.g., CPD, PDag, and severe and moderate periodontitis), there are concordant results about the influence of dental treatment on the pattern of inflammatory mediators [12, 69–72].

Given the complex interrelation between inflammatory mediators and immune system cells, studies have suggested the participation of other cellular subtypes such as regulatory T cells, Th3 cells (which have a immunosuppressant profile), and Th17 cells (which have a proinflammatory profile) for better understanding periodontal infections [35, 57, 73–76] and the evolution of the infection in leprosy reactional states [10, 62, 67, 77–79].

There is evidence that Th17 cells, once stimulated in PDs, produce a variety of mediators such as IL-17 and TNF-α, correlated to the formation of osteoclasts, bone resorption, and loss of clinical attachment. This is associated with CPD and PDag [47, 57, 58, 65, 70, 73–75].

In reactional episodes, IL-17 seems to be involved in the development of T1R [77, 79].

4.3. TNF-α and IL-1β. As previously described, TNF-α and IL-1β are produced by macrophages mainly activated by lipopolysaccharides in the cell wall of gram-negative bacteria; they are among the main mediators responsible for an acute inflammatory response. These mediators participate in tissue remodeling and bone resorption in addition to stimulating angiogenesis and promoting fibroblast activation. Both mediators were identified in biopsy specimens and serum of individuals with PD during the occurrence of reactional episodes [12, 30, 39, 40, 64], whereas TNF-α was detected in saliva and the gingival fluid of individuals with dental infections [12, 40, 56, 80–84].

In dental infections, TNF-α was associated with periapical granulomas [85], radicular cysts [86], acute periapical lesions [87, 88], and, mainly, PDs [42, 49, 50, 56, 70, 89–93].

On the one hand, high serum TNF-α levels were shown to be associated with the extent and severity of disease [82, 94] and with the clinical parameters of individuals with PDagG and CPD [42, 49, 70, 90, 91, 95–99]; on the other hand, Tokoro et al. [89] reported high serum TNF-α and IL-1β levels in gingivitis. Even after dental therapy, the TNF-α levels remained high compared to the control group.

On studying the immune pattern in cases of leprosy infection, Foss [61] found an increase in TNF-α production associated with high levels of C-reactive protein, suggesting that TNF-α was involved in the inflammatory reaction of erythema nodosum.

Elevated levels of TNF-α, IL-6, and IL-1β in serum and in the lesions of patients with T2R seem to be associated with the clinical manifestations of T2R [9, 100]. Motta et al. [12] suggested that the systemic inflammatory effects triggered by IL-1β in individuals with leprosy and dental infection can also contribute to the triggering of erythema nodosum leprosum. In this review, we found that TNF-α was associated with both T1Rs and T2Rs [30, 39, 40, 64].

The dynamic interaction between the cells of innate and adaptive immunity, the balance between Th1 and Th2 lymphocyte subpopulations, and the presence of molecular mediators and their receptors seem to determine the pattern of the immunological response of PDs and leprosy reactions, controlling or amplifying the inflammatory processes [26, 36, 70, 101].

Although most studies considered in this systematic review have used the classification defined by the American Academy of Periodontology, including gingivitis, CPD, and PDag (localized or generalized), there were differences in the definition of periodontitis, the criteria to establish the depth of the periodontal pocket and the loss of clinical attachment, and the age group of the participants. For example, 28% of the articles described PD as soft, moderate, or severe. Further, some studies had a small sample size for both PDs and leprosy reactions.

According to Buduneli and Kinane [102], the depth of the periodontal pocket and gingival bleeding after probing are the most reliable parameters, not only for diagnosis, as they are key indicators of periodontal tissue destruction, but also for disease prognostication. Some authors suggest that the heterogeneity in the definitions of periodontitis hinders the comparison of results between studies [103, 104]. Based on the literature analyzed, the need for longitudinal studies with better standardization of the population presented and a greater uniformity between techniques and experiments becomes evident. The development of molecular and chemical biomarkers with predictive and prognostic value can help in the early identification of patients at an increased risk of periodontal or leprosy diseases.

In this regard, monitoring of treatment effectiveness and the development of new instruments to monitor these infections would decrease the incidence of neural injuries and disabilities in individuals with leprosy and the early loss of dental function.

In summary, regardless of the laboratory technique used and the type of sample analyzed, the identified proinflammatory mediators involved in the immune pathologic process of dental infections and leprosy reactions, particularly IL-6 and TNF-α, were similar in the studies reviewed. Specifically, for leprosy reactions and PDs, IFN-γ and IL-β-1 were significant. This pattern was reflected in serum, and the presence of IFN-γ and IL-1β was associated with CPD.

Conflict of Interests

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

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Research Article

The Influence of Interleukin *17A* and *IL17F* Polymorphisms on Chronic Periodontitis Disease in Brazilian Patients

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A case-control study was conducted on patients with chronic periodontitis (CP) and healthy controls with the aim of evaluating possible association between interleukin *17A (IL17A)* G197A (rs2275913) and *IL17F* T7488C (rs763780) polymorphisms and periodontitis. Genotypes were determined by PCR-RFLP method. Statistical analyses were conducted using the OpenEpi and SNPStas software to calculate Chi-square with Yates correction or Fisher's exact tests, odds ratios (OR), and 95% confidence intervals (CIs). SNPStas software was used to calculate Hardy-Weinberg equilibrium. *IL17A* AA genotype was more frequent in patients with chronic periodontitis (CP) in the codominant and recessive models ($P = 0.09$; OR = 2.53 and $P = 0.03$; OR = 2.46, resp.), the females with CP ($P = 0.01$, OR = 4.34), Caucasoid patients with CP ($P = 0.01$, OR = 3.45), and nonsmoking Caucasian patients with CP ($P = 0.04$, OR = 3.51). The *IL17A* A allele was also more frequent in Caucasians with CP ($P = 0.04$, OR = 1.59). *IL17F* T7488C polymorphism was not associated with chronic periodontitis. In these patients from Southern Brazil, the *IL17A* rs2275913 polymorphisms, *IL17A* AA genotype, and the A allele were associated with a susceptibility to chronic periodontitis.

1. Introduction

Periodontitis is a chronic inflammatory disease that affects the tooth supporting tissue and destroys alveolar bone. It is the most frequent cause of tooth loss in the adult [1]. Epidemiologic studies suggest that up to 60% of the population is affected by the common form of the disease, termed chronic periodontitis (CP) [1, 2]. Periodontitis has been said to have interaction with a number of common human diseases like diabetes mellitus or rheumatoid arthritis. Epidemiological data has confirmed that diabetes has been a major risk factor for the onset of periodontitis and showed a clear relationship between degree of hyperglycemia and severity of periodontitis [3]. Other studies show that patients with rheumatoid arthritis had a higher incidence of periodontitis compared to healthy controls [4, 5], though the mechanism of periodontitis that interacted with other diseases was not very clear.

Periodontitis is a multifactorial disease and as such, the significant elements include not only the presence of pathogenic bacteria and the immune mechanism, but also the genetic predisposition [6]. From a pathophysiology perspective, periodontitis is the result of host-mediated inflammatory damage of the supporting tissues triggered in response to the microbial infection [7, 8]. More than 700 different bacterial species have been shown to inhabit periodontal biofilms [9] and some species are currently considered to be causally associated with periodontitis; these include Gram-negative species, such as anaerobic *Porphyromonas gingivalis*, a principal pathogen in chronic periodontitis [10].

Specific cytokines are important in the pathogenic process of the periodontitis [8, 11–13]. Interleukin-17 (IL-17) is a

proinflammatory cytokine secreted by activated T cells [14]. The IL-17 family contains six members, IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (or IL-25), and IL-17F, and five receptors, IL-17RA–RD and SEF. Interleukin-17A is most homologous to IL-17F and the genes encoding them are proximally located on chromosome 6p12 [15]. The IL-17F activity is similar to IL-17A but significantly weaker and is related to inducing the expression of various cytokines, chemokines, matrix metalloproteinases, antimicrobial peptides, and adhesion molecules by human fibroblasts, airway epithelial cells, and vein endothelial cells [16].

Th17 cells were a distinct T lineage that do not share developmental pathways with either Th1 or Th2 cells [17]. Th17 cells have been linked to several autoimmune disorders and are also linked to the development of pathological inflammatory disorders. However Th17 cells are physiologically found in the lamina propria of the intestine [17, 18]. Cytokines related with Th17, such as IL-17 and IL-22, are crucial for host protection against many extracellular pathogens. IL-17 stimulates the production and expression of TNF-alpha and IL-1 beta by human macrophages [19] and induces production of IL-1 beta in osteoblasts [20]. Thus, IL-17 was found to contribute to inflammatory bone pathology as in rheumatoid arthritis and inflammatory bowel diseases and was centrally involved in numerous autoimmune disorders [21–25].

IL-17 cytokine can stimulate fibroblasts, epithelial and endothelial cells, to produce IL-6, CXCL8/IL-8, and prostaglandin E2 (PGE2) [26]. IL-17 also induces the expression of receptor activator of nuclear factor kappa B ligand (RANKL) on osteoblasts and stimulates the differentiation and activation of osteoclasts, which can influence bone resorption mediated by these cells [22]. Many studies have demonstrated the presence of IL-17 in periodontal tissues, crevicular gingival fluid, saliva, and plasma of patients with periodontal disease [27–30].

In order to investigate whether *IL17A* and the *IL17F* polymorphisms are associated with chronic periodontal disease and understand its immunopathogenesis, this study aimed at evaluating the *IL17A* G197A (rs2275913) and *IL17F* T7488C (His161Arg, rs763780) polymorphisms in patients with chronic periodontitis and in a healthy group who had undergone dental care in the North/Northwest of the state of Paraná, Southern Brazil.

2. Materials and Methods

2.1. Sample Selection. A total of 313 individuals were selected from those who sought dental treatment in the dental clinics of the Maringa State University (UEM) and Inga University (UNINGÁ) from January 2012 to December 2014. After taking patient's medical records, clinical periodontal examinations were conducted by two examiners. Clinical parameters of probing depth (PD) and clinical attachment level (CAL) were examined at six sites (mesiovestibular, vestibular, distovestibular, mesiolingual, lingual, and distolingual) of each tooth, as was bleeding on probing (BOP).

After the periodontal examination, participants were assigned to two different groups: the chronic periodontitis group ($N = 140$) composed of individuals who had at least 5 sites in different teeth with PD ≥5 mm, CAL ≥3 mm, and more than 25% of BOP and the control group ($N = 173$), formed by individuals who did not have sites with reduced CAL, displayed a PD of less than 4 mm, and exhibited less than 25% of BOP. Both patients and controls were from the North and Northwest regions of the state of Paraná (between 22°29′30″–26°42′59″S and 48°02′24″–54°37′38″W), Southern Brazil, over 30 years of age, from all ethnic groups, and with at least 20 teeth in the oral cavity. Information on the patient's ethnic background and smoking history was obtained by interviewing the individual (anamnesis). Exclusion criterions were patients and control subjects with diabetes mellitus and acute infections and patients with aggressive periodontitis and who had periodontal treatment in the last 6 months.

All individuals who agreed to participate in this research were informed about the nature of the study and signed an informed consent form. This study was approved by the Human Research Ethics Committee of the Maringa State University (UEM: number 719/2011, 02/12/2011).

2.2. DNA Extraction. To extract the DNA, the buffy coat was obtained from 4 mL of peripheral blood collected in EDTA by centrifugation (210 g for 15 min). The DNA was extracted using the salting-out method [31]. The concentration and quality of the DNA were analyzed by optical density in a Thermo Scientific Nanodrop 2000 apparatus (Wilmington, USA).

2.3. Genotyping Analysis. Single nucleotide polymorphisms (SNP) to *IL17A* G197A (rs2275913) and *IL17F* T7488C (rs763780, His161Arg) genotyping were performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) [32] with some modifications. Primer sequences for *IL17A* G197A were sense 5′-AACAAGTAAGAATGAAAAGAGGACATGGT-3′ and antisense 5′-CCCCCAATGAGGTCATAGAAGAATC-3′ and for *IL17F* T7488C were sense 5′-ACCAAGGCTGCTCTGTTTCT-3′ and antisense 5′-GGTAAGGAGTGGCATTTCTA-3′. PCR amplification was performed in a total volume of 30 μL mixture containing 100 ng genomic DNA, 1.0 μM of each primer, 200 μM of each dNTP, 2.0 mM of $MgCl_2$, 3 μL 10x PCR buffer, and 1.5 U Taq DNA polymerase (Invitrogen Life Technologies, Grand Island, NY, USA). PCR products were digested for one hour at 37°C with *XagI* (Fermentas, Canada) for *IL17A* G197A and *NlaIII* (New England Biolabs) for *IL17F* T7488C and then separated by electrophoresis on 3% agarose with SYBR Green (Invitrogen Life Technologies, Grand Island, NY, USA).

2.4. Statistical Analysis. Allele and genotype frequencies of *IL17A* G197A and *IL17F* T7488C were obtained by direct counts. The association between genetic polymorphisms and chronic periodontitis was evaluated using the Chi-square test with Yates correction or the Fisher's exact test and the correlation was deemed present by an odds ratio with 95% confidence intervals only for significant P values. Adjusting the

Table 1: Characteristics of patients with chronic periodontitis (CP) and controls.

	CP patients *n* (%)	Controls *n* (%)	*P*	OR (95% CI)
	N = 140	*N* = 173		
Gender				
Male	66 (47)	56 (32)		
Female	74 (53)	117 (68)		
Age				
Mean ± SD (year)	47.03 ± 9.21	45.61 ± 9.18		
Ethnic origin				
Caucasian	84 (60)	118 (68)		
Mixed	36 (26)	40 (23)		
Black	18 (13)	15 (9)		
Not declared	2 (1)	0		
Smoking				
Nonsmokers	56 (40)	127 (73)	<0.001	0.24 (0.149–0.39)
Smokers	33 (24)	20 (12)	0.008	2.78 (1.33–5.78)
Ex-smokers	51 (36)	26 (15)	<0.001	3.24 (1.89–5.56)
Nonsmoker	*N* = 56	*N* = 127		
Gender				
Male	20 (35.7)	33 (26.0)		
Female	36 (64.28)	94 (74.0)		
Age				
Mean ± SD (year)	46.5 ± 9.3	45.5 ± 9.9		
Ethnic origin				
Caucasian	36 (64.28)	86 (67.7)		
Mixed	16 (28.6)	28 (22.0)		
Black	3 (5.4)	13 (10.3)		
Not declared	1 (1.8)	0		

n: number; *P* = *P* value; OR: odds ratio; CI: confidence interval.

genotypic differences for the effect of age, gender, and smoking status was applied. All tests were carried out using a significance level of 5%. For these analyses and calculating Hardy-Weinberg equilibrium OpenEpi program Version 2.3.1 and SNPStas software (http://bioinfo.iconcologia.net/index.php) were used.

3. Results

Polymorphisms in the *IL17A* G197A (rs2275913) and *IL17F* T7488C (rs763780) were analyzed in 140 CP patients and 173 control subjects. Most participants were female (61.0%), Caucasian (64.5%), and nonsmokers (58.5%). The characteristics of patient and control subjects are described in Table 1.

Differences were noted in smoking history when the groups were compared, and to eliminate the smoking as a confounding factor, all analyses were also done in the nonsmoker patients versus nonsmoker controls. There was no significant difference with respect to gender, age, and ethnic background distributions.

Genotype distribution of *IL17A* G197A and *IL17F* T7488C in CP patient and control groups was consistent with the Hardy-Weinberg equilibrium ($P > 0.05$). The *IL17A* and *IL17F* were not in linkage disequilibrium ($P = 0.51$).

The genotype and allele frequencies distributions are summarized in Table 2. There were significant differences in the codominant and recessive models for *IL17A* AA genotype between all CP patients and controls. No significant difference was observed for *IL17F* in the recessive, dominant, and codominant models.

We analyzed *IL17A* and *IL17F* genotype and allele frequencies in CP and controls after stratifying according to gender and ethnic background. No significant difference was observed for *IL17F* polymorphism after stratification. However *IL17A* AA genotype was more frequent in female CP patients (16.2% *versus* 4.3%, $P = 0.01$, OR = 4.34, and 95% CI: 1.46–12.87) (Table 3). *IL17A* AA genotype and A allele were more frequent in Caucasoid CP patients than in controls (17.8% *versus* 5.9%, $P = 0.01$, OR = 3.45, and 95% CI: 1.34–8.88, and 38.7% *versus* 28.4%, $P = 0.04$, OR = 1.59, and 95% CI: 1.05–2.42, resp.). Difference was also found for *IL17A* AA genotype that was more frequent in the Caucasian and nonsmoking CP patients than in the Caucasian nonsmoking controls (22.2% *versus* 8.1%, $P = 0.048$, OR = 3.51, and 95% CI: 1.17–10.55) (Table 4).

Table 2: Genotypes and allele distribution of *IL17A* rs2275913 and *IL17F* rs763780 in the patients with chronic periodontitis and controls from Southern Brazil.

Genotype	All subjects			Nonsmokers		
	Patients $N = 140$ n (%)	Controls $N = 173$ n (%)		Patients $N = 56$ n (%)	Controls $N = 127$ n (%)	
rs2275913						
GG	67 (47.9)	87 (50.3)	Ref.	25 (44.6)	61 (48.0)	Ref.
AA	18 (12.9)	12 (7.0)	*	9 (16.1)	10 (7.9)	
AG	55 (39.2)	74 (42.7)		22 (39.3)	56 (44.1)	
AA/AG	73 (52.1)	86 (49.7)		31 (55.4)	66 (52.0)	
GG/AG	122 (87.1)	99 (57.2)		47 (83.9)	117 (92.1)	
Allele						
A	91 (32.5)	98 (28.3)		40 (35.7)	76 (30.0)	
G	189 (67.5)	248 (71.7)		72 (64.3)	178 (70.0)	
rs763780						
TT	125 (89.3)	158 (91.3)	Ref.	49 (87.5)	116 (91.3)	Ref.
CC	1 (0.7)	0		1 (1.8)	0	
TC	14 (10.0)	15 (8.7)		6 (10.7)	11 (8.7)	
CC/TC	15 (10.7)	15 (8.7)		7 (92.9)	11 (8.7)	
TT/TC	139 (99.3)	173 (100)		55 (98.2)	127 (100)	
Allele						
T	264 (94.3)	331 (95.7)		104 (92.9)	243 (95.7)	
C	16 (5.7)	15 (4.3)		8 (7.1)	11 (4.3)	

*Codominant model: $P = 0.09$; OR = 2.53; 95% CI = 1.07–5.99; recessive model: $P = 0.03$; OR = 2.46; 95% CI = 1.08–5.59.

Table 3: *IL17A* genotype and allele frequencies in chronic periodontitis Brazilian patients and controls stratified according to gender* in the total samples and nonsmoking individuals.

	IL17A rs2275913	CP patients n (%)	Controls n (%)
Female	Genotype	$N = 74$	$N = 117$
	GG	36 (48.7)	63 (53.8)
	AA**	12 (16.2)	5 (4.3)
	GA	26 (35.1)	49 (41.9)
	Allele		
	A	50 (33.8)	59 (25.2)
	G	98 (66.2)	175 (74.8)
Nonsmoker female	Genotype	$N = 14$	$N = 33$
	GG	6 (42.9)	11 (33.3)
	AA	2 (14.2)	5 (15.2)
	GA	6 (42.9)	17 (51.5)
	Allele		
	A	10 (35.7)	27 (40.9)
	G	18 (64.3)	39 (59.1)

CP: chronic periodontitis; *only significant differences were showed; **$P = 0.01$, OR = 4.34, and 95% CI: 1.46–12.87.

4. Discussion

In this study we investigated a possible role of *IL17A* and *IL17F* polymorphisms in immunopathogenic mechanism for CP in a Southern Brazilian population. We observed that the *IL17A* 197AA genotype was more frequent in patients with chronic periodontitis (CP), females with CP, and the Caucasian and nonsmoking Caucasian patients with CP than in respective controls, and this could be correlated to the risk of disease. The *IL17F* T7488C polymorphism was not associated with CP risk in this population.

In a case-control study, pairing between study subjects is necessary in order to avoid bias in the final results. CP can be related to individual risk factors such as stress, diabetes, osteoporosis, and arthritis [3–5, 33–37] and, in this studied population, they were considered confounding factors and were an exclusion criterion. On the other hand, smoking habits, also a predisposition factor with oral diseases and especially in the chronic periodontitis [38–41], were more frequent in CP patients (smoker + ex-smoker: 60% *versus* 40%; $P < 0.001$, OR = 4.12, and 95% CI: 2.56–6.69); thus, to exclude smoking as a predisposing factor, statistical analyses were performed in all the individuals as well as in the nonsmokers CP versus nonsmokers in control group, and stratifying by smoking habits was not considered. Some studies alert that gender can be a confounding factor to periodontitis [42] although others showed the higher frequency in male was principally related to personal hygiene habits [43]; furthermore, it was revealed that there was no meaningful relation to the role of IL-17 polymorphism in men and in women when compared to each other [44]. With regard to ethnic background, the distributions of patients and controls were similar: this is important because *IL17A* and *IL17F* alleles and genotypes differ in different populations (http://www.snpedia.com/index.php/Rs2275913 and /Rs763780) and Brazilians are an admixed population. Although ethnicity was not a confounding variable, the analyses were done after stratification by the Caucasian, Black, and mixed population.

In the present report *IL17F* was not associated with periodontal disease in all the patients nor in nonsmoking patients and controls who were investigated in the recessive, dominant, and codominant models. However, *IL17A* 197AA genotype was more frequent in CP patients, in female patients, and in the Caucasian CP patients as well as in the nonsmoking Caucasian patients with CP. Furthermore, *IL17A* 197A allele was also more frequent in Caucasians with CP. These results suggest that *IL17A* 197AA genotype and A allele could be related to higher risk for the development of chronic periodontitis. In our Caucasian group with CP the *IL17A* 197G allele was less frequent and could be a resistant factor. The polymorphism in the promoter region of cytokines may be related to higher expression of the specific cytokine. According to Espinoza et al. [45] the *IL17A* 197A allele correlates to more efficient IL-17 secretion and higher affinity for the nuclear factor of activated T cells (NFAT), which is a critical regulator of the *IL17* promoter gene [46].

The *IL17A* 197AA genotype was associated with tumorigenesis susceptibility as in gastric cancer [47, 48], breast

Table 4: *IL17A* genotype and allele frequencies in chronic periodontitis Brazilian patients and controls stratified according to ethnic group*.

	IL17A rs2275913	CP patients *n* (%)	Controls *n* (%)	*P*	OR (95% CI)
Caucasian	Genotype	*N* = 84	*N* = 118		
	GG	34 (40.5)	58 (49.2)		
	AA	15 (17.8)	7 (5.9)	0.01	3.45 (1.34–8.88)
	GA	35 (41.7)	53 (44.9)		
	Allele				
	A	65 (38.7)	67 (28.4)	0.04	1.59 (1.05–2.42)
	G	103 (61.3)	169 (71.6)	0.04	0.63 (0.41–0.96)
Nonsmoker Caucasian	Genotype	*N* = 36	*N* = 86		
	GG	15 (41.7)	41 (47.7)		
	AA	8 (22.2)	7 (8.1)	0.048	3.51 (1.17–10.55)
	GA	13 (36.1)	38 (44.2)		
	Allele				
	A	29 (40.3)	52 (30.2)		
	G	43 (59.7)	120 (69.8)		

CP: chronic periodontitis; *only significant differences were showed.

cancer [49], and cervical cancer [50], as well as autoimmunity diseases such as ulcerative colitis [51, 52] and rheumatoid arthritis [53]. As for periodontitis diseases, only two previous studies in the Brazilian patients were reported. Corrêa et al. [54] showed similar results to our study: *IL17A* 197AA genotype and A allele were associated with worse clinical and inflammatory periodontal parameters. Saraiva et al. [55] conducted another study on chronic periodontitis in Brazil and severe periodontitis patients with the aim of investigating the phenotypic expression of *IL17A* and the polymorphisms of *IL17A* and *IL17F* within different clinical forms and severity of the disease. However, differently to our and Corrêa et al.'s results [54], their data suggested that the IL-17 and *IL17A* A allele were associated with the absence of periodontal disease, and the *IL17A* GG genotype and G allele were associated with risk factors.

According to the distribution of *IL17A* genotypes frequencies in our control group, *IL17A* AA was 7.0%, *IL17A* AG was 42.7%, and *IL17A* GG was 50.3%, similar to those in the study conducted by Saraiva et al. [55] which were 10% for AA, 44% for AG, and 46% for GG but very different from those of Corrêa et al. [54] which were 25.9% for AA, 14.81% for AG, and 59.26% for GG.

The *IL17F* T7488C polymorphism was not associated with chronic periodontitis in this study. This result was similar to Corrêa et al. [54] and Saraiva et al. [55] in CP Brazilian patients. *IL17F* has been associated with several diseases like asthma, Crohn's disease, multiple sclerosis, inflammatory bowel disease, autoimmune thyroid diseases, tuberculosis, and dilated cardiomyopathy as well as a high risk of recurrent pregnancy loss [51, 56–61]. According to the literature, the IL-17F activity is similar to IL-17A but significantly weaker, and the variant form of IL-17 protein (His121Arg) suppresses the expression and the activity of wild type [16, 62].

According to the *IL17F* genotype frequency distribution in our control group, we had 8.7% for *IL17F* TC and 91.3% for *IL17F* TT; the *IL17F* CC genotype was absent in our control population. Again, it was similar to the study conducted by Saraiva et al. [55] which found 6.4% for TC and 93.6% for TT and did not find the *IL17F* CC genotype. However, the distribution of genotype frequencies found by Corrêa et al. [54] was also very different: they found 16.66% for *IL17F* TC, 56.66% for *IL17F* TT, and 23.33% for *IL17F* CC in their Brazilian control group. The distribution of genotype frequency in our populations was consistent with a low frequency of polymorphic genotype of *IL17F* T7488C found in other populations (http://www.snpedia.com/index.php/Rs763780).

CP progression was related to a host inflammatory response that mediates tissue damage, and several previous studies had been relating immune genetic factors to CP disease, especially cytokines genes polymorphism [63–69]. The IL-17, a proinflammatory cytokine, was detected in periodontal tissues, crevicular gingival fluid, saliva, and plasma of patients with periodontal disease [27–30, 70]. IL-17, especially when combined with IFN-gamma, may have a role in immune modulation through stimulation of human gingival fibroblasts in periodontal disease [71]. This occurs by triggering the release of other proinflammatory, metalloproteinase, and neutrophil-mobilizing cytokines [47, 49, 51, 72] and having effects on osteoclasts maturity as a stimulating factor [73, 74]. IL-17A and IL-17F have a very similar amino acid sequence and both play similar functions and have the ability to induce chemokines which is crucial to the neutrophil recruitment and activation [75], the first wall in the periodontal diseases. Thus, understanding *IL17* polymorphism is necessary in order to infer the role of IL-17 in the CP immunopathogenesis.

In these Southern Brazilian patients, the *IL17A* rs2275913 polymorphisms, AA genotype, and A allele were associated with a susceptibility to chronic periodontitis disease, in females with CP, and in the Caucasian and nonsmoking Caucasian patients. Furthermore, the possible immunopathogenic mechanism would be investigated in the future through histological studies.

5. Conclusion

In conclusion we can infer that *IL17A* G197A rs2275913 polymorphism, *IL17A* AA genotype, and A allele could be associated with a susceptibility to chronic periodontitis but no evidence showed for risk or protection associations for *IL17F* T7488C rs763780. Additional studies are necessary for understanding the functional role of rs2275913 polymorphisms in chronic periodontitis.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Review Article

The Two-Way Association of Periodontal Infection with Systemic Disorders: An Overview

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Oral cavity that harbors diverse bacterial populations could also act as a site of origin for spread of pathogenic microorganisms to different body sites, particularly in immunocompromised hosts, patients, the elderly, or the underprivileged. A number of recent publications have advocated that patients with periodontal diseases are more susceptible to metabolic endotoxemia, inflammation, obesity, type 2 diabetes, and other related systemic complications, concluding that periodontal diseases could be a potential contributing risk factor for a wide array of clinically important systemic diseases. However, despite a significant increase in the prevalence of periodontal infections and systemic diseases in the past few decades, the fundamental biological mechanisms of connection between these ailments are still not fully explicated. Consequently, the mechanisms by which this bidirectional damage occurs are being explored with a concentric vision to develop strategies that could prevent or control the complications of these ailments. This paper attempts to summarize and hypothesize the diverse mechanisms that hint to a certain connection between the two prevalent chronic situations.

1. Introduction

Periodontitis is a multifactorial disease with numerous systemic or local risk factors playing a part in its clinical sequences. Periodontal diseases are influenced by various risk factors including ageing, smoking, oral hygiene, socioeconomic status, genetics, race, gender, psychosocial stress, osteopenia, osteoporosis, and other medical conditions including obesity and type 2 diabetes mellitus (T2DM) [1, 2], signifying that periodontitis does not occur merely as a consequence of plaque accretion but is also coupled with various host factors which could alter the consequence of the plaque on a particular individual. Recent findings have suggested that chronic low-grade inflammation is directly involved not only in the pathogenesis of obesity, diabetes, and their complications but also in the pathogenesis of periodontal diseases [3, 4], where cytokines play a central role in the host's responses to the periodontal biofilms. A number of diverse studies have indicated that periodontal diseases may also be associated with a wide array of systemic diseases and conditions (Figure 1). The primary putative facts that support the biological connection between periodontitis and systemic diseases are (a) usual implication of infection in the pathogenesis of both diseases, (b) transient and low-grade bacteremia and endotoxemia caused by periodontal diseases, (c) systemic immune responses and inflammation triggered by periodontal diseases, (d) expression of virulence factors by periodontal pathogens, and (e) presence of periodontal pathogens in nonoral tissues like atheromatous plaques [5–7]. Although the detailed mechanisms underlying this association are still unclear, available reports evidently demonstrate a bidirectional link between the mechanism of periodontal diseases and systemic/metabolic diseases where both conditions could aggravate each other [1, 8, 9].

2. Periodontitis and Obesity/Diabetes: The Two-Way Complication

Most of the mechanisms that support the influence of obesity and/or T2DM on periodontium generally share similar

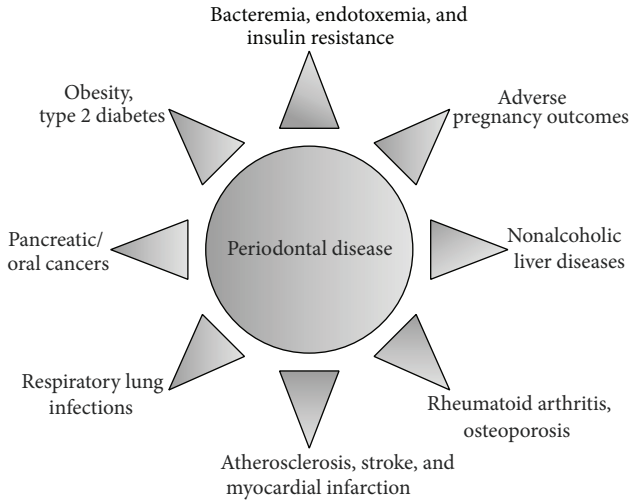

Figure 1: Diagram of periodontal disease leading to other complications.

characteristics with those implicated in the typical complications of the diabetes [10]. For instance, in T2DM patients, hyperglycemia leads to a higher deposition of advanced glycation end products (AGEs) in tissues where these AGEs bind to the neutrophils and impair their normal functions. Further, these AGEs may also activate several unsought cell-surface receptors (RAGEs) which may alter the macrophages to a destructive phenotype. Both of these situations aggravate an uncontrolled production of proinflammatory cytokines and eventually lead to an increased vascular permeability, collagen fiber breakdown, and destruction of connective tissues and bones through increased lipid peroxidation and raised levels of IgA, IgG, and so forth, thereby making the diabetic patients more prone to periodontitis (Figure 2). Likewise, in patients with periodontal infections, the penetration of pathogen(s) (mainly *Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Treponema denticola,* and *Aggregatibacter actinomycetemcomitans*) or their products in lamina propria may lead to endotoxemia and a state of systemic chronic inflammation through the leakage of endotoxins such as lipopolysaccharides (LPS) into the serum. This hyperinflammation may further affect the expression and functioning of important immunoinflammatory molecules such as IL-1β, IL-6, TNF-α, PGE2, IL-8, IL-12, and IL-18, thereby contributing to insulin resistance and an altered lipid and glucose metabolism [11]. Eventually, the functioning of various tissues and cells such as adipocytes, hepatocytes, and endothelial and muscle cells may get impaired, thereby leading to more chronic metabolic states, that is, obesity, T2DM, and so forth in these periodontitis patients (Figure 2).

Since periodontal diseases are infectious diseases, earlier studies emphasized primarily the possible variations in subgingival microflora of patients with and without T2DM. However, the findings of altered functions of neutrophils, monocytes, and macrophages in people with T2DM gradually shifted the research focus towards possible discrepancies in the immunoinflammatory responses between people with and without T2DM [10]. Impaired adherence, chemotaxis, and phagocytosis capacities of neutrophils (the first line of host defense) may avoid the destruction of bacteria in the periodontal cavity and lead to an increased periodontal damage. Since altered wound healing is another frequent problem suffered by people with T2DM, distorted periodontal wound healing responses to persistent microbial encounters in patients with persistent hyperglycemia may also contribute to an increased bone and attachment loss. Given that the inflammatory cells such as monocytes and macrophages harbor receptors for AGEs, the accumulation of AGEs in T2DM patients may also intensify the proinflammatory responses to periodontal pathogens. Further, the interactions between AGEs and their receptors on inflammatory cells could stimulate hyperproduction of proinflammatory cytokines such as IL-1β and TNF-α, consequently increasing the risk or occurrence of periodontal diseases in T2DM patients (Figure 2).

Patients with inflammatory periodontal diseases usually have higher serum levels of proinflammatory cytokines [12]. Since the hyperinflammatory immune cells could intensify the production of proinflammatory cytokines in T2DM patients, this could also increase the insulin resistance and complicate the control of diabetes. Nonetheless, hyperglycemia and AGEs are only a few of the numerous potential factors that are implicated in the complications of obesity/T2DM as well as in the pathophysiology of periodontitis in people with the diabetes [4]. Polymorphonuclear leukocytes and alterations in the collagen metabolism could be another possible reason for higher predisposition of T2DM patients towards periodontal diseases. The formation of AGEs may influence the collagen stability and vascular integrity and could also aggregate macrophage and monocyte receptors, thereby aggravating the susceptibility to periodontitis through the stimulation of IL-1 and TNF-α [13] (Figure 2). These inflammatory cytokines are known to stimulate the insulin resistance and several other chronic inflammatory complications including periodontitis [14]. Moreover, the fact that TNF-α and IL-6 are produced in the adipose tissues could also support the shared link between obesity, T2DM, and periodontitis [15].

3. Periodontitis and Obesity/Diabetes: Underlying Mechanisms

The principal mechanisms that link oral infection with systemic diseases are (a) metastatic spread of infection from the oral cavity as a consequence of transient bacteremia, (b) metastatic spread of cellular injuries because of the circulation of oral bacterial toxins, and (c) metastatic spread of inflammation through the immunological injuries triggered by oral bacteria [16]. The association between periodontal diseases and systemic inflammation is also supported by the observation that the chronic inflammation is a significant factor in the fundamental pathophysiology of both of these ailments and that the local/systemic variations triggered by periodontitis may also lead to a chronic inflammatory state

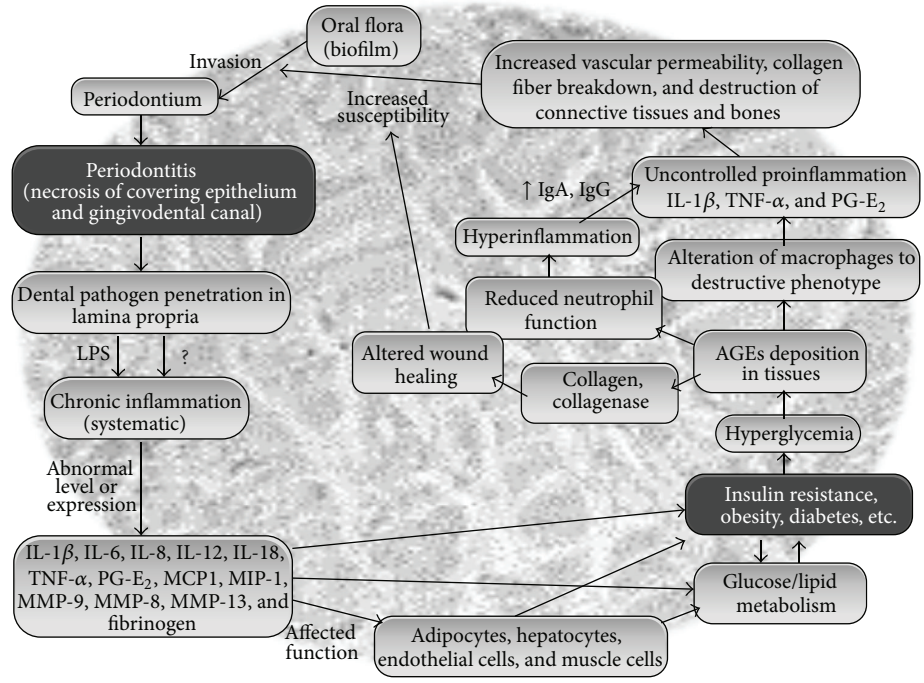

Figure 2: A summary of proposed connections between periodontal diseases and metabolic disorders such as obesity, insulin resistance, and type 2 diabetes (LPS: lipopolysaccharide; IL: interleukins; TNF: tumor necrosis factor; PGE2: prostaglandin E2; MCP: monocyte chemoattractant protein; MIP: macrophage inflammatory protein; MMP: matrix metalloproteinase; AGEs: advanced glycation end products; Ig: immunoglobulin).

that can increase the susceptibility to metabolic syndromes [11] (Figure 2).

Given that the adipose tissue, particularly the white adipose tissue (WAT), acts as a main endocrine organ secreting a number of bioactive substances such as adipocytokines, TNF-α, leptin, adiponectin, and resistin, it can also affect the periodontal response or can also be affected during periodontal infections [12]. For instance, a negative correlation of the degree of periodontal damage with leptin concentration in gingival crevicular fluid of periodontitis patients and a positive correlation with leptin concentration in the serum indicate a negative correlation of gingival crevicular leptin concentration and a positive correlation of serum leptin during the progression of clinical attachment level [17]. Since the gingival inflammation could also cause vasodilatation, it may also increase the serum levels of leptin which would further act as a defense mechanism of the body to battle the periodontal inflammation [18]. The serum levels of resistin have also been observed to be elevated in people with periodontitis, indicating that it may also play a role in periodontitis [19]. It has been observed that in patients with periodontitis and T2DM, effective glycemic control may improve bleeding on probe lesions by improving the inflammation at gingival sites of periodontal tissues [20], while the treatment of periodontitis with topical antibiotics may ameliorate the periodontal status and glycemic control with an elevation of serum adiponectin and reduced HbA1c [21].

Because of the predominated role of gram-negative anaerobic bacteria in periodontal infections, the ulcerated pocket epithelium turns into a chronic source of systemic challenge from bacteria, bacterial products, and locally produced inflammatory mediators. Further, as a consequence of the high vascularity, the inflamed periodontium may act as an endocrine-like source of inflammatory mediators (such as TNF-α, IL-6, and IL-1) which are significant in periodontal inflammation and may also influence glucose and lipid metabolism [22] (Figure 2). In view of the fact that osteoblasts, which are involved in bone turnover, also express Toll-like receptors- (TLRs-) 1, 4, 5, 6, and 9, while osteoclasts express TLRs-1, 2, 3, 4, 5, 6, 7, 8, and 9 [23, 24], it is also likely that the TLRs signaling within the alveolar bone may cause an inflammatory response to invading pathogens, and this initiation of a cascade of proinflammatory cytokines within the alveolar bone could lead to a pathological resorption of bone through excessive or extended production of osteolytic host molecules such as IL-1, TNF-α, and prostaglandin E2 (PGE2) which may further stimulate the osteoblast inhibition and osteoclast activation through the receptor activator of nuclear factor kappa-β (NFk-β) ligand.

Since the serum levels of total cholesterol, low-density lipoprotein cholesterol, and triglycerides have been observed to be higher in periodontal patients, periodontitis may also be a risk factor for hyperlipidemia [25]. The hyperactivity of white blood cells which is caused by the hyperlipidemia may also increase the production of oxygen radicals that are often linked with the development of periodontitis, and this decline in the antioxidant ability in periodontitis patients could also trigger the development of insulin resistance [18].

Such variations in the phenotype of immune cells due to the elevated levels of lipids and serum proinflammatory cytokines in chronic periodontitis may also support the two-way correlation between the two diseases [26] (Figure 2). However, it still remains to be fully revealed if (and how) periodontitis provokes the higher lipid levels or higher lipid levels influence the periodontitis.

4. Periodontal Infections and Other Systemic Diseases

There has been a significant interest in the possible association between oral and systemic diseases in the past few decades [27–29], especially after the case-control study by Mattila et al. [30] who noticed a significant association between poor dental health and acute myocardial infarction in the patients, as compared to control subjects. Subsequently, various epidemiological studies have investigated and supported a causal association of periodontitis with several clinical systemic diseases, including cardiovascular disease [31, 32], diabetes [33], respiratory disease [34], adverse pregnancy outcomes [35], Alzheimer's disease [36], pancreatic cancer [37], and cerebral infarction [38]. In addition to the chronic inflammation triggered in response to the oral pathogens, periodontal infection may also result in tooth loss, oral pain, poor mastication, and several nutritional defects and may also be expected to be related with Alzheimer's disease and dementia [39–41]. Decreased mastication due to oral pain and tooth loss could also result in reduced acetylcholine synthesis which may cause several learning and memory problems [42]. In addition to the incidences of hypertension and diabetes mellitus, the number of lost teeth has also been found to be higher in patients with silent infarctions and cerebral white matter changes, as compared to healthy group, thereby hinting that periodontal infections may also be a predictor of stroke and cognitive impairment [43].

Although the precise role and underlying mechanisms of periodontal infections in the pathology of systemic diseases still remain to be completely established, several hypotheses have been proposed based on the findings of various clinical and epidemiological investigations (Figure 3) [5, 44–46]. The primary factor includes the shared risk factors among oral infection and systemic diseases, such as genetic or environmental factors including age, smoking, lifestyle, and socioeconomic status. Another mechanism is the systemic inflammation against the local infection or circulating bacteria and associated higher levels of circulating inflammatory biomarkers which could play a contributing role in systemic disease. Also, the significant role of infection and inflammation in diseases such as atherosclerosis, cardiovascular disease (CVD), and coronary heart disease (CHD) also underscores the possible etiological role of periodontal infections in these diseases [28, 30, 47–50]. The pathogens from periodontal pockets may also enter into the connective tissues, endothelial cells, and the bloodstream and thus could lead to the formation of thrombus by platelet aggregation degrading collagen [51–53]. Chronic periodontal infections can contribute to atherogenesis either directly

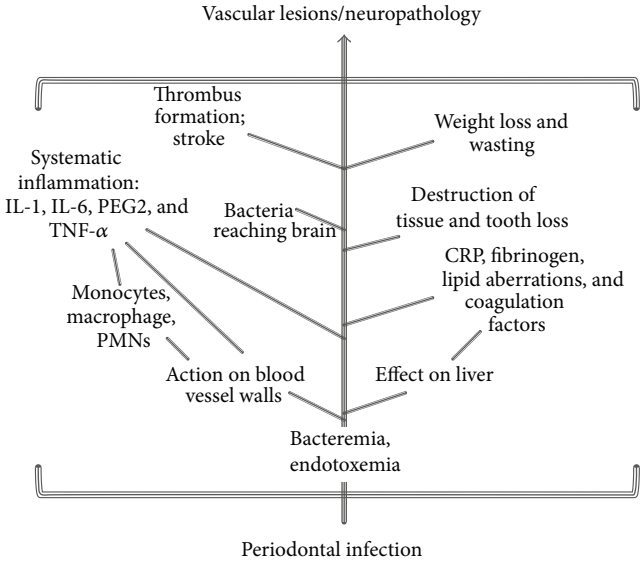

Figure 3: Potential consequences of periodontal disease leading to stroke, infarction, atherosclerosis, and other neuropathological complications.

by triggering the platelet aggregation and invasion causing damage to endothelial cells or indirectly by stimulating the synthesis of intracellular adhesion molecules and production of antibodies against bacterial LPS thereby causing a discrepancy of the immune system [54, 55]. Moreover, *P. gingivalis* and *A. actinomycetemcomitans* have also been detected in atheromatous plaques of CVD patients, indicating a connection between periodontal infections and the formation of atherogenic lesions [56–59]. A recent systematic meta-analysis of epidemiologic literature has also suggested that periodontal infection could be an independent risk factor for CHD (although relatively weak) and that various measures of periodontal infections could explicate 30% increase in risk of CHD [60].

4.1. Periodontitis and Fatty Liver. In addition to insulin resistance, obesity, diabetes, and oxidative stress, periodontal diseases may also be implicated in the pathogenesis of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). Since periodontal pathogens, their endotoxins, and/or cytokines released from the organisms could invade into the blood circulation and cause bacteremia, endotoxemia, and inflammation, such periodontal infections may also be implicated as an independent risk factor for NAFLD/NASH. For instance, the incidences of *P. gingivalis* infection have been found to be significantly higher in NAFLD patients as compared to healthy subjects [61], hinting at the involvement of *P. gingivalis* infection in the onset of NAFLD. Further, observation of a lower serum albumin levels in *P. gingivalis*-positive NASH/NAFLD patients indicates that *P. gingivalis* infection may lead to a decreased liver function thereby progressing to the pathogenesis for NAFL or NASH. Interestingly, periodontal treatment has been found to improve the liver functional parameters such as serum

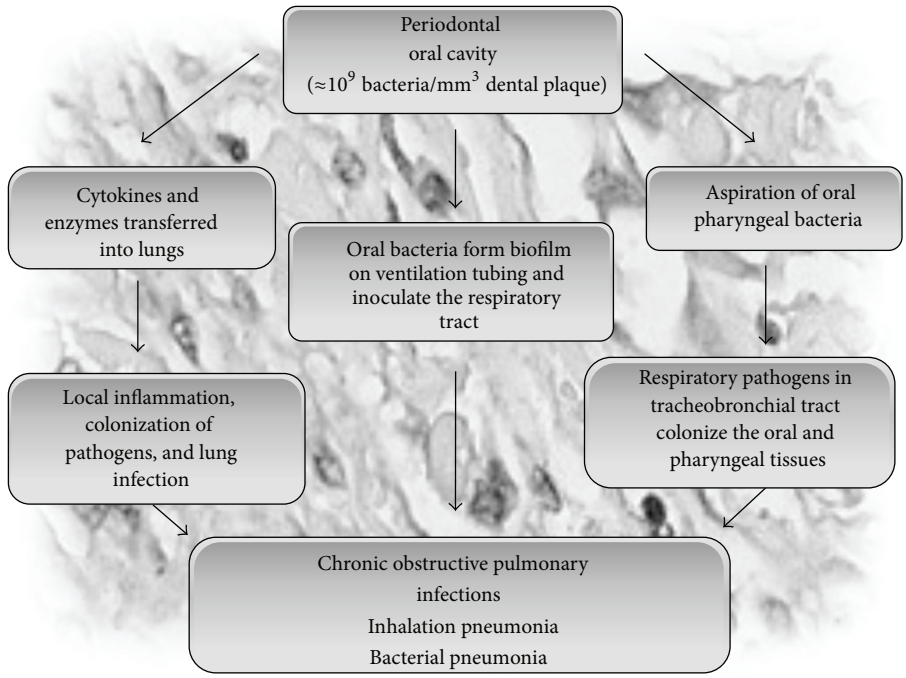

Figure 4: Possible role of periodontal infection in respiratory diseases.

aspartate aminotransferase and alanine aminotransaminase in NAFLD patients, again signifying the fact that *P. gingivalis*-positive periodontitis may be a risk factor for the progression of NAFLD. Since *P. gingivalis* virulence strains could release LPS and TNF-α, its infection may lead to the inflammation of other systemic organs, besides the local gingiva. *P. gingivalis* may also enter into the blood circulation from the gingiva after widespread periodontal processes such as chewing, tooth-brushing, subgingival irrigation, and dental extractions again supporting the hypotheses that *P. gingivalis* or other similar periodontal infections may also be an infrequent risk factor for the progression of NAFLD or NASH [62].

4.2. Periodontal Disease and Respiratory Infections. Poor oral health may also predispose the host to respiratory diseases, particularly in high-risk patients such as residential nursing patients, hospitalized patients, elderly, smokers, and the underprivileged. In periodontal infections, the aspiration or hematogenous spread of bacteria from the oropharynx into the lower respiratory tract and the consequent infection of respiratory ducts can easily cause respiratory infections such as pneumonia and chronic obstructive pulmonary diseases [63, 64] (Figure 4). Since the oral cavity is adjacent to the trachea, it could be an easy entrance for the immigration and colonization of respiratory pathogen. Respiratory pathogens may infrequently populate dental plaques and may also be aspirated/inhaled from the oropharynx into the upper airway and then the lower airway where they may adhere to the alveolar and bronchial epithelium [65–67]. In periodontal patients, one mm^3 of dental plaque may contain about 10^9 bacteria and hence could serve as a persistent pool for potential oral/respiratory pathogens which could be shed into the saliva and aspirated into the lower respiratory tract and the lungs to cause infection [68] (Figure 4). Further, the cytokines and enzymes induced from the inflamed periodontal tissues may also relocate into the lungs and trigger local inflammatory processes and lung infections [34]. Also, in periodontal diseases, poor oral hygiene may result in a higher concentration of oral pathogens in the saliva, and these pathogens may be aspirated into the lung overcoming the immune defenses and assist the pulmonary pathogens in inhabiting the upper airways. Generally, in healthy scenarios, the respiratory tract is capable of defending against aspirated bacteria. However, in periodontal diseases, the disturbed oral hygiene, reduced salivary flow, decreased cough reflex, dysphagia, and other disabilities can predispose the patients to a high risk for pulmonary infections [69–74].

4.3. Periodontal Diseases and Cancer(s). A number of clinical and epidemiological studies have observed higher risks of oral, gastrointestinal, lung, and pancreatic cancers in subjects with periodontal disease, thereby linking oral bacteria with the etiology of these cancers. In addition to tobacco and alcohol consumption, a poor oral hygiene could also be a possible risk factor for oral cancers [75, 76]. Several case-control studies have found tooth loss to be associated with a higher oral cancer risk [77, 78], indicating that tooth loss may contribute to oral cancers either by promoting the initiated tumors or by some other complex mechanism(s). Several reports have also suggested that oral bacteria could contribute to the cancers of upper gastrointestinal tract including aerodigestive tract, esophagus, and stomach, possibly through the similar inflammatory mechanisms as that

of *Helicobacter pylori* [79–82]. However, available evidences are inadequate, and hence further studies are awaited to validate a definite association between periodontal diseases and gastrointestinal cancers.

4.4. Periodontal Diseases and Adverse Pregnancy Outcomes. Approximately half of the perinatal deaths or congenital neurological deficits are caused as a result of premature births [83]. Incidences of intrauterine infection and inflammation are known to be a significant contributor to majority of the preterm deliveries [83]. Several studies have speculated that periodontal diseases (besides appendicitis, pneumonia, or other remote infections) may also trigger preterm labor, prematurity, and low birth-weight, primarily through (a) the possible hematogenous invasion of oral pathogens and/or their metabolites/toxins, (b) circulation of the inflammation by-products through the bloodstream, and (c) subsequent maternal/fetal immune responses against the invading pathogens, toxins, inflammatory inducers, and so forth [84–89]. Several clinical and observational investigations, however, have failed to observe any significant association between periodontal disease and the occurrence of preterm births or low-birth weight, and hence more investigations are requisite to resolve this paradox [90–93].

5. Periodontal Diseases and Overall Health: *The Nonclinical Links*

In addition to the various clinical, immunological, or molecular mechanisms that link periodontal infection with the systemic health, periodontal diseases may also have an indirect effect on the overall health status of the patient which could further exaggerate the health complications. Since periodontal disease leads to oral pain and teeth loss, it may result in poor mastication, less appetite, and less food intake which can cause nutritional deprivations. The oral pain may also cause sleep deprivation, thereby causing an upset behavior and hypertension. Bad breath and oral pain may also negatively affect the social routine of the patient and reduce the social and physical activities of the patient. The high cost of treatment regimen may also disturb the socioeconomic status of the patient. All these factors such as oral pain, teeth loss, bad breath, deprived nutrition and sleep, reduced physical and social activities, and depression may altogether make the patient more vulnerable to low self-esteem, hypersensitivity, and weakened immune system and hence may adversely affect the overall health.

6. Concluding Remarks

Although the recent evidences have supported the role of periodontal infection and consequent inflammation in diseases such as obesity, type 2 diabetes, cardiovascular disease, and gastrointestinal and pancreatic cancers, the precise etiological role of periodontal infections still needs to be deciphered completely. Yet, the available literature is sufficient to establish that the periodontal diseases may be a significant risk factor for various systemic disorders, and hence future studies are anticipated to elucidate the mechanisms through which the periodontal diseases and systemic diseases affect each other. Nevertheless, it is only after the precise understanding of these diseases that the attention could be shifted from the treatment of these ailments to their prevention for a healthier socioclinical scenario.

Disclosure

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Conflict of Interests

The authors declare that there is no conflict of interests relevant to this paper.

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Research Article

HLA Haplotypes and Genotypes Frequencies in Brazilian Chronic Periodontitis Patients

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Human leukocyte antigens (HLA) have a pivotal role in immune response and may be involved in antigen recognition of periodontal pathogens. However, the associations of HLA with chronic periodontitis (CP) have not been previously studied in the Brazilian population. In an attempt to clarify the issue of genetic predisposition to CP, we examined the distribution of HLA alleles, genotypes, and haplotypes in patients from Southern Brazil. One hundred and eight CP patients and 151 healthy and unrelated controls with age-, gender-, and ethnicity-matched were HLA investigated by polymerase chain reaction with sequence specific oligonucleotides. To exclude smoking as a predisposing factor, statistical analyses were performed in the total sample and in nonsmoking individuals. The significant results showed a positive association of the A*02/HLA-B*40 haplotype with CP (total samples: 4.2% versus 0%, P_c = 0.03; nonsmokers: 4.3% versus 0%, P_c = 0.23) and a lower frequency of HLA-B*15/HLA-DRB1*11 haplotype in CP compared to controls (total samples: 0.0% versus 4.3%, P_c = 0.04; nonsmokers: 0 versus 5.1%, P_c = 1.0). In conclusion, the HLA-A*02/B*40 haplotype may contribute to the development of CP, while HLA-B*15/DRB1*11 haplotype might indicate resistance to disease among Brazilians.

1. Introduction

Chronic periodontitis (CP) is a common complex disease of the oral cavity that is characterized by an inflammatory response to commensal and pathogenic oral bacteria [1, 2]. Due to bacterial infection, periodontal tissues become inflamed and are slowly destroyed by the action of the inflammatory process. If the disease is left untreated, teeth lose their ligamentous supporting structure to the alveolar bone, the alveolar bone is resorbed, and the teeth become mobile, finally resulting in teeth loss [1]. CP is considered the main cause of tooth loss among adults and is associated with severe quality of life impact [3].

The inflammatory response of the periodontal tissues to infection is influenced by environmental factors as well as by genetic factors [4, 5]. It is estimated that 50% of the expression of periodontitis in CP could be attributed to genetic factors [5]. The observation that periodontitis is a complex disease entity with a multifactorial etiology has led to the search for risk factors that predispose to periodontitis in general as well as distinctive risk factors that might predispose to different clinical presentations of this group of diseases.

The human leukocyte antigens (HLA) play an important role in immune responsiveness and may be involved in antigen recognition of periodontal pathogens [6]. These cell-surface molecules have a key role in antigen presentation and activation of T cells. The polymorphisms of HLA can directly affect the binding capability of antigen peptides and thus affect the antigen-specific T-cell response [7]. Hence, these polymorphisms could represent an important susceptibility or resistance factor to periodontitis.

For many years, researchers have periodically screened populations of patients with different forms of periodontitis for associations with HLA antigens [8–14] and consistent results in relation to CP could not be obtained up to now. This study aimed to investigate differences in allelic group, genotype, and haplotype frequencies of HLA classes I and II in a sample of Brazilian patients with CP compared with a control group without CP.

2. Materials and Methods

2.1. Sample Selection. Between January and September 2012, a total of 259 individuals were selected from those who sought dental treatment at the dental clinics of Maringa State University (UEM) and Inga University (UNINGÁ) at Maringá, PR, Brazil (north/northwest region of the State of Parana, located in the southern region of Brazil, between 22°29′30″–26°42′59″S and 48°02′24″–54°37′38″W). Males and females, ethnically similar, aged over 34 years and with at least 20 teeth in the buccal cavity participated in this study. The criteria for exclusion were as follows: individuals with acute infections or diseases with known associations to HLA alleles such as diabetes, rheumatic diseases, systemic lupus erythematosus or narcolepsy, use of antibiotics during the last six months, and chronic usage of anti-inflammatory drugs or lactations and those who were pregnant.

After taking the patient's history, clinical periodontal examinations were conducted by two examiners. Clinical parameters of probing depth (PD) and clinical attachment level (CAL) were examined at six sites (mesiovestibular, vestibular, distovestibular, mesiolingual, lingual, and distolingual) of each tooth, as was bleeding on probing (BOP). After the periodontal examination, participants were categorized into two different groups: the CP group ($n = 108$) composed of individuals who had at least 5 sites in different teeth with PD ≥ 5 mm, CAL ≥ 3 mm, and more than 25% of BOP; and the control group ($n = 191$), formed by individuals who did not have sites with reduced CAL, displayed a PD of less than 4 mm, and exhibited less than 25% of BOP. Therefore, the control group was matched to the case group according to epidemiological characteristics such as ethnicity, gender, and geographical region. Information on the habit of smoking was obtained by interviewing the individual (anamnesis).

2.2. Ethics Information. All individuals who agreed to participate in this research were informed regarding the nature of the study and signed an informed consent form authorizing the use of their samples in the study, which was approved by the Ethics Committee for Human Research at Maringa State University (UEM-N°.719/2011, 02/12/2011).

2.3. Sample Collection and DNA Extraction. Blood samples (4 mL) were collected from the subjects in tubes containing anticoagulant (EDTA) and centrifuged at 210 g for 15 minutes, and the buffy-coat was conserved at −20°C until use. The genomic DNA was extracted using the salting-out method described by Miller et al. [15]. The concentration and quality of the DNA were analyzed by optical density in a Thermo Scientific Nanodrop 2000 apparatus (Wilmington, USA).

2.4. HLA Genotyping. HLA typing (HLA-A, HLA-B, HLA-C, DRB1, DQA1, and DQB1) was carried out using the polymerase chain reaction-sequence specific oligonucleotides technique (PCR-SSO; One Lambda, Canoga Park, CA, USA), low resolution to HLA-A, HLA-B, HLA-C, and DRB1, and high definition to HLA-DQA1 and DQB1. First, target DNA was PCR-amplified using group specific primers set, after the amplified product was biotinylated, which allowed later detection using R-phycoerythrin-conjugated streptavidin (SAPE), and hybridized with microspheres linked to specific conjugated fluorescent probes for HLA allele groups (One Lambda, Canoga Park, CA, USA). The fluorescent intensity varied based on reaction outcome and was expected to be 1000 or above for control positive probes. Reaction readings were carried out by flow cytometry using Luminex technology (One Lambda). Samples were analyzed through the HLA FUSION software (One Lambda Inc., San Diego, CA, USA).

2.5. Statistics. HLA specificity frequencies between the groups were compared using Fisher exact test. A two-sided P value (P) of <0.05 was considered statistically significant. Odds ratio (OR) values with a 95% confidence interval (95% CI) were also calculated to evaluate the risk of the individual developing the disease when having a particular HLA type. All the statistical analyses were performed using the *R* statistical environment [16]. However, to account for multiple comparisons, the observed P values were corrected (P_c) for the number of alleles when one locus was considered alone (Bonferroni correction). As the gametic phase of alleles from the different loci was not known and segregation analysis into families was not carried out, haplotypes and the calculation of their frequency were determined by the likelihood ratio test, through the Arlequin statistical software program [17]. Hardy-Weinberg equilibrium [18] was achieved by calculating the expected genotype frequencies and comparing them to the observed values, also using Arlequin statistical software program [17]. To exclude smoking as a predisposing factor statistical analyses were performed in the total sample (smokers, ex-smokers, and nonsmokers: patientsversus control) as well as in nonsmoking patients versus nonsmoking controls. Only the haplotypes with frequencies above 1.0% were considered in this study.

3. Results

The study involved 259 individuals, 108 of whom were patients with CP, while 151 controls were healthy individuals. At the time of sample collection, the ages of the patients and controls ranged between 34 and 81 years (47.22% males and 52.78% females by patients and 35.10% males and 64.90% females by controls). Regarding the ethnic background, 59.26% of the patients with CP were reported to be Caucasians, 27.78% racially mixed, and 12.96% Afro-Brazilian. Concerning the control individuals, 69.54% were reported to be Caucasians, 23.84% racially mixed, and 6.62% Afro-Brazilian. No differences were observed between patients and controls according to gender, age, and ethnicity. Smoking was associated with CP and was more frequent in patients than in

Table 1: Characteristics of patients with chronic periodontitis and controls.

Category	Subcategory	Patients ($N = 108$) n (%)	Controls ($N = 151$) n (%)	Total ($N = 259$) n (%)
Gender	Female	57 (52.78)	98 (64.90)	157 (60.62)
	Male	51 (47.22)	53 (35.10)	102 (39.38)
Age	34–49 years	65 (60.18)	97 (64.24)	162 (62.55)
	50–65 years	35 (32.41)	50 (33.11)	85 (32.82)
	66–81 years	8 (7.41)	4 (2.65)	12 (4.63)
Ethnic background	Caucasian	64 (59.26)	105 (69.54)	169 (65.25)
	Afro-Brazilian	14 (12.96)	10 (6.62)	24 (9.27)
	Racially mixed	30 (27.78)	36 (23.84)	66 (25.48)
Smoking	Smoker (**A**)	23 (21.30)	17 (11.26)	40 (15.44)
	Nonsmoker (**B**)	46 (42.59)	108 (71.52)	154 (59.46)
	Ex-smoker (**C**)	39 (36.11)	26 (17.22)	65 (25.10)

B versus **A**: $P \leq 0.002$; OR = 0.32; 95% CI = 0.14–0.68.
B versus **C**: $P \leq 0.001$; OR = 0.29; 95% CI = 0.15–0.54.
A versus **C**: $P = 0.84$.

Table 2: HLA associations between chronic periodontitis patients and controls.

HLA	Total*					Nonsmokers**				
	Patients $N = 216$ n (%)	Controls $N = 302$ n (%)	P	P_c	OR (95% CI)	Patients $N = 92$ n (%)	Controls $N = 216$ n (%)	P	P_c	OR (95% CI)
A*02	49 (22.7)	86 (28.5)				17 (18.5)	66 (30.6)	0.035	0.63	0.52 (0.26–0.97)
A*32	3 (1.4)	15 (5.0)	0.030	0.45	0.27 (0.05–0.97)	1 (1.1)	15 (6.0)			
B*40	16 (7.4)	8 (2.6)	0.018	0.38	2.93 (1.16–8.07)	9 (9.8)	7 (3.2)	0.025	0.60	3.22 (1.03–10.55)
DRB1*08	17 (7.9)	11 (3.6)	0.048	0.58	2.26 (0.97–5.45)	8 (8.7)	8 (3.7)			
DQB1*06:01	0 (0)	7 (2.3)	0.045	0.63	0 (0–0.96)	0 (0.0)	5 (2.3)			
DQB1*06:09	5 (2.3)	0 (0.0)	0.012	0.17	undf (1.29–undf)	0 (0.0)	0 (0.0)			

undf = undefined.
*Total group (smokers, nonsmokers, and ex-smokers patients and controls).
**Nonsmoking group (patients and controls).

controls ($P = 0.043$; OR = 2.12; 95% CI = 1.02–4.51) as well as in ex-smokers compared to controls ($P = 0.001$, OR = 2.7; 95% IC = 1.46, 5.05) (Table 1).

In all groups, the distribution of HLA genotypes was confirmed to be in Hardy-Weinberg equilibrium ($P \geq 0.05$ in the patients and in the controls).

The most common HLA types were the following: HLA-A*01, *02, *03, and *24 and HLA-B*15, *35, *44, and *51, similar to other previous reports in the north/northwest region of the State of Parana [19]; this was an important control to demonstrate that the population was representative.

Table 2 presents the HLA classes I and II group allele frequencies distribution only for the significant differences between patients and controls. When comparing the HLA class I frequencies between CP and controls, HLA-A*32 had a lower frequency in patients with CP (smokers, nonsmokers, and ex-smokers) and HLA-A*02 in nonsmokers group; HLA-B*40 had a higher frequency in CP total patients and also in nonsmokers patients group; no significant differences were found for HLA-C allelic groups. In relation to class II, HLA-DRB1*08 allelic group and DQB1*06:09 were overrepresented in patients compared to controls, and HLA-DQB1*06:01 was less frequent in patients compared with controls; however, neither of these associations was confirmed in the nonsmoker group.

Differences between CP and controls were found comparing the HLA genotypes frequencies for homo- and heterozygosis. HLA-C*03-C*06 genotype was increased in CP, whereas HLA-DQB1*03:01 homozygous genotype and DQB1*03:01/DQB1*03:02 were significantly less frequent in CP (Table 3). After Bonferroni correction, the associations lost the significance.

Table 3: HLA genotype frequency associations between chronic periodontitis patients and controls*.

HLA genotypes	Patients $N = 108$ n (%)	Controls $N = 151$ n (%)	P	P_c	OR (CI 95%)
C*03-C*06	4 (4.6)	0 (0)	0.012	0.56	undf (1.31–undf)
DQB1*03:01-DQB1*03:01	0 (0.0)	7 (4.6)	0.044	1.00	0 (0–0.95)
DQB1*03:01-DQB1*03:02	0 (0.0)	7 (4.6)	0.044	1.00	0 (0–0.95)

undf = undefined.
*Total group (smokers, nonsmokers, and ex-smoking patients and controls).

Table 4: HLA haplotype associations between chronic periodontitis patients and controls*.

HLA haplotypes	Patients $N = 216$ n (%)	Controls $N = 302$ n (%)	P	P_c	OR (95% CI)
A*02/B*40	**9 (4.2)**	**0 (0.0)**	**<0.001**	**0.03**	undf (2.83–undf)
A*03/B*51	5 (2.5)	0 (0.0)	0.012	0.36	undf (1.29–undf)
A*26/B*38	5 (2.3)	0 (0.0)	0.012	0.36	undf (1.29–undf)
B*40/C*03	12 (5.6)	5 (1.7)	0.022	0.62	3.49 (1.12–12.83)
B*18/C*12	4 (1.9)	0 (0.0)	0.03	0.93	undf (0.93–undf)
B*51/C*01	4 (1.9)	0 (0.0)	0.30	0.93	undf (0.93–undf)
B*15/DRB1*11	**0 (0.0)**	**13 (4.3)**	**0.001**	**0.04**	undf (0–0.45)
B*40/DRB1*07	6 (2.8)	0 (0.0)	0.005	0.19	undf (1.67–undf)
B*50/DRB1*04	4 (1.9)	0 (0.0)	0.030	1.14	undf (0.93–undf)
DQA1*01:02/DQB1*06:09	5 (2.3)	0 (0.0)	0.012	0.23	undf (1.30–undf)
DRB1*13/DQA1*01:02/DQB1*06:09	5 (2.3)	0 (0.0)	0.012	0.25	undf (1.29–undf)

undf = undefined.
*Total group (smokers, nonsmokers, and ex-smoking patients and controls).
Bold font: significant after Bonferroni correction.

Regarding haplotype frequencies (Tables 4 and 5) patients with CP had significantly higher frequencies for HLA-A*02/HLA-B*40 and a lower frequency for HLA-B*15/HLA-DRB1*11. Furthermore, patients with CP (only total samples) had higher haplotypes frequencies for B*18/C*12, B*51/C*01, B*50/DRB1*04, DQA1*01:02/DQB1*06:09, and DRB1*13/DQA1*01:02/DQB1*06:09 (Table 4); for nonsmokers B*44/DRB1*07, B*35/DRB1*11, and B*14/DRB1*01 were more frequent and HLA-A*02/HLA-B*35 was less frequent in patients compared to controls (Table 5); A*03/B*51, A*26/B*38, B*40/C*03, and B*40/DRB1*07 (Tables 4 and 5) had higher frequencies for both groups, although for all of them the significance was lost after Bonferroni correction.

4. Discussion

While many studies have shown associations of HLA polymorphisms with aggressive periodontitis in different populations [8, 10–14, 20–24] there are few studies of HLA association with CP and the results are inconsistent [9–11, 20, 25–27]. To the best of our knowledge, this is the first study of HLA association with CP in the Brazilian population. Our results provide evidence that HLA classes I and II are associated with chronic periodontitis.

In general, discrepancies in results are observed in HLA case-control studies, and these discrepancies could be caused by differences in the choice of controls, disease diagnostic, racial background, and statistical analyses [28]. In this study, in order to avoid bias in the final results, the control population was selected after clinical examination with the same criteria used for patient exclusion, such as gestation and manifestations of infectious disease or known medical conditions that may contribute to development of CP. Patients with aggressive periodontitis were also excluded. Other confounding variables as gender, age, and ethnicity were considered. Gender and age ratio was similar between patients and controls. Reichert et al. [20] warned against the fact that gender could represent a confounding variable that should be considered in HLA and periodontitis studies; however, as HLA antigen expression has been reported to not vary as a function of gender [29, 30], this expression was probably not taken into consideration in many of the earlier studies on HLA association for periodontal diseases [10, 11, 13, 27, 31]. Age was not related to HLA expression but can be related to the disease, although severity and prevalence were mostly related to past disease history, social and behavior factors [32], and altered inflammatory responses [33].

Important to emphasize is that HLA varies according to population and ethnic group and that Brazil has an admixed

Table 5: HLA haplotype associations between nonsmokers, chronic periodontitis patients, and controls.

HLA haplotypes	Patients $N = 92$ n (%)	Controls $N = 216$ n (%)	P	P_c	OR (95% CI)
A*02/B*40	4 (4.3)	0 (0.0)	0.008	0.23	undf (1.58–undf)
A*02/B*35	0 (0.0)	11 (5.1)	0.038	1.00	0 (0–0.91)
A*03/B*51	3 (3.3)	0 (0.0)	0.026	0.75	undf (0.98–undf)
A*26/B*38	3 (3.3)	0 (0.0)	0.026	0.75	undf (0.98–undf)
B*40/C*03	7 (7.6)	4 (1.9)	0.019	0.51	4.34 (1.07–20.75)
B*44/B*07	4 (4.3)	1 (0.5)	0.029	0.78	9.69 (0.94–482.15)
B*15/DRB1*11	0 (0.0)	11 (5.1)	0.038	1.00	0 (0–0.91)
B*40/DRB1*07	4 (4.3)	1 (0.5)	0.029	0.81	9.69 (0.94–482.15)
B*35/DRB1*11	4 (4.3)	1 (0.5)	0.029	0.81	9.69 (0.94–482.15)
B*14/DRB1*01	4 (4.3)	0 (0.0)	0.008	0.22	undf (1.58–undf)

undf = undefined.

population. The population in Parana has been well defined regarding ethnicity and HLA distribution: in accordance with HLA phenotypic classification, the white population (majority) is predominantly of European origin (80.6%), with a smaller contribution of African (12.5%) and Amerindian (7.0%) genes [34]. Thus, regarding ethnic backgrounds, periodontitis patients and controls were similar, not representing a confounding variable. For this reason added to the high polymorphism of HLA, stratification by ethnicity was not realized.

In this study, as expected, smoking was associated with CP and having stopped smoking maintained susceptibility to disease. As smoking is a risk factor for the onset and progression of CP and the habit can obscure genetic risk factors [35] we analyzed nonsmoking patients and nonsmoking controls in addition to the total group (smokers, ex-smokers, and nonsmokers).

In the current study some trends to associations of HLA class I antigens with CP were found. HLA-A*32 was less frequent in total samples and HLA-A*02 was less frequent in nonsmoker patients representing possible protection to CP; however, significance was lost after Bonferroni correction. Added to these results, the HLA-A*02/B*35 haplotype was significantly less frequent in nonsmoker patients. Similar to our results, pioneering and recent investigations involving periodontitis and HLA have also found HLA*02 associated with protection to periodontitis [25, 27, 36, 37]. Thus, our results indicate a protective role of HLA-A*02. A2 could yield an efficient antimicrobial T-cell response reducing the disease [25].

Otherwise, HLA-B*40 was more frequent in both groups representing a risk effect against CP, although significance was also lost after Bonferroni correction. However, HLA-A*02/B*40 haplotype was significantly associated with susceptibility to CP. Added to this result, HLA-B*40/C*03 and HLA-B*40/DRB1*07 haplotypes tend to be associated with CP susceptibility. As HLA-A*02/B*40 was in linkage disequilibrium ($\Delta' > 0.70$, for all analyzed population: patients and controls) and HLA-A*02 has been associated as a protection factor, the susceptibility found for patients with CP may be related to HLA-B*40. Individuals carrying B*40 (B60 and B61 antigens) had a three-fold higher risk of developing the disease. Contrarily, in another reporter, HLA-B*40 was associated with a lower clinical attachment loss in CP; however, this association was lost after Bonferroni correction [31].

Taking into account other haplotypes that were significantly associated in our population, the HLA-B*15-DRB1*11 combination must be highlighted conferring protection against CP in the total group of patients. HLA-B*15-DRB1*11 was in linkage disequilibrium ($\Delta' > 0.80$ for all analyzed population). HLA-B*15 could be associated with the disease: no associations between DRB1*11 and CP have been previously reported; however, in agreement with our results, Mauramo et al. [31] found that patients expressing HLA-B*15 had less clinical periodontal disease manifestation and better periodontal health over a long period of time. HLA-B*15 positive individuals might have a somewhat peculiar genetic response towards periodontal bacteria challenge contributing to CP development [31].

Another haplotype that lost the significance after Bonferroni correction in our study but must be highlighted was HLA-B*50/DRB1*04. This haplotype was a susceptibility factor to CP in our population and it is possible that the effect was associated with DRB1*04. According to previous reports, and in agreement with our results, HLA-DRB1*04 was associated with risk to CP [38, 39] and aggressive periodontitis [9, 12, 23, 30], as well as their alleles [8, 30]. DRB1*04:04 was considered a risk factor for bone loss [30, 40]. Contrarily, in a recent investigation, HLA-DRB1*04 or HLA-DRB1*04/DRB4*(DR53)/DQB1*03:02 haplotype had a decreased colonization risk of *Aggregatibacter actinomycetemcomitans* [9]. DRB1*04 was an immunogenetic susceptibility factor for type 1 diabetes [41] and for rheumatoid arthritis [30]; however, both diseases were included in our exclusion criteria.

According to other association studies between periodontitis and HLA, a meta-analysis focusing on Caucasian case-control studies conducted by Stein et al. [25] demonstrated no associations between HLA and CP, although for

aggressive periodontitis HLA-A*09 and B*15 appeared to represent susceptibility factors and HLA-A*02 and B*05 were potential protective factors. Other HLA-A, HLA-B, and HLA-C associations with periodontal disease previously described in the literature were as follows: susceptibility mediated by HLA-A*11, A*29, B*14, and C*08 and protection by HLA-A*03, HLA-A*31 and -A*30/A*31 genotype observed in German patients with CP [11] and HLA-B*57 as a protecting factor in German patients with CP or generalized aggressive periodontitis [9]; HLA-A*02 and HLA-B*05 associated with protection in the CP patients from USA [27, 36, 37]; HLA-A*09 positively associated with CP from France [26]; and HLA-B*51 related to fewer deep periodontal pockets in the CP in Swiss adults. HLA-DQA1*03 and HLA-DRB1*04 were higher and HLA-DQB1*06:03 might have protective effects against aggressive periodontitis in Iranian patients [8].

A possible role of HLA in the immune response and development of periodontal disease may be related to their ability to bind some processed peptides from bacteria antigens and expressing them on the surface of antigen presenting cells (peptide-HLA class II) or target cells (peptide-HLA class I) in order to present them to T cells (CD4 or CD8) [42]. The binding capacity of the bacteria peptide depends on HLA allotypic structure of their paratope. Failures in this link capacity can compromise the immune response and could be a risk of disease. The large individual capacity of immune response occurs due to the fact that HLA is highly polymorphic: each gene has multiple alleles and each individual has many expressed genes. These preliminary results indicate that HLA haplotypes might be involved in the susceptibility or risk for periodontal disease. However, further investigations of HLA haplotypes markers in relation to antigenic peptide-binding motifs are necessary in order to understand its relation in periodontitis.

The weaknesses of the case-control studies were related to the reproduction of results in function of ethnicity background of the individuals, sample size, diagnostic tolls of disease, and HLA genotyping methods. Consequently, no strong association could be found, especially in CP. In addition, CP is a complex and multifactorial disease and several other genetic polymorphisms have been reported to be associated with bacterium response, inflammation, chronicity, and wide ranging systemic effects and disease development, such as *IL1, IL4, TNF, IL8*, and *IL10* cytokines genes, immunoglobulin G Fc receptor (FcyR), and TLR [35, 43–60]. Despite this fact, all information regarding genetic susceptibility to diseases is valuable and can be applied to therapy intervention or individual approach. For better results, we have tried to be very critical in the choice of the study population. CP inclusion criteria and CP and control exclusion criteria were defined in order to avoid confounding factors and were a representative of generally healthy and CP adults. As independent multiple comparisons were carried out and we considered that all genotypes examined had the same chance of being increased or decreased in CP, Bonferroni correction was applied for all significant P values.

The present study has potential limitations. We focused on the HLA allele group, not on alleles and epitopes analysis, and split antigens were not investigated. The major limitation of this study is the relatively small sample size regarding the analysis of the nonsmoker group.

In these Brazilian CP patients, the HLA-A*02/B*40 haplotype was considered a risk factor for CP and HLA-B*15/DRB1*11 haplotype was considered a protective factor for disease. Susceptibility may possibly be associated with B*40 molecules and protection may be associated with B*15 and A*02. Further investigation relating antigenic peptide-binding motifs and their immunopathogenesis involving CP development should be investigated.

5. Conclusion

These results provide evidence that class I and II HLA polymorphisms are associated with chronic periodontitis. HLA-A*02/B*40 haplotype seems to represent susceptibility factors and HLA-B*15/DRB1*11 haplotype was potential protective factors against disease.

Conflict of Interests

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

Authors' Contribution

Emília Ângela Sippert, Christiane Maria Ayo, and Silvia Barbosa Dutra Marques carried out the molecular genetic studies, Cléverson de Oliveira e Silva participated in the clinical diagnostic of chronic periodontitis, Ana Maria Sell and Jeane Eliete Laguila Visentainer designed and coordinated the study, and Emília Ângela Sippert, Ana Maria Sell, and Jeane Eliete Laguila Visentainer wrote the paper. All authors read and approved the final paper.

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