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Salivary cytokines as biomarkers of periodontal diseases

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(Guest Editors: John J. Taylor & Philip M. Preshaw)
Abstract

Research into biomarkers of periodontitis is driven by mainly three targets: to identify “at risk” patients before periodontal tissue destruction occurs, to determine disease activity and progression, and to build up our understanding of this complex disease with the purpose of finding new therapeutic targets. Whilst previously blood and gingival crevicular fluid were the biological samples of choice, recently saliva has gained more attention as a readily accessible oral fluid and which has a mediator profile similar to that of serum and gingival crevicular fluid. The aim of this paper is to give a comprehensive overview of salivary cytokines in periodontitis, highlighting extensively studied cytokines such as interleukin-1β and interleukin-6, but also cytokines which only have been the subject of few studies and which warrant further investigation. Cross-sectional and longitudinal studies of salivary cytokines and the potential of cytokines as periodontitis biomarkers are evaluated. Finally, a discussion of potential confounding factors such as concurrent systemic diseases and smoking is presented.
Periodontal disease is time consuming and expensive to treat and therefore its prevention, early detection and management are issues which, if effectively addressed, are likely to yield considerable healthcare benefit [97]. However, despite numerous advances in our understanding of the pathogenesis of chronic inflammatory diseases, periodontitis is still only diagnosed once connective tissue and bone destruction has occurred. Furthermore, monitoring disease progression is a highly skilled and technically demanding process involving measurement of bleeding on probing, probing depth and attachment loss coupled with radiographic assessment and (subjective) visual observations [76]. The development of biomarkers for early detection of periodontal disease and to identify progression would be highly desirable as current diagnostic approaches do not reflect current disease activity, but simply assess the cumulative effects of historical tissue destruction [53]. Rational diagnosis would also have concomitant patient benefit as the paucity of evidence-based knowledge of disease progression in individual patients may lead to unintentional clinical mismanagement [97]. In addition, studies of the salivary mediators associated with disease may help in the development of novel therapies aimed at controlling cytokine bioavailability (e.g. through anti-cytokine antibodies, antagonists or soluble receptors) or targeting the intra-cellular signalling pathways they activate, approaches which have been successful in the treatment of other chronic inflammatory diseases such as rheumatoid arthritis [60, 91, 95].

Cytokines have been defined as soluble factors produced by one (immune) cell that act on another cell within the same milieu [26]. However, it is now recognised that the range of molecules with cytokine-like activity can be extended to include, for example growth factors and adipokines which also have immunoregulatory functions. Importantly, cytokine functions often overlap or merge, building a complex immunoregulatory network in the immune system that is often perturbed in disease. It is increasingly appreciated that
cytokines have vital roles in the development and homeostasis of numerous cell types and, in a wide range of tissues, have roles in resolution of inflammation, wound healing, repair and regeneration. In the following review, the term “cytokine” will be used in this broad context. In addition to direct analysis of cytokines, the levels of molecules such as matrix metalloproteinases and tissue inhibitors of metalloproteinases which are regulated by cytokines have also been given considerable attention as potential periodontitis biomarkers, as reviewed elsewhere in this issue.

**Cytokines and periodontal diseases**

It is now widely accepted that although the initiating factors in gingivitis and periodontitis are the microbial elements of the dental plaque biofilm, the pathogenesis and concomitant tissue destruction is driven by the development of a chronic, inflammatory host immune response, the nature and extent of which are fundamental determinants of susceptibility and progression. The function of immune cells and periodontal tissue cells in orchestrating periodontal disease pathogenesis is underpinned by the functional diversity of cytokines [56, 79]. The earliest cytokine studies in periodontal research can be dated back to the 1980s [20, 62] and coincide with the detailed characterisation of interleukin-1, interleukin-2 and tumor necrosis factor-α [26]. These experiments showed increased thymocyte proliferation induced by gingival crevicular fluid from inflamed sites compared to non-inflamed sites, which was presumed to be due to cytokine-like activity, although the precise molecular nature of the mediators remained to be determined [20, 62]. With the establishment of enzyme-linked immunosorbant assays, interleukin-1 β was the first cytokine to be specifically measured in the gingival tissue of patients with periodontal
disease [44]; since then, many publications have reported the measurement of cytokines in periodontal tissues, periodontal cell culture supernatants and oral fluids [17, 56, 79, 102].

While early studies focused on serum and gingival crevicular fluid samples for investigations of periodontitis-related cytokines, saliva has come into focus as an alternative source of biomarkers in recent years [17, 102, 115]. Saliva is made up of the exocrine secretions of the salivary glands in addition to gingival crevicular fluid, microbial components and food remnants [10, 38]. Analysis of saliva, similar to gingival crevicular fluid, gives a better representation of the local pathological changes in the mouth than analysis of serum. However, saliva has several advantages over gingival crevicular fluid, as it is more easily accessible than gingival crevicular fluid, can be sampled in a much larger volume without the need for clinical facilities and there are no complex skills necessary for saliva sampling. Furthermore, whereas gingival crevicular fluid content reflects inflammatory processes at individual disease sites, it is reasonable to suggest that saliva content reflects a consensus ‘whole of mouth’ inflammatory status, which is likely to be much more clinically relevant.

The “Biomarkers Definitions Working Group” defines a biomarker as: “A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” [12]. Individual biomarkers should be discriminatory and robust (see below). Also, the utility of a biomarker needs to be assessed using appropriate study design and methodology [57]. Most studies are simply designed to be cross-sectional, comparing the levels of cytokines in groups of healthy volunteers and periodontal disease patients. Superficially, such studies merely report associations of biomarkers with the presence of disease and therefore test
whether or not a particular marker is discriminatory. Many, but not by any means all, studies attempt statistical correlation with clinical parameters of periodontitis such as clinical attachment loss and probing depth; this approach is weakened by the knowledge that these parameters may merely reflect historical disease activity rather than contemporary processes. Cross-sectional studies do not indicate whether or not a particular salivary biomarker mirrors disease progression and/or response to treatment. Studies of longitudinal changes in salivary biomarkers to date have been rather limited and have used highly variable clinical protocols [17, 102]. Although these studies help identify candidate disease markers and many have replicated data derived from studies of gingival crevicular fluid, the majority of studies relate to a limited number of popular biomarkers and a significant number of studies have not been reproduced independently, at least using comparable methodologies [102]. It has also been noted that many clinical research studies of periodontal disease fail to report examiner calibrating procedures for the measurement of parameters such as probing depth, attachment loss or bleeding on probing [42]. The lack of uniformity in periodontitis definitions is another factor that makes comparisons of different studies challenging [78]. Another potential flaw in study design is the quality of the methodology to assess cytokine concentrations in saliva. Enzyme-linked immunosorbant assays clearly have the advantage in terms of high throughput and high reproducibility. Most research studies use commercially available enzyme-linked immunosorbant assays kits for sample analysis, however the efficacy of these kits in measuring mediators in saliva has not been reported in any study; most assays have been optimised by the manufacturers for use with serum samples. Failing to validate an enzyme-linked immunosorbant assay for its suitability for use with saliva samples can lead to erroneous results and could partly explain inconsistencies in findings between different studies; for example we have noted same
assays exhibit poor sample recovery from saliva and poor inter-assay variation for measurement of salivary mediators [47]. Similar data have been reported in a study comparing the performance of multiplex cytokines assays although recovery can, in some cases, be improved by sample pre-treatment [15].

Concerns regarding the widespread adoption of inappropriate statistical approaches in biomedical research have been raised in the literature [30, 72]. Our review of the literature on cytokine biomarkers for periodontal disease (see tables) revealed that whilst the majority of studies apply correct statistical analysis procedures, there remain not infrequent examples of statistical errors: e.g. the use of parametric tests (e.g. t-tests) when data are not parametric; the use of dependent samples tests when data are independent (for example paired samples t-tests instead of independent samples t-test); inappropriately using a mixture of parametric and non-parametric tests (for example, performing an analysis of variance followed by Mann-Whitney-U comparisons). In addition, few studies report a formal power calculation for sample size and likely many of the reviewed studies are underpowered. Furthermore, although most studies did take normal distribution of their sample groups into account, many used the Kolmogorov–Smirnov test to assess normal distribution and only a minority of studies used the Shapiro-Wilk test. This is of concern as the Kolmogorov–Smirnov test is valid for a sample sizes of n>2000 whereas the Shapiro-Wilk test of normal distributions is valid for a smaller sample sizes and has superior statistical power [83]. Some studies have reported data from receiver-operator characteristic analysis [29, 80] which establishes the efficacy of biomarker analysis in identifying individuals in an unselected population sample with or without a particular condition such as periodontitis by comparing sensitivity and specificity of a particular technique [112]. The value of receiver-
operator characteristic statistics clearly depends on the application of the biomarker analysis: it has value to confirm the robustness of an approach to identifying disease (e.g. for use by untrained workers in the field) but does not indicate whether the test outcome is relevant for disease progression which is of greater interest in disease management. Clearly, much remains to be done to improve study designs, analysis, and reporting in studies of biomarkers levels in periodontal diseases.

**Salivary cytokines in oral health and periodontal disease**

**Interleukin-1 family**

The interleukin-1 family of cytokines is defined on the basis of structural similarity and comprises 11 members, further divided into 3 sub-families: the interleukin-1 sub-family (interleukin-1\(\alpha\), interleukin-1\(\beta\), \(\beta\) interleukin-36 receptor antagonist), the interleukin-18 sub-family (interleukin-18, interleukin-37) and the interleukin-36 sub-family (interleukin-36\(\alpha\), interleukin-36\(\beta\), \(\beta\) interleukin-36 receptor antagonist, interleukin-36 receptor antagonist) [36, 109]. The interleukin-1 cytokines are produced by a wide variety of cell types and perform critical functions in innate and adaptive immune responses to infection [36, 109]. In terms of pathogenesis, the interleukin-1 cytokines are generally pro-inflammatory but interleukin-36Ra have antagonistic functions [36, 109].

Interleukin-1\(\beta\) is the prototypical interleukin-1 cytokine and as such is the most extensively studied cytokine in clinical studies of salivary biomarker in periodontal disease. Interleukin-1\(\beta\) has a multitude of immunological functions including activation of neutrophils, T- and B-cells during infection as well as mediating liver acute phase protein release and febrile responses (reviewed in [27]). In periodontitis, interleukin 1\(\beta\) is associated
with neutrophil recruitment and activation of osteoclasts through its ability to induce chemokines and activate osteoclasts [79]. Furthermore, the association between periodontitis and elevated interleukin-1β in gingival crevicular fluid is well established [7, 17]. In case control studies, 9 out of the 12 studies show a significantly higher interleukin-1β level in periodontitis compared to healthy controls (Table 1). In other studies (n=3), interleukin-1β levels are significantly correlated with clinical parameters of periodontitis (Table 1). In addition, there are consistent findings from 3 independent studies that levels of salivary interleukin-1β decrease after periodontal treatment (Table 1). Interleukin-1β concentrations in saliva are not affected by systemic conditions such as diabetes or smoking (see below). The potential of salivary interleukin-1β to discriminate between periodontal health and disease with high specificity and sensitivity in receiver operator curve analysis has been investigated but with conflicting results [29, 80]. It is noteworthy that there is considerable variation in the levels of salivary interleukin-1β reported; levels in health range from an average of 7.24 ± 7.69 pg/ml [29] to as much as 633.91 ± 91.0 pg/ml [103] and levels in periodontitis patients range from an average of 90.94 ± 85.22 pg/ml [29] to 1312 ± 691.22 pg/ml [50]. Notwithstanding differences in patient selection criteria, these findings make precise comparisons between studies challenging. Thus, it would seem there is substantial but not unanimous support for the notion that salivary interleukin-1β is a robust choice of analyte for studies of periodontal disease biomarkers.

Only a limited number of studies have investigated salivary concentrations of other members of the interleukin-1 family of cytokines in periodontitis. Interleukin-1α has been detected in saliva but the levels are not altered in periodontitis [31]. Although interleukin-1 receptor antagonist has well-defined antagonist roles in immunoregulation [36, 109], there are currently no reports of the investigation of salivary interleukin-1 receptor antagonist in
periodontitis. Interleukin-18 is secreted by a number of cell types (myeloid cells, keratinocytes, epithelial cells) and by activating neutrophils and Th1 cells potentially plays an important role in periodontal inflammation (reviewed in [7]). Increased concentrations of interleukin-18 were found in the saliva of periodontitis patients compared to healthy controls [74]. Salivary concentrations of interleukin-33, an interleukin-1 family member that drives Th2 cytokine responses, appear to remain unaffected by periodontitis [18] (Table 1). Although the role of other, newly identified members of the interleukin-1 family in chronic inflammatory diseases is receiving increasing attention [79] nothing is known about salivary concentrations of these novel cytokines.

**Tumor necrosis factor-α**

The tumor necrosis factor-α superfamily comprises some 19 ligands identified on the basis of gene sequence homology [1]. A survey of the various physiological roles of these ligands reveals wide-ranging roles in cell proliferation, apoptosis and morphogenesis as well as host immune defence [1]. However, with the exception of tumour necrosis factor-α and receptor activator of nuclear factor kappa-B ligand (see below) there is little or no information about the potential of the tumour necrosis factor superfamily members as biomarkers of periodontitis. Tumor necrosis factor-α is a key pro-inflammatory cytokine and central to anti-microbial immunity at a number of levels. The fundamental physiological role of this cytokine beyond the immune system is now established and tumor necrosis factor-α has numerous homeostatic functions in human physiology including a role in neurological regulation [91]. Tumor necrosis factor-α is produced mainly by macrophages as a primary response of Toll-like receptor signalling, by activated T cells and NK cells and also by non-immune cells including endothelial cells and fibroblasts [91]. The long-standing success of
anti-tumor necrosis factor-α therapy in rheumatoid arthritis has heightened interest in this important cytokine in inflammatory diseases such as periodontitis.

Tumor necrosis factor-α and receptor activator of nuclear factor kappa-B ligand both belong to the tumour necrosis factor superfamily, and both are potent activators of osteoclasts and thereby mediate bone resorption [39]. Some 7 studies have investigated salivary tumour necrosis factor-α in periodontal health and disease (Table 2) however, tumor necrosis factor-α is either undetectable or the levels detected in saliva are very low. Frodge et al [33] reported significantly higher salivary tumour necrosis factor-α concentrations in periodontitis compared to periodontal health using a highly sensitive detection methodology, but the levels detected in the different patient groups were nonetheless very low (<4.3pg/ml). Although after periodontal treatment salivary tumor necrosis factor-α concentrations were reported to decrease [92] there is no evidence that salivary tumour necrosis factor-α levels correlate with clinical changes in the periodontium either during disease progression [89] or after treatment [92]. Currently, therefore, it seems unlikely that salivary tumor necrosis factor-α can be regarded as a good candidate biomarker for periodontal disease.

**Interleukin-6**

Interleukin-6 is produced by innate immune cells such as macrophages and dendritic cells but also by some CD4+ T-cells as well as non-immune cells such as fibroblasts and endothelial cells [84]. Interleukin-6 is elevated in many inflammatory diseases and mainly functions to activate B-lymphocytes in addition to having a role in influencing the balance of CD4+ effector T-cell populations and potentially influencing myeloid cell differentiation [84]. Interest in interleukin-6 has recently increased as anti-interleukin-6 blockade has proven to
be efficacious in the treatment of rheumatoid arthritis [48, 84]. Levels of interleukin-6 measured in saliva are low in both periodontal health and disease [29]. Also, while some studies have shown that salivary interleukin-6 concentrations are significantly higher in periodontitis than in healthy individuals, others found no differences between the two groups (Table 2). Reports on post-treatment evaluations of salivary interleukin-6 concentrations showed no significant changes [54, 77]. Therefore, interleukin-6 is not a robust salivary periodontitis biomarker. Interleukin-6 is a member of a family of cytokines which have overlapping biological functions and binding receptors which share a common subunit (gp130); these include leukaemia inhibitory factor, interleukin-11 and oncostatin M [84] although these mediators are yet to be assessed as potential salivary biomarkers for periodontal disease.

**Interleukin-4**

Interleukin-4 is mainly produced by T-cells, specifically the Th2 subset of T-cells and has important functions in the regulation of cell proliferation and apoptosis in numerous cell types, including lymphocytes, myeloid cells, fibroblasts and epithelial cells [59]. Interleukin-4 is a critical mediator in regulating T-cell development and plasticity in periodontal disease [32]. However, interleukin-4 is only present at very low concentrations in saliva and findings relating to salivary concentrations of interleukin-4 in periodontitis are inconsistent: one study reported significantly higher interleukin-4 concentrations in periodontitis [77] and two studies find no differences in salivary interleukin-4 concentrations between periodontal health and disease [80, 103]. Similarly, although one study reported a treatment-related reduction in salivary interleukin-4 levels [77], other studies do not report any evidence for post-treatment changes in salivary interleukin-4 in periodontitis patients [31, 89]. Therefore
interleukin-4 cannot, as yet, be considered a viable salivary biomarker for periodontal disease.

**Interleukin-10**

The interleukin-10 family of cytokines has some nine members of which interleukin-10 itself is the most studied [73, 88]. Interleukin-10 is widely produced by innate and adaptive immune cells alike and is a key anti-inflammatory cytokine, involved in inhibiting and regulating pro-inflammatory immune responses and promoting resolution of inflammation [73]. However, data from clinical studies have been inconsistent (Table 2): one study reported significantly lower levels in periodontitis compared to controls [77] and others have found no differences in salivary interleukin-10 concentrations between periodontal health and disease [80, 103] (Table 2). Periodontal treatment does not appear to affect salivary interleukin-10 concentrations [54, 77] and initial levels do not correlate with disease progression [89]. Thus, evidence from clinical studies does not support the notion that interleukin-10 is a useful salivary biomarker in periodontitis.

**Interleukin-17**

Since the discovery of the interleukin-17 secreting T-cell subset Th17, the dogma of the central importance of Th1 cytokines driving the “early”, stable and reversible gingivitis lesions versus Th2 cytokines driving the “later”, progressive periodontitis lesions has been challenged [34]. Interleukin-17 is produced by Th17 cells after activation of the adaptive immunity and serves to reinforce pro-inflammatory immune responses [35]. Although salivary interleukin-17 concentrations are minute in both periodontal health and disease [74], two studies comparing interleukin-17 in periodontal health and disease reported significantly lower concentrations in the latter [74, 77] (Table 2). However, salivary
interleukin-17 concentrations did not change after periodontal treatment [77]. In contrast, in a comparative analysis of different IL-17 isoforms, interleukin-17A, interleukin 17E and the interleukin-17A/F ratio was found to be significantly elevated in periodontitis patients [6]. It is currently unclear, therefore, whether interleukin-17 is a suitable periodontitis biomarker, although this seems unlikely given the very low concentrations present in saliva.

Other immunoregulatory cytokines

No differences between salivary concentrations of granulocyte-macrophage colony-stimulating factor, interleukin-2, interleukin-5, interleukin-13 or interferon-γ between periodontal health and disease, or changes after periodontal treatment, have been reported (Table 2).

Chemokines

Chemokines target a wide range of immune cells, including neutrophils, lymphocytes and macrophages to facilitate their recruitment to the site of inflammation, and they are a key player in the pathogenesis of periodontitis [94, 107]. At least human 44 chemokines have thus far been identified and these are divided into 4 families on the basis of structural homology [107]. Chemokines such as macrophage inflammatory protein-1α (CCL3), interleukin-8 (CXCL8) and CXCL10 are produced by oral cells and tissues in response to pro-inflammatory cytokines such as interleukin-1, tumour necrosis factor-α or interferon-γ, and bacterial products such as lipopolysaccharide or fimbriae [8, 79, 94]. Salivary concentrations of the neutrophil chemotactic factor interleukin-8 do not appear to be altered in periodontitis or following periodontitis treatment [81, 92, 103] (Table 2). Significantly higher salivary concentrations of the Th1-cell chemotactic factor macrophage inflammatory protein 1α and the monocyte chemotactic protein-1 (also called CCL2) and have been found in
periodontitis compared to periodontal health [2, 40]. A decrease in salivary macrophage chemotactic protein-1 concentrations was found after periodontal treatment [40]; however, no change was observed for macrophage inflammatory protein-1α [92]. Currently, therefore, only macrophage chemotactic protein-1 seems to hold promise as a potential biomarker for periodontitis, but for the vast majority of chemokines there is no information available on salivary concentrations in periodontitis.

Cytokines involved in bone cell regulation

The receptor activator of nuclear factor kappa-B ligand-osteoprotegerin system has been the focus of many recent investigations in periodontal research. Receptor activator of nuclear factor kappa-B ligand is mainly produced by osteoblasts, fibroblasts and activated T and B cells. When binding to its receptor on pre-osteoclasts, receptor activator of nuclear factor kappa-B ligand induces osteoclast differentiation and bone resorption [39]. The decoy receptor osteoprotegerin is largely produced by fibroblasts and, through binding of receptor activator of nuclear factor kappa-B ligand, inhibits osteoclastogenesis [9]. In addition to roles in bone metabolism, these cytokines influence immune responses, emphasising the emerging paradigm that these two systems share common regulatory factors [9, 39]. Generally, it appears that receptor activator of nuclear factor kappa-B ligand is upregulated and osteoprotegerin is downregulated in periodontal disease [11, 17]. However, the results of the analysis of salivary concentrations of osteoprotegerin and receptor activator of nuclear factor kappa-B ligand in periodontal health and disease are not consistent. Out of eight studies, only three [16, 99, 105] simultaneously measured salivary concentrations of osteoprotegerin and receptor activator of nuclear factor kappa-B ligand (Table 3). Two of these studies showed a significant increase in receptor activator of nuclear factor kappa-B
ligand concentrations in periodontitis compared to periodontal health, but, of these two studies only Tobon-Arroyave et al. [105] noted lower levels of osteoprotegerin in periodontitis. Only one other study [80] recorded lower osteoprotegerin concentrations in periodontal disease as compared to health (Table 3). Two studies have investigated post-treatment salivary concentrations of osteoprotegerin and both found a significant reduction after periodontal treatment [54, 92]. Salivary concentrations of receptor activator of nuclear factor kappa-B ligand have not been investigated after periodontitis treatment to date. Osteoprotegerin and receptor activator of nuclear factor kappa-B ligand show great promise as biomarkers of rheumatoid arthritis [110]. However, salivary concentrations of both osteoprotegerin and receptor activator of nuclear factor kappa-B ligand are relatively small and results from studies of periodontal disease are inconsistent. More data are needed to judge if salivary levels of receptor activator of nuclear factor kappa-B ligand and osteoprotegerin are suitable periodontitis biomarkers. Calprotectin [80] may be elevated in periodontitis but these studies are yet to be independently replicated.

**Growth factors**

So-called growth factors have been regarded as multifunctional, being involved in several physiological processes such as tissue development, regeneration and wound healing. More recently, these molecules have been revealed to have more specific roles in immune responses and inflammatory diseases. The paradigm for this has been transforming growth factor-β that has numerous clearly defined roles in immunoregulation including regulation of T-cell subsets [32]. Although elevated levels of transforming growth factor-β and other growth factors have been reported in gingival crevicular fluid [37] there are only very limited similar studies of saliva (and no available data on salivary transforming growth factor-β in
periodontitis). One exception is hepatocyte growth factor which has been shown to enhance the production of matrix metalloproteinases and stimulates processes of wound healing such as vascularisation and keratinocyte proliferation [28, 61] and, more recently, numerous roles in inflammation and immunity have been revealed [65]. In oral tissues, hepatocyte growth factor is produced by periodontal ligament cells and gingival fibroblasts [71] in response to pro-inflammatory cytokines such as interleukin-1, tumor necrosis factor-α as well as prostaglandin E₂ and bacterial lipoteichoic acid [70, 98]. It is suggested the hepatocyte growth factor may drive apical migration of epithelial cells in periodontitis [49, 70, 71]. Cross-sectional studies investigating salivary concentrations of hepatocyte growth factor consistently report elevated concentrations in periodontal disease compared to periodontal health and 2 longitudinal studies provide further substantial evidence in support of the potential of hepatocyte growth factor as a biomarker for periodontitis (Table 4).

**Adipokines**

Adipokines comprise over 600 biologically active molecules produced by adipose tissue which include a wide range of pro- and anti-inflammatory cytokines (interleukin-1β, tumour necrosis factor-α, interleukin-6, interleukin-10), growth factors (such as vascular endothelial growth factor and transforming growth factor-β), hormones (leptin, adiponectin, visfatin, resistin) and chemokines (monocyte chemotactic protein-1, interleukin-8) [58]. These molecules have wide-ranging and overlapping roles in physiology and there is an increasing recognition of their importance in immune-regulation and inflammatory disease [25, 69]. Many of the aforementioned adipokines are also synthesised by other cell types and are considered in other sections of this review.
The archetypal adipocyte-derived adipokine is leptin, which has well-defined pro-inflammatory functions and is one potential mediator linking metabolic disease with common co-morbidities such as periodontitis and rheumatoid arthritis [22, 69, 101]. However, although there is some information implicating leptin in the pathogenesis of periodontitis there have been no studies of salivary leptin levels. However, the adipokines visfatin and chemerin have been found in significantly higher salivary concentrations in periodontitis compared to periodontal health [75, 100] (Table 5). Interestingly, although there is little direct evidence for a role of these adipokines in periodontitis pathogenesis, they do have relevant biological immunoregulatory functions; for example, visfatin does induces tumour necrosis factor-α, interleukin-6 and interleukin-1β in monocytes and fibroblasts and also acts as a chemoattractant for monocytes and B-cells [14, 66] and chemerin acts as a chemoattractant for various immune cells such as macrophages, dendritic cells and NK cells during inflammation [118].

Identification of cytokine biomarkers from proteomic analysis of saliva

The majority of studies investigating possible biomarkers for periodontal disease have used a ‘candidate’ biomarker approach exploiting existing information about disease pathogenesis. However, the proteome of human saliva has been characterised and is providing information underpinning a global, unbiased analytical approach to biomarker identification, an endeavour enhanced by recent developments in protein separation and mass spectrometry [24, 90]. This approach has been exploited in the identification of biomarkers for oral diseases including Sjogren’s syndrome and oral cancer [45, 46]. Although such proteomic studies have identified elevated levels of two of the S100 family of calcium binding proteins (which have cytokine-like activity in the regulation of
inflammation) [55, 86, 116] problems of sensitivity, recovery and processivity likely explain the limited data from proteomic studies relating to low abundance proteins such as cytokines [102].

**Cytokines as an element of biomarker signature analysis**

It is generally accepted that the immunopathogenesis of periodontal disease is driven by complex and dynamic networks of cytokine interactions, which exhibit considerable inter-individual variation rather than being dominated by the action of individual cytokines [52, 79]. However cross-sectional analysis of multiple cytokines has generally not revealed consistent evidence for a ‘biomarker signature’ (e.g. Table 2). Cytokines are present in significantly lower concentrations in saliva than other mediators, for example enzymes such as matrix metalloproteinases, and therefore subtle changes may not always be detected. Indeed there is some evidence that analytical techniques with greater sensitivity and linear range may unmask periodontitis-associated biomarkers not detected by conventional enzyme-linked immunosorbant assays, which are almost universally used in research in this field [21]. Nonetheless, there have been some encouraging recent studies, which suggest that simultaneous analysis of a combination of interleukin-1β, interleukin-6 and matrix metalloproteinase-8 strongly distinguished periodontitis patients from healthy individuals [29]. Also, differences in periodontal disease severity were efficiently detected by a combination of matrix metalloproteinase-8, matrix metalloproteinase-9, osteoprotegerin and calprotectin assays coupled with quantification of the periodontal pathogens *Porphyromonas gingivalis* and *Treponema denticola* in dental plaque [80]. Further studies revealed the combined levels of plaque bacterial biomarkers and salivary mediators (matrix metalloproteinase-8, matrix metalloproteinase-9, osteoprotegerin and interleukin-1β)
successfully predicted progression of periodontal disease for the majority of patients studied [54].

The intriguing concept that the biomarker tests required to screen for disease susceptibility and those which will be useful for monitoring response to therapy will be different has been raised, although this remains to be systematically investigated [108]. There is a wide variety of multiplex analysis platforms available but, unlike the popular sandwich enzyme-linked immunosorbant assays, no one multiplex approach has been universally adopted. Indeed, concerns about imprecision and analyte recovery for some of these platforms have been highlighted and careful comparison with ‘gold standard’ ELISA methods is recommended before multiplex approaches are used in quantitative analysis of salivary mediators [15]. It is likely that many of these techniques are semi-quantitative at best and might only be considered of use for biomarker discovery.

**The effect of systemic diseases on salivary cytokine concentrations**

Few studies have investigated the impact of systemic diseases on salivary cytokines in periodontitis and none have been designed specifically with this aim in mind. Therefore, one has to be cautious when drawing conclusions from the presented study results. Reports on salivary interleukin-1β concentrations in heart disease, hypertension, diabetes, inflammatory bowel disease or rheumatoid arthritis show no significant differences between patients and systemically healthy controls [64, 81, 117] even though oral health status was not always considered in the analysis [81]. A comparable theme can be seen for salivary interleukin-6 and tumour necrosis factor-α; concentrations of both do not appear to be affected by systemic conditions [3, 23, 64, 81, 96]. An investigation of the salivary concentrations of interleukin-1 receptor antagonist in diabetes revealed lower
concentrations in patients with periodontal disease as compared to those with periodontal health [19]. Since a non-diabetic control group was not included in this study, it is not possible to estimate whether local or systemic conditions influenced the levels of salivary interleukin-1 receptor antagonist. Inflammatory bowel disease was associated with higher salivary interleukin-8 concentrations in one study, however since oral health status was not considered, it is not possible to evaluate local or systemic effects independently [81]. Similarly, an increase in salivary monocyte chemoattractant protein-1 concentrations in Sjögren’s syndrome cannot be accounted for without evaluation of oral health [43]. The same study also investigated salivary macrophage inhibitory protein-1α concentrations and found no differences between patients with Sjögren's syndrome and systemically healthy controls [43].

**The effect of smoking on salivary cytokine concentrations**

Smoking is an established risk factor for periodontal disease [106] and it is reasonable to hypothesise that smoking may influence salivary cytokine levels. Salivary concentrations of cytokines such as interleukin-1β and interleukin-6 do not appear to be affected by smoking although the concentrations of other salivary proteins were lower in smokers [51, 68, 81, 82]. However, decreased concentrations of interleukin-8 in smokers compared to non-smokers have been noted [81, 82]. One study found no association between receptor activator of nuclear factor kappa-B ligand or osteoprotegerin concentrations and smoking [105]. However, in contrast, higher receptor activator of nuclear factor kappa-B ligand and lower osteoprotegerin concentrations in the saliva of smokers with periodontitis compared to non-smokers with periodontitis have been reported [16]. Significantly elevated levels of hepatocyte growth factor were found in smokers with periodontitis as compared to non-
smokers with periodontitis [4]. Considering the available data, it appears that smoking only has a limited impact on salivary cytokine concentrations. However, more research, which specifically aims to evaluate these relationships, is needed to come to firm conclusions.

Concluding remarks

The present review demonstrates that saliva is a valid local source for investigating periodontal cytokines, with many advantages over other sources such as gingival crevicular fluid. Care has to be taken when considering which methodology to choose for analysis; thus, not all commercial manufacturers of enzyme-linked immunosorbant assay kits provide quality control data for use with saliva samples and the utility of multiplex analysis remains somewhat uncertain. Also, despite the fact that it is established that cytokines are elements of complex and dynamic networks [93], precise interpretation of quantitative data above and beyond correlation analyses remains challenging.

The majority of studies of salivary cytokines in periodontitis have either not yielded evidence for disease associations, or have yielded inconsistent results or positive correlations, which remain to be substantially replicated in independent studies. Nevertheless, current evidence suggests that of the molecules with cytokine-like activity studied thus far, interleukin-1β and hepatocyte growth factor are the most robust salivary biomarkers for periodontal disease. High quality research designs specifically targeting sensitivity and specificity and confounding factors such as smoking and systemic diseases should be the next step in salivary periodontitis biomarker research. This would help to evaluate if the biomarker knowledge can be implemented in a diagnostic test to detect periodontitis (or risk thereof) before periodontal tissue breakdown occurs. Although there is much interest in developing ‘chairside’ tests for salivary mediators using novel
technologies, it is critical that such research is supported by substantial laboratory and clinical data confirming the clinical utility of individual candidate biomarkers.

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Table 1. Studies of IL-1 family cytokines as candidate salivary biomarkers for periodontitis.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Type</th>
<th>Patient groups</th>
<th>IL-1 cytokine</th>
<th>Principal findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>[63]</td>
<td>Case-control, cross-sectional</td>
<td>28 periodontitis patients; 29 healthy controls</td>
<td>IL-1β</td>
<td>Significantly higher levels in periodontitis group (mean ± SD; 753.7 ± 1022.4 pg/ml) as compared to controls (212.8 ± 167.4 pg/ml); significant positive correlation with bleeding on probing and clinical attachment loss measurements.</td>
</tr>
<tr>
<td>[21]</td>
<td>Case-control, cross-sectional</td>
<td>10 periodontitis patients; 9 healthy controls</td>
<td>IL-1β</td>
<td>No significant difference between groups.</td>
</tr>
<tr>
<td>[67]</td>
<td>Cross-sectional with no controls</td>
<td>98 periodontitis patients</td>
<td>IL-1β</td>
<td>Positive association between levels of IL-1β and alveolar bone loss.</td>
</tr>
<tr>
<td>[89]</td>
<td>Case-control, longitudinal study</td>
<td>40 periodontitis patients with bone loss after 5 years; 40 controls with no bone loss</td>
<td>IL-1β</td>
<td>Positive association with IL-1β and extent of alveolar bone loss over a 5-year period.</td>
</tr>
<tr>
<td>[104]</td>
<td>Case-control, cross-sectional</td>
<td>28 periodontitis patients; 29 healthy controls</td>
<td>IL-1β</td>
<td>Significantly higher levels in periodontitis group as compared to controls.</td>
</tr>
<tr>
<td>[103]</td>
<td>Case-control, cross-sectional</td>
<td>74 periodontitis patients; 44 healthy controls</td>
<td>IL-1β</td>
<td>No significant difference between groups</td>
</tr>
<tr>
<td>[31]</td>
<td>Case-control, longitudinal study</td>
<td>8 localised aggressive periodontitis patients with <em>Aggregatibacter actinomycetemcomitans</em>, IL-1α, IL-1β</td>
<td>IL-1β</td>
<td>IL-1β elevated in children who developed bone loss over a 2-3 year period compared to those who remained healthy.</td>
</tr>
<tr>
<td>Reference</td>
<td>Study Design</td>
<td>Study Population</td>
<td>Inflammatory Marker</td>
<td>Findings</td>
</tr>
<tr>
<td>-----------</td>
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<td>---------------------</td>
<td>----------</td>
</tr>
<tr>
<td>[41]</td>
<td>Case-control, cross-sectional</td>
<td>84 periodontitis patients; 81 healthy controls</td>
<td>IL-1β</td>
<td>Significantly higher levels in periodontitis group (Mean ± SD; 665.7 ± 267.5 pg/ml) as compared to controls (467.8 ± 279.8 pg/ml).</td>
</tr>
<tr>
<td>[80]</td>
<td>Case-control, cross-sectional</td>
<td>49 periodontitis patients; 32 gingivitis patients; 18 healthy controls</td>
<td>IL-1β</td>
<td>No significant difference between groups.</td>
</tr>
<tr>
<td>[64]</td>
<td>Case-control, cross-sectional</td>
<td>35 periodontitis patients; 35 healthy controls</td>
<td>IL-1β</td>
<td>Significantly higher levels in periodontitis group as compared to controls (mean; 33.11 pg/ml). Precise values for IL-1β in the periodontitis group not presented.</td>
</tr>
<tr>
<td>[74]</td>
<td>Case-control, cross-sectional</td>
<td>21 healthy adults; 52 chronic periodontitis patients</td>
<td>IL-18</td>
<td>Significantly increased concentration of IL-18 in periodontitis (mean ± SD; 275.05 ± 289.46 pg/ml) as compared to controls (143.71 ± 103.68 pg/ml). Significant positive correlation of IL-18 levels with bleeding on probing measurements.</td>
</tr>
<tr>
<td>[50]</td>
<td>Case-control, longitudinal study</td>
<td>28 periodontitis patients; 24 healthy controls. Patients monitored 1 month after treatment (oral health instruction, scaling and</td>
<td>IL-1β</td>
<td>Significantly higher levels in periodontitis group (mean ± SD; 1312.75 ± 691.22 pg/ml) as compared to controls (161.51 ± 149.6 pg/ml). Significant positive correlation of IL-1β levels with bleeding on probing, gingival index and plaque</td>
</tr>
<tr>
<td>Reference</td>
<td>Design</td>
<td>Participants</td>
<td>Index</td>
<td>Description</td>
</tr>
<tr>
<td>-----------</td>
<td>--------</td>
<td>--------------</td>
<td>-------</td>
<td>-------------</td>
</tr>
<tr>
<td>[54]</td>
<td>Case-control, longitudinal study</td>
<td>17 moderate to severe periodontitis patients; 24 mild periodontitis patients, 23 gingivitis patients; 15 healthy controls</td>
<td>IL-1β</td>
<td>Disease monitoring over 6 months (no treatment) then 6 months during which time treatment (oral health instruction, scaling and root planing as appropriate) was provided. No changes during monitoring phase but levels of IL-1β declined significantly after treatment in volunteers with moderate to severe periodontitis.</td>
</tr>
<tr>
<td>[117]</td>
<td>Cross-sectional with no controls</td>
<td>192 subjects with and without type 2 diabetes mellitus</td>
<td>IL-1β</td>
<td>Levels correlate with clinical parameters of periodontal disease.</td>
</tr>
<tr>
<td>[92]</td>
<td>Longitudinal</td>
<td>68 periodontitis patients. 35 patients undergoing both scaling and root-planing and oral hygiene instruction as compared to 33 patients having oral hygiene instruction alone</td>
<td>IL-1β</td>
<td>IL-1β declined only in the group who received both scaling and root planing and oral hygiene instruction.</td>
</tr>
<tr>
<td>[18]</td>
<td>Case-control, cross-sectional</td>
<td>25 healthy adults; 32 chronic periodontitis patients</td>
<td>IL-33</td>
<td>No significant difference in IL-33 levels between groups.</td>
</tr>
<tr>
<td>[82]</td>
<td>Case-control,</td>
<td>49 severe periodontitis</td>
<td>IL-1β</td>
<td>Significantly higher levels in severe</td>
</tr>
<tr>
<td>Study</td>
<td>Study Type</td>
<td>Population</td>
<td>IL-1β</td>
<td>Description</td>
</tr>
<tr>
<td>-------</td>
<td>------------</td>
<td>------------</td>
<td>-------</td>
<td>-------------</td>
</tr>
<tr>
<td>[29]</td>
<td>Case-control, cross-sectional</td>
<td>30 healthy adults; 50 severe periodontitis patients</td>
<td>IL-1β</td>
<td>Significantly higher levels in periodontitis group (mean ± SD; 90.94 ± 85.22 pg/ml) as compared to controls (7.24 ± 7.69 pg/ml).</td>
</tr>
<tr>
<td>[87]</td>
<td>Cross-sectional with no controls</td>
<td>340 no to mild periodontitis; 123 moderate to severe periodontitis; 30 edentulous</td>
<td>IL-1β</td>
<td>Levels significantly associated with all periodontitis parameters, presence of coronary artery disease and diabetes (but not smoking or ex-smokers). Significantly lower levels in edentulous patients.</td>
</tr>
</tbody>
</table>

IL-1α, interleukin-1α; IL-1β, interleukin-1β; IL-18, interleukin-18; IL-33, interleukin-33.
Table 2. Studies of immunoregulatory cytokines as candidate salivary biomarkers for periodontitis.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study type</th>
<th>Patient groups</th>
<th>Cytokines</th>
<th>Principal findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>[111]</td>
<td>Cross-sectional with no controls</td>
<td>30 moderate periodontitis patients and 9 severe periodontitis patients. Both groups were HIV+</td>
<td>IL-2, IL-4, IL-10, IL-12, IFN-γ</td>
<td>No significant differences between groups for any cytokine.</td>
</tr>
<tr>
<td>[5]</td>
<td>Cross-sectional, case-control</td>
<td>10 periodontitis patients; 14 healthy controls</td>
<td>TNF-α</td>
<td>TNF-α not detected.</td>
</tr>
<tr>
<td>[67]</td>
<td>Longitudinal</td>
<td>98 periodontitis patients</td>
<td>IL-6</td>
<td>No association with alveolar bone loss.</td>
</tr>
<tr>
<td>[89]</td>
<td>Longitudinal, case-control</td>
<td>40 periodontitis patients with bone loss after 5 years; 40 controls with no bone loss</td>
<td>IFN-γ, TNF-α, IL-4, IL-6, IL-8</td>
<td>No association with alveolar bone loss.</td>
</tr>
<tr>
<td>[33]</td>
<td>Cross-sectional, case-control</td>
<td>35 periodontitis patients; 39 healthy controls</td>
<td>TNF-α</td>
<td>Significantly higher levels in periodontitis group (mean ± SEM; 4.33 ± 0.73 pg/ml) as compared to controls (2.03 ± 0.49 pg/ml).</td>
</tr>
<tr>
<td>[31]</td>
<td>Longitudinal, case-control</td>
<td>8 localised aggressive periodontitis patients with <em>A. actinomycetemcomitans</em>, 20 healthy controls with <em>A. actinomycetemcomitans</em> and 20 healthy controls without <em>A.</em></td>
<td>21 cytokines including IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12, IL-13, IL-17, RANTES, MCP-1, IFN-MIP-1α</td>
<td>MIP-1α elevated in children who developed bone loss compared to those who remained healthy.</td>
</tr>
<tr>
<td>Study</td>
<td>Study Design</td>
<td>Participants</td>
<td>Cytokines Assessed</td>
<td>Findings</td>
</tr>
<tr>
<td>-------</td>
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<td>-------------------</td>
<td>----------</td>
</tr>
<tr>
<td>[41]</td>
<td>Cross-sectional, case-control</td>
<td>84 periodontitis patients; 81 healthy controls</td>
<td>IL-6; TNF-α</td>
<td>No significant differences between groups for either cytokine.</td>
</tr>
<tr>
<td>[103]</td>
<td>Cross-sectional, case-control</td>
<td>74 periodontitis patients; 44 healthy controls</td>
<td>GM-CSF; IL-2; IL-4; IL-5; IL-6; IL-8; IL-10; IFN-γ; TNF-α</td>
<td>No significant differences between groups for any cytokine. Significant negative correlation between IL-10 levels and bleeding on probing measurements. Significantly positive correlation between IL-8 and bleeding on probing measurements.</td>
</tr>
<tr>
<td>[80]</td>
<td>Cross-sectional, case-control</td>
<td>49 periodontitis patients; 32 gingivitis patients; 18 healthy controls</td>
<td>IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, TNF-α, IFN-γ</td>
<td>IL-5 and IFN-γ not detected, no significant differences between groups for the other cytokines.</td>
</tr>
<tr>
<td>[23]</td>
<td>Cross-sectional, case-control</td>
<td>24 periodontitis patients; 22 healthy controls</td>
<td>IL-6</td>
<td>Significantly higher levels in periodontitis group as compared to controls. Values for IL-6 concentrations not presented.</td>
</tr>
<tr>
<td>[74]</td>
<td>Cross-sectional, case-control</td>
<td>21 healthy adults; 52 chronic periodontitis patients</td>
<td>IL-17</td>
<td>Significantly lower levels of IL-17 in periodontitis group as compared to controls.</td>
</tr>
<tr>
<td>[64]</td>
<td>Cross-sectional, case-control</td>
<td>35 periodontitis patients; 35 healthy controls</td>
<td>TNF-α</td>
<td>No significant differences between groups.</td>
</tr>
<tr>
<td>[92]</td>
<td>Longitudinal</td>
<td>68 periodontitis patients. 35 patients undergoing both scaling</td>
<td>TNF-α, IL-8, MIP-1α</td>
<td>TNF-α levels declined in both treatment groups, MIP-1α declined in responders compared to non-responders.</td>
</tr>
</tbody>
</table>
and root-planing and oral hygiene instruction as compared to 33 patients having oral hygiene instruction alone.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design, Disease Details</th>
<th>Group Details</th>
<th>Cytokine</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>[2]</td>
<td>Cross-sectional, case-control</td>
<td>70 periodontitis patients; 40 healthy controls</td>
<td>MIP-1α</td>
<td>Significantly higher levels of MIP-1α in periodontitis group as compared to controls. No values for MIP-1α levels presented. Significantly positive correlation between MIP-1α levels and both bleeding on probing and clinical attachment loss measurements.</td>
</tr>
<tr>
<td>[77]</td>
<td>Longitudinal study, cross-sectional</td>
<td>20 periodontitis patients; 20 healthy controls. Screening 6 weeks after scaling and root-planing.</td>
<td>IL-4, IL-6, IL-10, IL-17</td>
<td>Significantly higher levels of IL-4, IL-6, and significantly lower levels of IL-10 and IL-17 in periodontitis group as compared to controls. IL-4 levels reduced to those of healthy volunteers 6 weeks after treatment. Cytokine concentrations not presented.</td>
</tr>
<tr>
<td>[82]</td>
<td>Cross-sectional, case-control</td>
<td>49 severe periodontitis patients; 89 periodontitis patients; 303 healthy controls</td>
<td>IL-6, IL-8</td>
<td>No significant differences between groups. Significantly positive correlation between IL-8 and bleeding on probing measurements.</td>
</tr>
<tr>
<td>[29]</td>
<td>Cross-sectional, case-control</td>
<td>30 healthy adults; 50 severe periodontitis patients</td>
<td>TNF-α, IL-6, IFN-α</td>
<td>Significantly higher levels of IL-6 in periodontitis group (mean ± SD; 35.57 ± 48.17 pg/ml) as compared to controls (3.30 ± 2.32 pg/ml); significantly lower</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>Sample Size</td>
<td>Cytokines</td>
<td>Findings</td>
</tr>
<tr>
<td>-------</td>
<td>--------</td>
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<td>-----------</td>
<td>----------</td>
</tr>
<tr>
<td>[40]</td>
<td>Cross-sectional, longitudinal</td>
<td>30 periodontitis patients; 15 healthy controls</td>
<td>MCP-1</td>
<td>Significantly higher levels of MCP-1 in periodontitis group as compared to controls. Values for MCP-1 concentrations not presented.</td>
</tr>
<tr>
<td>[6]</td>
<td>Cross-sectional, Case-control</td>
<td>97 periodontitis patients; 77 healthy controls</td>
<td>IL-17A, IL-17E, IL-17F</td>
<td>IL-17A, IL-17 E and IL-17A/F ratio significantly elevated in periodontitis patients and positively correlated with various clinical parameters of periodontitis.</td>
</tr>
</tbody>
</table>

GM-CSF, granulocyte macrophage-colony stimulating factor; IFN-α, interferon-α; IFN-γ, interferon-γ; IL-2, interleukin-2; IL-3, interleukin-3; IL-4, interleukin-4; IL-5, interleukin-5; IL-6, interleukin-6; IL-8, interleukin-8; IL-9, interleukin-9; IL-10, interleukin-10; IL-12, interleukin-12; IL-13, interleukin-13; IL-17, interleukin-17; RANTES, regulated on activation, normal T expressed and secreted; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein1α; RANTES, regulated on activation, normal T expressed and secreted; TNF-α, tumor necrosis factor-α.
Table 3. Studies of bone regulating cytokines as candidate salivary biomarkers for periodontitis.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study type</th>
<th>Patient groups</th>
<th>Cytokines analysed</th>
<th>Principal findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>[63]</td>
<td>Cross sectional, case control</td>
<td>28 periodontitis patients; 29 healthy controls</td>
<td>OPG</td>
<td>No significant difference between groups.</td>
</tr>
<tr>
<td>[16]</td>
<td>Cross sectional</td>
<td>67 untreated chronic periodontitis patients; 44 chronic periodontitis patients on maintenance therapy</td>
<td>OPG, RANKL</td>
<td>OPG levels correlated with clinical measures of periodontitis.</td>
</tr>
<tr>
<td>[33]</td>
<td>Cross sectional, case control</td>
<td>21 periodontitis patients; 21 healthy controls</td>
<td>RANKL</td>
<td>Only detected in a minority of subjects and no significant difference between groups.</td>
</tr>
</tbody>
</table>
| [80]      | Cross sectional, case control  | 21 moderate-severe periodontitis patients; 28 mild periodontitis patients; 32 gingivitis patients; 18 healthy controls | OPG, calprotectin  | Significantly lower levels of both cytokines in periodontitis (mild and severe) as compared to controls (gingivitis and healthy). OPG levels were (median, range): moderate-severe periodontitis 1.9 pg/ml (0.2-10); mild periodontitis: 1.6 pg/ml; (0.5-11.8); gingivitis: 2.7 pg/ml (1.2-6.2); healthy: 2.3 pg/ml (1.4-6.6). Calprotectin levels were moderate-severe periodontitis 5.4 pg/ml (1.7-97.6); mild periodontitis: 4.3 pg/ml (0-
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Study Population</th>
<th>Biomarkers</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>[23]</td>
<td>Cross sectional, case control</td>
<td>24 periodontitis patients; 22 healthy controls</td>
<td>OPG</td>
<td>No significant difference between groups</td>
</tr>
<tr>
<td>[54]</td>
<td>Longitudinal, case control</td>
<td>17 moderate to severe periodontitis patients; 24 mild periodontitis patients, 23 gingivitis patients; 15 healthy controls</td>
<td>OPG, Calprotectin</td>
<td>No changes during monitoring phase but levels of OPG declined significantly after 6 months treatment in volunteers with moderate to severe periodontitis. Data presented in graphical form only.</td>
</tr>
<tr>
<td>[92]</td>
<td>Cross sectional, case control</td>
<td>68 periodontitis patients: 35 patients undergoing both scaling and root planning and oral hygiene instruction as compared to 33 patients having oral hygiene instruction alone.</td>
<td>OPG</td>
<td>OPG levels declined in both treatment groups. Data presented in graphical form only.</td>
</tr>
<tr>
<td>[105]</td>
<td>Cross sectional, case control</td>
<td>97 periodontitis patients; 43 healthy controls</td>
<td>OPG, RANKL</td>
<td>Significantly higher levels of RANKL and significantly lower levels of OPG in periodontitis group as compared to controls. Levels are (median, inter-quartile range): OPG: periodontitis 95.2 pg/ml (49.8-145.2); healthy 131.6 pg/ml (82.2-202.4); RANKL: periodontitis 6.0 pg/ml (2.7-11.1);</td>
</tr>
</tbody>
</table>
**Table:**

| [99] | Cross sectional, case control | 25 chronic periodontitis patients; 25 healthy controls | OPG, RANKL | Significantly higher levels of RANKL in the periodontitis group (266 ± 48 pg/ml; median ± interquartile range) as compared to controls (207 ± 83 pg/ml). No significant difference in OPG levels between groups. |

OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor-kappaB ligand.
Table 4. Studies of growth factors as candidate salivary biomarkers for periodontitis.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study type</th>
<th>Patient groups</th>
<th>Growth factor</th>
<th>Salivary growth factor levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>[13]</td>
<td>Cross sectional, case control</td>
<td>32 periodontitis patients and 12 healthy controls</td>
<td>VEGF</td>
<td>Significantly higher levels in periodontitis group as compared to controls. Data presented in graphical form only.</td>
</tr>
<tr>
<td>[89]</td>
<td>Longitudinal, case control</td>
<td>40 periodontitis patients with bone loss after 5 years; 40 controls with no bone loss</td>
<td>HGF</td>
<td>Positive association with HGF and extent of alveolar bone loss over a 5 year period.</td>
</tr>
<tr>
<td>[113]</td>
<td>Cross sectional, case control</td>
<td>26 periodontitis patients; 20 healthy controls</td>
<td>HGF</td>
<td>Significantly higher levels in periodontitis group (Mean, range: 1.87 ng/ml; 0.06-5.38) as compared to controls (0.68 ± ng/ml; 0-7.33). HGF levels correlated with gingival index, plaque index and papillary bleeding index.</td>
</tr>
<tr>
<td>[85]</td>
<td>Cross sectional, case control</td>
<td>12 severe periodontitis patients; 12 moderate periodontitis; 12 healthy controls</td>
<td>HGF</td>
<td>Significantly higher levels in severe periodontitis group (mean ± SD; 3430.8 ± 1640.2) and moderate periodontitis group (1878.99 ± 1713.54 pg/ml) as compared to controls (443.82 ± 295.14 pg/ml).</td>
</tr>
<tr>
<td>[114]</td>
<td>Cross sectional, case control</td>
<td>26 periodontitis patients; 20 healthy controls</td>
<td>HGF</td>
<td>Significantly higher levels in periodontitis group as compared to controls. Data presented previously (see above)</td>
</tr>
</tbody>
</table>

HGF, hepatocyte growth factor; VEGF, vascular endothelial growth factor.
Table 5. Studies of adipokines as candidate salivary biomarkers for periodontitis.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study type</th>
<th>Patient groups</th>
<th>Adipokine</th>
<th>Principal findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>[75]</td>
<td>Cross sectional, case-control</td>
<td>25 patients with chronic periodontitis, 24 patients with gingivitis, 25 healthy controls</td>
<td>Chemerin, visfatin, progranulin and TNF-α</td>
<td>Chemerin was significantly elevated in the periodontitis group (median, range: 0.084ng/ml, 0.063-0.105) but not the gingivitis group (0.042ng/ml, 0.042-0.063) as compared to controls (0.042ng/ml, 0.023-0.063). Visfatin was significantly elevated in both the periodontitis group (589 ng/ml, 302-1195) and the gingivitis group (791 ng/ml, 267-1127) as compared to controls (267 ng/ml (125-616). Levels of chemerin but not visfatin correlated with clinical attachment loss measurements.</td>
</tr>
<tr>
<td>[100]</td>
<td>Cross sectional, case-control</td>
<td>20 patients with chronic periodontitis, 20 healthy controls</td>
<td>Visfatin</td>
<td>Visfatin significantly elevated in periodontitis (mean ± SD: 33.43 ± 15.72 ng/ml) as compared to controls (23.38 ± 7.58 ng/ml).</td>
</tr>
</tbody>
</table>

TNF-α, tumor necrosis factor-α.