Perio 2000
Salivary analysis of cytokines in periodontal disease.

Contents

Abstract

Periodontitis biomarker research is driven by mainly two targets: to discover biomarkers which can identify “at risk” patients before actual bone and periodontal tissue destruction occurs and to build up the knowledge of understanding this complex disease with the purpose of finding new drug targets. Whilst previously systemic blood samples and local GCF samples were the analytes of choice, recently saliva has gained more attention as an easy and readily accessible oral fluid, with many advantages over GCF and blood. The aim of this paper is to give a comprehensive overview of the current literature of salivary cytokines in periodontitis, highlighting extensively studied cytokines such as IL-1β and IL-6, but also cytokines such as HGF which only have been the target of few studies and which warrant further investigation. Salivary cytokines are considered in both cross-sectional and longitudinal studies and their potential as a salivary periodontitis biomarker is evaluated against criteria of what marks a robust biomarker. Finally, this evaluation will expand to include how robust these salivary biomarkers are in light of confounding factors such as systemic diseases and smoking.

I Introduction

Despite numerous advances in diseases with comparable etiology such as rheumatoid arthritis, periodontitis is still only diagnosed once oral tissue and bone destruction has occurred. There is therefore a definite need for developing biomarkers for an earlier detection of the disease and to further understanding of the underlying complex cytokine patterns in order to develop new drug targets.

Most commonly, cytokines are considered as “hormones” of the immune system e.g. a soluble factor produced by one cell which acts on another cell (Dinarello, 2007). However, as Dinarello (2007) highlights, hormones are more associated with being products of whole organs rather than individual cells and certain cytokines are never secreted and have intracellular functions. Thus, not only classic “cytokines” such as interleukins can be considered, but the range of cytokines can be extended to include for example chemokines, growth factors, adipokines, acute phase proteins or MMPs. All these molecules have signalling capacities in one way or another, often acting in an autocrine and/or paracrine manner. Importantly, there signalling pathways often overlap or merge, building a complex network resulting in a high signalling redundancy in the immune system. In the following, the term “cytokine” will be used in this broad context. The reader is reminded that salivary MMPs and TIMPs are reviewed in another chapter of this issue.

Functions and applications of cytokines

As “messengers” of the immune system, cytokines have a multitude of functions. For example, they facilitate wound healing, act against pathogens and cancer but also are vital in cell lineage developments and embryogenesis. Clinically relevant, some cytokines are either used as drugs or drug targets for treatment of a variety of conditions. For example, targeting TNF-α in rheumatoid arthritis is a recognized treatment for this autoimmune disorder and IL-2 and interferons have been successfully applied in cancer treatment.

Apart from their use as drugs or drug targets, cytokines have received much attention for causing life threatening conditions such as sepsis and multiple organ failure. One of the most well-known

examples of the detrimental effects of cytokines is the phase 1 clinical trial of TGN1412, a monoclonal antibody targeting the T-cell receptor CD28 (Suntharalingam *et al.*, 2006). 6 subjects receiving the new drug suffered a so-called "cytokine storm", a sudden increase in pro-inflammatory cytokines resulting in severe cell and tissue destruction and loss of organ function (Suntharalingam *et al.*, 2006). Whilst all subjects survived, review of the trial conduction let to worldwide changes in regulations of clinical trial conducts (Hunig, 2012).

Cytokines and periodontal diseases

The earliest cytokine studies in periodontal research can be dated back to the 1980s (Charon *et al.*, 1982; Mergenhagen, 1984) and coincide with the discovery of IL-1, IL-2 and TNF (Dinarello, 2007). These first studies were conducted using murine thymocyte bioassays. In these assays, thymocytes from mice were cultured and stimulated with GCF from inflamed or non-inflamed sites of human periodontitis patients. The results of the experiments showed increased thymocyte proliferation with GCF from inflamed sites compared to non-inflamed sites (Charon *et al.*, 1982; Mergenhagen, 1984). However, being non-specific, which factor(s) in GCF caused the proliferation remained undetermined. With the establishment of ELISAs IL-1 β was the first specifically measured cytokine in gingival tissue of patients with periodontal disease (Honig *et al.*, 1989) and the field of studying cytokine concentrations in periodontal research using ELISAs multiplied from there. Other techniques such as immunohistochemistry and *in situ* hybridization (ISH) are used to investigate specifically the localization of cytokines at the protein or DNA/RNA level in gingival and periodontal tissues. However, acquisition of suitable tissues poses ethical complications as the aim of periodontitis treatment is to repair as much tissue as possible and additionally, the disease per se causes tissue destruction. Accurate quantification and suitable controls in these techniques therefore remains a challenge and ELISA clearly has the advantage in terms of high throughput, high reproducibility and relatively easy accessibility of oral fluids such as saliva or GCF.

Utility of measurement of salivary cytokines in periodontitis

Comparisons of cytokine concentrations between health and disease have been investigated for virtually any inflammatory condition and literature on this topic in periodontitis research is extensive. The aims of this research are to gain a better understanding of disease aetiology and susceptibility, to discover new treatment opportunities and to detect periodontitis early enough to avoid permanent tissue and bone destruction. Especially the last aim of detecting early changes of "biomarker" concentrations is of high importance in periodontal research as currently, there is no diagnostic method for periodontitis available other than a physical inspection of the oral cavity. Being able to identify the risk of developing periodontitis or very early stages of periodontitis and relapses would be an immense advantage to prevent permanent tissue and bone destruction. Additionally, the targeted anti-TNF α treatment in rheumatoid arthritis raised hopes that potentially a similarly effective drug treatment could be developed for periodontitis if the right target cytokine would be found.

While early studies focused on serum and GCF samples for investigations of periodontitis related cytokines, saliva came into focus as an alternative sample in recent years and several manufacturers now offer ELISA kits specified for salivary detection of cytokines. Saliva has several advantages over GCF as it is easier accessible than GCF, can be sampled in a much larger volume and virtually anywhere and there is no special skill necessary for saliva sampling. Also, equally to GCF saliva gives a better representation of the local situation than analysis of serum.

Considerations for biomarker identification

To evaluate if any of the below described salivary cytokines have potential as a periodontitis biomarker, it is necessary to first give a definition of what a biomarker is and what criteria it will be assessed on. 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." (Biomarkers Definitions Working, 2001). The quality of a biomarker should be assessed on a number of criteria, including study design (e.g. study quality) and statistical analysis (Lassere, 2008).

Quality and design of the study

Reviewing the current literature on analysis of salivary cytokines, potential methodological errors can be highlighted. For example, only few studies report calibrating procedures (reviewed in Hefti and Preshaw (2012)) for evaluating clinical parameters such as pocket depth (PD), clinical attachment loss (CAL) or bleeding on probing (BOP). Also, definitions of periodontitis or periodontal health groups appear to resemble more a lottery than a defined universal approach, with a total of 16 different definitions found in 27 evaluated studies. The lack of uniformity in periodontitis definitions has been observed before (Preshaw, 2009) and makes it challenging to compare different study results. Another potential flaw in study design is the quality of the methodology to assess cytokine concentrations in saliva. Most research studies use commercially available ELISA kits for sample analysis, however none of them report any form of evaluation if these kits are suitable for analysis of saliva (despite manufacturers clearly indicating that any other sample type than the one specified for the kit has to be validated by the user themselves). ELISA validation procedures are well established in the literature and if successful, give great credibility to research results (Jaedicke *et al.*, 2012). Whereas failing to validate an ELISA for the suitability with saliva samples can lead to erroneous results.

Statistical evaluation

Whilst the majority of studies apply correct statistical analysis procedures, some common examples of statistical errors are made: Using parametric tests when data are not parametric, using dependent samples test when data are independent (for example paired samples t-test instead of Student's independent sample t-test) or having a mixture of parametric and non-parametric tests (for example doing an ANOVA followed by Mann-Whitney-*U* comparisons). In addition, only few studies report a formal power calculation for sample size and likely many of the reviewed studies are underpowered. A key finding of this review was that although most studies did take normal distribution of their sample groups into account, the majority 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 size of $n>2000$ whereas the Shapiro-Wilk test of normal distribution is valid for a smaller sample size, too and has superior statistical power (Razali and Wah, 2011).

II Salivary cytokines in oral health and periodontal disease

IL-1 family

IL-1β (IL-1F2) undoubtedly is one of the most extensively studied cytokines in periodontitis and it is the one which is most referred to in salivary biomarker analysis. In general, IL-1β has a multitude of metabolic and immunological functions such as inducing fever and neutrophil development, activation of neutrophils, T-and B-cells and inducing liver acute phase protein release (reviewed in Dinarello, 2011). In periodontitis, IL-1β is recognised as an important inducer of other pro-inflammatory cytokines, MMPs and chemokines. Additionally, it is associated with recruitment of PMNLs and activation of osteoclasts (Preshaw and Taylor, 2011). Seven out of the nine studies that

have investigated salivary IL-1β concentrations in periodontitis show a significant increase of IL-1β concentrations in periodontitis compared to healthy controls (see table 1). The other two studies do not report any significant changes. In addition, there are consistent findings that levels of salivary IL-1β decrease after periodontal treatment (see table 1). Taking concentrations (mean±SD) described in the literature, salivary IL-1β concentrations in oral health range from 7.24±7.69 pg/ml (Ebersole *et al.*, 2013) to 633±91 pg/ml (Teles *et al.*, 2009). In periodontal disease, a range of 90.94±85.22 pg/ml (Ebersole *et al.*, 2013) to 1312.75±691.22 pg/ml (Kaushik *et al.*, 2011) can be found.
Only a limited number of studies have investigated salivary concentrations of other members of the IL-1 family of cytokines in periodontitis. A study on IL-18 (IL-1F4) found increased concentrations in periodontitis patients (275.05±289.46 pg/ml) compared to healthy controls (143.71±103.68 pg/ml) (Ozcaka *et al.*, 2011). IL-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 Barksby *et al.,* (2007)). Salivary concentrations of IL-33 (IL-1F11), an IL-1 family member which drives Th2 cytokine responses (Schmitz *et al.*, 2005), appear to remain unaffected by periodontitis. Concentrations of 1.2±0.6 ng/ml and 1.1±0.5 ng/ml have been measured in periodontal health and disease, respectively (Buduneli *et al.*, 2012).
Nothing is known on salivary concentrations in systemically healthy patients with periodontitis of IL-1 family members IL-1α (IL-1F1), IL-1RA (F3), F5, F6, F7, F8, F9 and F10. Except for IL-1β, no further information is available on post-treatment salivary concentrations of the IL-1 family of cytokines.

IL-1β is highly relevant in periodontal disease etiology and with most studies consistently showing increased salivary IL-1β concentrations in periodontitis and decreased concentrations after periodontal treatment, this cytokine lends itself as a suitable periodontitis biomarker. In addition, reports show that its concentrations are not overly affected by systemic conditions or smoking (see section III and IV), making IL-1β a robust analyte. Furthermore, IL-1β concentrations in periodontitis are easily high enough to be detected by standard ELISA techniques, making widespread assays possible and avoiding the need for specialised centres. Both Ebersole *et al.* (2013) and Ramseier *et al.* (2009) demonstrate potential of IL-1β to discriminate between periodontal health and disease with high specificity and sensitivity in ROC (receiver operator curve) analysis. Taken together, all evidence points towards changes in salivary IL-1β concentrations as a strong periodontitis biomarker. The only potential negative finding is a significant overlap of salivary IL-1β concentrations within healthy or periodontitis categories between studies (see table 1), which makes it difficult to compare results between different studies.

TNF-α

TNF-α is a key pro-inflammatory cytokine, produced mainly by macrophages and activated T cells as a first inflammatory response of TLR signalling (Bradley, 2008). TNF-α and RANKL both belong to the TNF superfamily and comparable to RANKL, TNF-α is a potent activator of osteoclasts and facilitates bone resorption (Romas *et al.*, 2002). Several studies have investigated salivary TNF-α concentrations in periodontal health and disease (see table 1) however the levels detected are very small (health: 1.85±2.11 pg/ml; periodontitis: 5.44±10.88 pg/ml (Ebersole *et al.*, 2013)). Out of seven studies only Frodge *et al.* (2008) report a significant increase in TNF-α concentrations in periodontitis compared to periodontal health, all other studies find no differences between the two groups (see table 1). After periodontal treatment, salivary TNF- α concentrations were reported to either decrease (Sexton *et al.*, 2011) or stay the same (Kinney *et al.*, 2011).
Salivary TNF-α concentrations have been investigated numerous times however often concentrations were below level of detection and therefore not suitable as a biomarker. However, differences between periodontal health and disease were found when using highly sensitive detection methodology (Frodge *et al.*, 2008) and therefore the potential of TNF-α as a periodontitis biomarker should be re-evaluated after more research with sensitive detection methodology is performed.

IL-6

After induction by pro-inflammatory cytokines and stimuli such as IL-1β,TNF-α or LPS, IL-6 is produced by a variety of cell types, including lymphocytes, myeloid cells and epithelial cells (reviewed in Irwin and Myrillas (1998)). Together with other cytokines, IL-6 induces T-cell proliferation and, importantly for periodontitis, is associated with bone resorption and regulation of MMP activity (Irwin and Myrillas, 1998).
In general, compared to for example salivary IL1-β concentrations, levels of IL-6 measured in saliva are low in both periodontal health (3.30±2.32 pg/ml) and disease (35.57±48.17 pg/ml) (Ebersole *et al.*, 2013). Also, while some studies show that salivary IL-6 concentrations are significantly higher in periodontitis than in healthy individuals (Costa *et al.*, 2010; Ebersole *et al.*, 2013; Prakasam and Srinivasan, 2013), the majority of studies find no differences between the two groups (see table 1). Reports on post-treatment evaluations of salivary IL-6 concentrations show no significant changes (Kinney *et al.*, 2011; Prakasam and Srinivasan, 2013).
Therefore, IL-6 does not receive a positive outcome as a salivary periodontitis biomarker in evaluation of the current evidence. Concentrations of IL-6 are small, often no differences between periodontal health and disease are detected and no post-treatment differences can be found.

IL-4

IL-4 is mainly produced by T-cells, specifically the Th2 subset of T-cells. It has important functions in the regulation of cell proliferation and apoptosis in numerous cell types, including lymphocytes, myeloid cells, fibroblasts and epithelial cells (reviewed in Luzina *et al.,* 2012). Findings for salivary concentrations of IL-4 are inconsistent. One study reports significantly lower levels in periodontitis compared to controls (Napimoga *et al.*, 2011), another study reports significantly higher IL-4 concentrations in periodontitis (Prakasam and Srinivasan, 2013) and two studies find no differences in salivary IL-4 concentrations between periodontal health and disease (Ramseier *et al.*, 2009; Teles *et al.*, 2009). In periodontal health, minimal levels of IL-4 can be detected (0.0 (0-5.315.1) pg/ml, given as median + range) and in periodontitis the amount is small as well (69.5 (0-11,714.3) pg/ml, given as median + range) (Ramseier *et al.*, 2009). After periodontal treatment, salivary IL-4 concentrations were shown to decrease or to not be affected (Kinney *et al.*, 2011; Prakasam and Srinivasan, 2013).
Considering the current evidence, the Th2 cytokine IL-4 is not a strong salivary periodontitis biomarker. Salivary IL-4 concentrations are small, often no differences between periodontal health and disease are detected and inconsistencies between studies evaluating post-treatment differences are seen.

IL-10

IL-10 is produced by a variety of cell types, including myeloid cells, T and B cells, NK-cells and neutrophils (reviewed in Ouyang *et al.,* 2011). It is a key anti-inflammatory cytokine, involved in inhibiting and regulating pro-inflammatory immune responses and promoting resolution of inflammation (Ouyang *et al.*, 2011). In comparison to IL-4, overall salivary IL-10 concentrations are considerably higher (periodontal health: 881.4 (0-11,088.8) pg/ml; periodontal disease: 1,445.1 (0-30,633.1) pg/ml given as median + range)) (Ramseier *et al.*, 2009). One study reports significantly lower levels in periodontitis compared to controls (Prakasam and Srinivasan, 2013), another study reports significantly higher IL-10 concentrations in periodontitis (Napimoga *et al.*, 2011) and two

studies find no differences in salivary IL-10 concentrations between periodontal health and disease (Ramseier *et al.*, 2009; Teles *et al.*, 2009). Periodontal treatment does not appear to affect salivary IL-10 concentrations (Kinney *et al.*, 2011; Prakasam and Srinivasan, 2013).
In terms of the amount found, salivary IL-10 concentrations show somewhat more potential as a periodontitis biomarker than IL-6 or IL-4. Levels in saliva per se are higher and readily detected with standard ELISA techniques. However, study findings are inconsistent and no changes are detected after periodontal treatment (see table 1 and 2).

IL-17

The role of the many T cell subsets expressing for example "Th1" cytokines such as IL-2, IL-10 and IFN-γ, "Th2" cytokines such as IL-4, IL-5, IL-6 and IL-13 or "Th17" cells expressing IL-17 is still not completely understood in periodontal disease. Since the discovery of Th17 cells, the classic roles of Th1 cytokines driving the "early", stable and reversible gingivitis lesions versus Th2 cytokines driving the "later", progressive periodontal lesions has been challenged (Gaffen and Hajishengallis, 2008). In their review on T cell subsets in periodontitis, Gaffen and Hajishengallis (2008) convincingly outline that rather than simply employing the Th1/Th2 paradigm to explain cell mediated immune responses in periodontitis, studies should interpret old and new findings taking Th17 cells into account, creating an ever more complex picture of the disease.
Although salivary IL-17 concentrations are minute in both periodontal health (2.9±0.82 pg/ml) and disease (2.22±1.87 pg/ml) (Ozcaka *et al.*, 2011), both studies comparing health and disease IL-17 levels find significantly lower concentrations in periodontitis compared to health (Ozcaka *et al.*, 2011; Prakasam and Srinivasan, 2013). However, these concentrations do not change after periodontal treatment (Prakasam and Srinivasan, 2013). There are currently not enough data available to judge if IL-17 is a suitable periodontitis biomarker.

GM-CSF

GM-CSF is a key haematopoietic growth factor and is produced by a variety of cell types, including macrophages and fibroblasts (Shi *et al.*, 2006). In oral epithelial cells, it is induced in response to *Candida* infections (Li and Dongari-Bagtzoglou, 2009) and it is used as a drug against severe mucositis associated with radiotherapy during cancer treatment (Worthington *et al.*, 2007).
A study investigating salivary concentrations of GM-CSF reports no differences between periodontal health and disease (no concentrations available) (Teles *et al.*, 2009). There are currently not enough data available to judge if GM-CSF is a suitable periodontitis biomarker.

Other cytokines

No differences between salivary concentrations of IL-2, IL-5, IL-13 or IFN-γ between periodontal health and disease can be detected and no changes in concentrations after periodontal treatment have been reported (see table 1 and 2). Salivary concentrations of other cytokines have not been investigated to date.

RANKL and OPG

The RANKL-OPG system has been the focus of many recent investigations in periodontal research. RANKL (receptor activator of NF-κB ligand) is produced mainly by osteoblasts, fibroblasts and activated T and B cells. When binding to its receptor RANK on pre-osteoclasts, it induces osteoclast differentiation and bone resorption. The decoy receptor OPG (osteoprotegerin) for RANKL is mainly produced by fibroblasts and through binding of RANKL inhibits osteoclastogenesis (reviewed in Belibasakis and Bostanci (2012)). Generally, it appears that RANKL is upregulated and OPG is downregulated in periodontal disease (Belibasakis and Bostanci, 2012). However, findings of salivary concentrations of OPG and RANKL in periodontal health and disease are not as clear. Out of seven

studies, only three (Buduneli *et al.*, 2008; Tobon-Arroyave *et al.*, 2012; Tabari *et al.*, 2013) simultaneously measured salivary concentrations of OPG and RANKL. These studies show a significant increase in RANKL concentrations in periodontitis compared to oral health (health: 4.0 (2.4-6.6) pg/ml, given as median+IQR; disease: 6.0 (2.7-11.1) pg/ml, given as median+IQR (Tobon-Arroyave *et al.*, 2012)) but only Tobon-Arroyave *et al.* (2012) show a decrease in OPG levels in periodontitis (95.2 (49.8-145.2) pg/ml, given as median+IQR) compared to periodontal health (131.6 (82.2-202.4) pg/ml, given as median+IQR). Buduneli *et al.* (2008) and Tabari *et al.* (2013) do not report any change in salivary OPG concentrations. Other studies report varied findings, showing both increases, decreases or no changes in salivary OPG and RANKL concentrations in periodontal health and disease (see table 1). Two studies have investigated post-treatment salivary concentrations of OPG and both find a significant reduction after periodontal treatment (Kinney *et al.*, 2011; Sexton *et al.*, 2011). Salivary concentrations of RANKL have not been investigated after periodontitis treatment to date.

Judging from biomarker research studies in rheumatoid arthritis (van Tuyl *et al.*, 2010) and the above discussed role in bone metabolism, salivary OPG and RANKL concentrations should be prime candidates as periodontitis biomarkers. However, salivary concentrations of both OPG and RANKL are relatively small and study results are inconsistent. It is noteworthy in this context that in our own laboratory a RANKL ELISA (BioSupply UK) failed standard validation procedures for saliva (unpublished observations). Since none of the evaluated studies state anything about testing suitability of their assays with saliva, potentially false positive or false negative detection of salivary OPG or RANKL concentrations could partly explain inconsistencies in findings between different studies. More data are needed to judge if RANKL and OPG are suitable periodontitis biomarkers.

CRP

The classical acute phase protein CRP has been investigated numerous times as a potential connection between periodontal inflammation and other systemic inflammatory conditions (Paraskevas *et al.*, 2008). CRP is produced in the liver in response to pro-inflammatory cytokines such as IL-1β or IL-6 and acts as an activator of the complement system (Black *et al.*, 2004). Most studies show an increase in salivary CRP concentrations between periodontal health (6.7±6.5 pg/ml) and disease (80.4±104.3 pg/ml) (Pederson *et al.*, 1995) (see table 1). However, one study using radial-immuno diffusion to measure CRP concentrations reports significant lower CRP levels in periodontitis (Aurer *et al.*, 2005). Post-treatment salivary CRP concentrations have not been investigated to date.

Although most studies show increased salivary CRP concentrations in periodontitis compared to health, there is also evidence that smoking potentially is a confounding factor (see section IV). Taken together with the lack of post-treatment studies, currently salivary CRP concentrations are likely not the most suitable periodontitis biomarker.

Chemokines

Chemokines are produced by oral tissues in response to pro-inflammatory cytokines such as IL-1, TNF-α or IFN-γ and bacterial products such as LPS or fimbria (reviewed in Silva *et al.*, 2007). 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 chronic periodontitis (Silva *et al.*, 2007).

Salivary concentrations of the neutrophil chemotactic factor IL-8 do not appear to be effected by periodontitis or periodontitis treatment (see table 1 and 2) and concentrations of 457±668 pg/ml and 664±1112 pg/ml have been reported for periodontal health and disease, respectively (Rathnayake *et al.*, 2013b). Significantly higher salivary concentrations of the macrophage chemotactic factor MCP-1 (CCL2) and the Th1-cell chemotactic factor MIP1-α (CCL3) have been found in periodontitis compared to periodontal health (no concentrations available) (Al-Sabbagh *et*

al., 2012; Gupta *et al.*, 2013). A decrease in salivary MCP-1 concentrations was found after periodontal treatment (Gupta *et al.*, 2013) however no change was observed for MIP-1-α (Sexton *et al.*, 2011). There are currently not enough data available to judge if either IL-8, MCP-1 or MIP-1-α are a suitable periodontitis biomarker. No further information is available on salivary concentrations of other chemokines.

HGF

HGF is a multifunctional growth factor, involved in several physiological processes such as tissue development, regeneration and wound healing. It enhances the production of MMPs and stimulates processes of wound healing such as vascularisation and keratinocyte proliferation (Matsumoto *et al.*, 1991; Dunsmore *et al.*, 1996). In oral tissues, HGF is produced by periodontal ligament cells and gingival fibroblasts (Ohshima *et al.*, 2001) in response to pro-inflammatory cytokines such as IL-1, TNF-α or PGE2 and bacterial LTA (Sugiyama *et al.*, 1996; Ohnishi *et al.*, 2000).
Studies investigating salivary concentrations of HGF consistently report increased concentrations in periodontal disease (3430.8±1640.2 pg/ml) compared to periodontal health (443.82±295.14 pg/ml) (Rudrakshi *et al.*, 2011) (see table 1). No further information is available on salivary concentrations of other growth factors.
Although only few studies have investigated salivary HGF concentrations, together with our own findings (unpublished data) all study results are very consistent, with clear differences between periodontal health and disease. Salivary HGF concentrations have not been evaluated yet in a longitudinal study but the consistency of the findings highlight its potential as a periodontitis biomarker on a similar scale as IL-1β.

Adipokines

Adipokines are a loosely termed group of pro-and anti-inflammatory cytokines, growth factors, hormones and chemokines, their main common feature being production by adipose tissue, although they are produced by other tissues and cell types, too. Adipokines such as leptin, adiponectin, visfatin or resistin have lately received some attention in periodontitis research however only two papers investigating salivary visfatin and chemerin concentrations in periodontal health and disease have been published to date.
Although visfatin can be secreted from adipocytes (Tanaka *et al.*, 2007), main sources include immune cells such as macrophages, neutrophils and lymphocytes (Jia *et al.*, 2004; Curat *et al.*, 2006). Visfatin induces pro-inflammatory cytokines such as TNF-α, IL-6 or IL-1β in monocytes and fibroblasts and also acts as a chemoattractant for monocytes and B-cells (Brentano *et al.*, 2007; Moschen *et al.*, 2007). Together with other pro-inflammatory cytokines it therefore potentially plays an important role in periodontal disease. Chemerin is produced by several cell types, including adipocytes, fibroblasts and epithelial cells. True to its name, its key function is to act as a chemoattractant for various immune cells such as macrophages, dendritic cells and NK cells during inflammation (reviewed in Zabel *et al.* (2014)).
Significantly higher salivary concentrations of visfatin and chemerin have been found in periodontitis compared to periodontal health (Ozcan *et al.*, 2014; Tabari *et al.*, 2014). Salivary concentrations of 23.38±7.58 ng/ml and 33.43±15.72 ng/ml for visfatin and of 0.042 ng/ml and 0.084 ng/ml for chemerin have been reported for periodontal health and disease, respectively (Ozcan *et al.*, 2014; Tabari *et al.*, 2014). There are currently not enough data available to judge if any of the adipokines are a suitable periodontitis biomarker.

III 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 with specifically this aim in mind. Therefore, one has to be cautious drawing too strong conclusions from the presented study results.
Reports on salivary IL-1β concentrations in heart disease, hypertension, diabetes, inflammatory bowel disease or rheumatoid arthritis show no significant differences between patients and systemically healthy controls (Mirrielees *et al.*, 2010; Yoon *et al.*, 2012; Rathnayake *et al.*, 2013a), even though oral health status was not always considered in the analysis (Rathnayake *et al.*, 2013a).
A comparable theme can be seen for salivary cytokines IL-6 and TNF-α, concentrations of both do not appear to be effected by systemic conditions (Aleksandra Nielsen *et al.*, 2005; Costa *et al.*, 2010; Mirrielees *et al.*, 2010; Rathnayake *et al.*, 2013a).
An investigation on IL-1RA salivary concentrations in diabetes revealed lower concentrations in patients with periodontal disease than those with periodontal health (Chan *et al.*, 2012). Since a non-diabetic control group was not included in this study, it is not possible to estimate whether local or systemic conditions influence salivary IL-1RA concentrations.
Inflammatory bowel disease was associated with higher salivary IL-8 concentrations in one study, however since oral health status was not considered, it is not possible to evaluate local or systemic effects independently (Rathnayake *et al.*, 2013a). Similar, an increase in salivary MCP-1 (CCL2) concentrations in Sjögren's syndrome cannot be accounted for without evaluation of oral health (Hernandez-Molina *et al.*, 2011). The same study also investigated salivary MIP-1α concentrations and found no differences between patients with Sjögren's syndrome and systemically healthy controls (Hernandez-Molina *et al.*, 2011).

IV The effect of smoking on salivary cytokine concentrations

Salivary concentrations of IL-1β and IL-6 do not appear to be effected by smoking (Nishida *et al.*, 2006; Rathnayake *et al.*, 2013b), however these studies did not account for the periodontal status of either smokers or non-smokers. Assessing salivary IL-8 concentrations in the same study, Rathnayake *et al.* (2013b) report decreased concentrations in smokers compared to non-smokers, indicating that smoking potentially has an effect on this chemokine.
Effects of smoking on salivary concentrations of RANKL and OPG have been investigated in two studies, with different outcomes. Tobon-Arroyave *et al.* (2012) find no difference in RANKL or OPG concentrations between smokers and non-smokers, however oral health was not taken into account. A study by Buduneli *et al.* (2008) reports lower salivary OPG concentrations in smokers with periodontitis than in non-smokers with periodontitis, indicating a possible effect of smoking on OPG concentrations. The same study does not find any differences in salivary RANKL concentrations between smokers and non-smokers (Buduneli *et al.*, 2008).
Lonn *et al.* (2014) do not account for smoking when they show increased salivary HGF concentrations in smokers with periodontitis than in a majority of non-smokers without periodontitis, making it impossible to elucidate effects of either periodontitis or smoking.
After excluding self-reported periodontitis cases, Azar and Richard (2011) conclude that salivary CRP concentrations are higher in smokers than in non-smokers.
Considering the available data, it appears that overall systemic conditions or smoking only have a limited impact on salivary cytokine concentrations. However, more research which specifically aims to evaluate these relationships is needed to come to firm conclusions.

V 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 GCF. Care has to be taken when considering which methodology to choose for analysis, however commercial manufacturers of ELISA kits have picked up on the "saliva" trend and several assays are available.
In conclusion, current evidence suggests that the two cytokines IL-1β and HGF are the most robust salivary periodontitis biomarkers. High quality research designs such as randomized controlled trials 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 permanent tissue and bone damage occurs.

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Table 1: Cross-sectional analysis of salivary cytokine concentrations in systemically healthy subjects in periodontal health and disease

Cytokine	Concentration health	Concentration disease	Main findings
IL-1 family			
IL-1β	n=29 (ELISA, LOC lab-on-a-chip system) (only graph, no numbers)	CP n=28 (ELISA, LOC lab-on-a-chip system) (only graph, no numbers) significantly higher*	significantly increased concentrations in periodontitis (Christodoulides *et al.*, 2007)
	n=29 212.8±167.4 pg/ml	CP n=28 753.7±1022.4 pg/ml*	significantly increased concentrations in periodontitis; significant positive correlation with BOP and CAL (Miller *et al.*, 2006)
	n=303 61±87 pg/ml	mild CP n=89 82±109 pg/ml* severe CP n= 49 144±220 pg/ml*	significantly increased concentrations in both mild and severe periodontitis; significant positive correlation with BOP (Rathnayake *et al.*, 2013b)
	n=44 633±91 (SEM) pg/ml	CP n=74 673±69 (SEM) pg/ml	no difference between periodontitis and health, no significant correlation with BOP (Teles *et al.*, 2009)

	n=30 7.24 ± 7.69 pg/ml	CP n=50 90.94 ± 85.22 pg/ml*	significantly increased concentrations in periodontitis (Ebersole *et al.*, 2013)
	n=81 467.8±279.8 pg/ml	CP n=84 665.7 ± 267.5 pg/ml*	significantly increased concentrations in periodontitis (Gursoy *et al.*, 2009)
	n=24 161.51±149.6 pg/ml	CP n=28 1,312.75 ± 691.22 pg/ml*	significantly increased concentrations in periodontitis; significant positive correlation with BOP (Kaushik *et al.*, 2011)
	n=35 (only graph, no numbers)	CP n=35 (only graph, no numbers)* significantly higher	significantly increased concentrations in periodontitis (Mirrielees *et al.*, 2010)
	n=18 (healthy) 158.6 (0 to 6,000) pg/ml (median + range) n= 23 (gingivitis) 206.7 (0 to 3,856.8) pg/ml (median + range)	n=28 (mild CP) 247.5 (24.1 to 3,120) pg/ml (median + range) n=21 (moderate-severe PD) 462.2 (15.7 to 6,000) pg/ml (median + range)	no difference between periodontitis and health (Ramseier *et al.*, 2009)
IL-18 (IL-1F4)	n=21 143.71±103.68 pg/ml	CP n=22 275. 05±289.46 pg/ml*	significantly increased concentrations in periodontitis; significant positive correlation with BOP

			(Ozcaka *et al.*, 2011)
IL-33 (IL-1F11)	n=25 1.2±0.6 ng/ml	CP n=32 1.1±0.5 ng/ml	no difference between periodontitis and health, no significant correlation with BOP or CAL (Buduneli *et al.*, 2012)
Other Interleukins			
IL-2	n=44 (only graph, no numbers)	CP n=74 (only graph, no numbers)	no difference between periodontitis and health, no significant correlation with BOP (Teles *et al.*, 2009)
	n=18 (healthy) 0.0 (0 to 3,718.1) pg/ml (median + range) n= 23 (gingivitis) 0.0 (0 to 6,000) pg/ml (median + range)	n=28 (mild CP) 8.0 (0 to 6,205.5) pg/ml (median + range) n=21 (moderate-severe PD) 0.0 (0 to 14,400.1) pg/ml (median + range)	no difference between periodontitis and health (Ramseier *et al.*, 2009)
IL-4	n=44 (only graph, no numbers)	CP n=74 (only graph, no numbers)	no difference between periodontitis and health, no significant correlation with BOP (Teles *et al.*, 2009)

	n=15 (only graph, no numbers)	CP n=15 (only graph, no numbers)* significantly lower	significantly decreased concentrations in periodontitis (Napimoga *et al.*, 2011)
	n=18 (healthy) 0.0 (0 to 5,315.1) pg/ml (median + range) n= 23 (gingivitis) 0.0 (0 to 6,579.3) pg/ml (median + range)	n=28 (mild CP) 54.4 (0 to 14,588) pg/ml (median + range) n=21 (moderate-severe PD) 69.5 (0 to 11,714.3) pg/ml (median + range)	no difference between periodontitis and health (Ramseier *et al.*, 2009)
	n=20 (only graph, no numbers)	CP n=20 (only graph, no numbers)* significantly higher	significantly increased concentrations in periodontitis (Prakasam and Srinivasan, 2013)
IL-5	n=44 (only graph, no numbers)	CP n=74 (only graph, no numbers)	no difference between periodontitis and health, no significant correlation with BOP (Teles *et al.*, 2009)
IL-6	n=22 (only graph, no numbers)	CP n=24 (only graph, no numbers)* significantly higher	significantly increased concentrations in periodontitis (Costa *et al.*, 2010)
	n=303 8.0±12 pg/ml	mild CP n=89 7.4±6.9 pg/ml severe CP n= 49	no difference between periodontitis and health, no significant correlation with BOP

		8.4±14.3 pg/ml	(Rathnayake *et al.*, 2013b)
	n=44 (only graph, no numbers)	CP n=74 (only graph, no numbers)	no difference between periodontitis and health, no significant correlation with BOP (Teles *et al.*, 2009)
	n=30 3.30 ± 2.32 pg/ml	CP n=50 35.57 ± 48.17 pg/ml*	significantly increased concentrations in periodontitis (Ebersole *et al.*, 2013)
	n=81 3.1 ± 3.6 pg/ml	CP n=84 3.6 ±5.9 pg/ml	no difference between periodontitis and health (Gursoy *et al.*, 2009)
	n=18 (healthy) 0.0 (0 to 1,915) pg/ml (median + range) n= 23 (gingivitis) 22.1 (0 to 8,784.9) pg/ml (median + range)	n=28 (mild CP) 14.6 (0 to 5,259.7) pg/ml (median + range) n=21 (moderate-severe PD) 88.7 (0 to 10,816.9) pg/ml (median + range)	no difference between periodontitis and health (Ramseier *et al.*, 2009)
	n=20 (only graph, no numbers)	CP n=20 (only graph, no numbers)* significantly higher	significantly increased concentrations in periodontitis (Prakasam and Srinivasan, 2013)

IL-10	n=44 (only graph, no numbers)	CP n=74 (only graph, no numbers)	no difference between periodontitis and health, significant negative correlation with BOP (Teles *et al.*, 2009)
	n=15 (only graph, no numbers)	CP n=15 (only graph, no numbers)* significantly higher	significantly increased concentrations in periodontitis (Napimoga *et al.*, 2011)
	n=18 (healthy) 881.4 (0 to 11,088.8) pg/ml (median + range) n= 23 (gingivitis) 120.6 (0 to 45,488.9) pg/ml (median + range)	n=28 (mild CP) 1,153.1 (0 to 24,581.4) pg/ml (median + range) n=21 (moderate-severe PD) 1,445.1 (0 to 30,633.1) pg/ml (median + range)	no difference between periodontitis and health (Ramseier *et al.*, 2009)
	n=20 (only graph, no numbers)	CP n=20 (only graph, no numbers)* significantly lower	significantly decreased concentrations in periodontitis (Prakasam and Srinivasan, 2013)
IL-13	n=18 (healthy) 14.3 (0 to 83,151.1) pg/ml (median + range) n= 23 (gingivitis) 0.0 (0 to 92,423.8) pg/ml (median +	n=28 (mild CP) 0.0 (0 to 76,046) pg/ml (median + range) n=21 (moderate-severe PD) 169.9 (0 to 75,445.2) pg/ml	no difference between periodontitis and health (Ramseier *et al.*, 2009)

	range)	(median + range)	
IL-17	n=20 (only graph, no numbers)	CP n=20 (only graph, no numbers)* significantly lower	significantly decreased concentrations in periodontitis (Prakasam and Srinivasan, 2013)
	n=21 2.9±0.82 pg/ml	CP n=22 2.22±1.87 pg/ml*	significantly decreased concentrations in periodontitis; no correlation with BOP (Ozcaka *et al.*, 2011)
IFN-γ	n=44 (only graph, no numbers)	CP n=74 (only graph, no numbers)	no difference between periodontitis and health, no significant correlation with BOP (Teles *et al.*, 2009)
RANKL and OPG			
OPG		on CP maintenance therapy n=22 CP (pre-treatment) n=33 (only graph, no numbers)	no difference between patients on periodontitis maintenance therapy and periodontitis patients (Buduneli *et al.*, 2008)
	n=22 (only graph, no numbers)	CP n=24 (only graph, no numbers)	no difference between periodontitis and health (Costa *et al.*, 2010)

	n=29 2.6±1.37 pmol/l	CP n=28 3.6±2.58 pmol/l	no difference between periodontitis and health, significant positive correlation with BOP but not with CAL (Miller *et al.*, 2006)
	n=43 131.6 (82.2-202.4) pg/ml (median + interquartile range)	CP n=97 95.2 (49.8-145.2) pg/ml* (median + interquartile range)	significantly decreased concentrations in periodontitis; significant negative correlation with CAL (Tobon-Arroyave *et al.*, 2012)
	n=40 (only graph, no numbers)	CP n=40 (only graph, no numbers) significantly higher*	significantly increased concentrations in periodontitis; significant positive correlation with BOP and with CAL (Al-Sabbagh *et al.*, 2012)
	n=18 (healthy) 2.3 (1.4 to 6.6) pg/ml (median + range) n= 23 (gingivitis) 2.7 (1.2 to 6.2) pg/ml (median + range)	n=28 (mild CP) 1.9 (0.2 to 10.1) pg/ml (median + range) n=21 (moderate-severe PD) 1.6 (0.5 to 11.8) pg/ml (median + range) * significantly lower	significantly decreased concentrations in periodontitis (Ramseier *et al.*, 2009)
	n=25 (healthy) 2.1±1.0 pg/ml (median + IQR)	n=25 (CP) 2.2 ±0.7 pg/ml (median + IQR)	no difference between periodontitis and health (Tabari *et al.*, 2013)

RANKL (OPG-l)	n=21 0.68±0.96 (SEM) pmol/l	CP n=21 1.15±0.25 (SEM) pmol/l	no difference between periodontitis and health (Frodge *et al.*, 2008)
		on CP maintenance therapy n=22 CP (pre-treatment) n=33 (only graph, no numbers) significantly higher pre-treatment*	significantly higher concentrations in periodontitis patients than in patients on periodontitis maintenance therapy (Buduneli *et al.*, 2008)
	n=43 4.0 (2.4–6.6) pg/ml (median + interquartile range)	CP n=97 6.0 (2.7–11.1) pg/ml* (median + interquartile range)	significantly increased concentrations in periodontitis; significant positive correlation with CAL (Tobon-Arroyave *et al.*, 2012)
	n=25 (healthy) 207±83 pg/ml (median + IQR)	n=25 (CP) 266 ±48 pg/ml* (median + IQR)	significantly increased concentrations in periodontitis; significant positive correlation with CAL (Tabari *et al.*, 2013)
TNF-α			
TNF-α	n=14 ELISA below level of detection	CP n=10 ELISA below level of detection AgP n=9 ELISA below level of detection	no TNF-α was detected (Aurer *et al.*, 2005)
	n=39 2.03±0.49 (SEM)	CP n=35 4.33±0.73 (SEM)	significantly increased concentrations in periodontitis (Frodge

	pg/ml	pg/ml*	*et al.*, 2008)
	n=44 (only graph, no numbers)	CP n=74 (only graph, no numbers)	no difference between periodontitis and health, no significant correlation with BOP (Teles *et al.*, 2009)
	n=30 1.85 ± 2.11 pg/ml	CP n=50 5.44 ± 10.88 pg/ml	no difference between periodontitis and health (Ebersole *et al.*, 2013)
	n=81 2.7 ± 2.8 pg/ml	CP n=84 2.9 ± 4.0 pg/ml	no difference between periodontitis and health (Gursoy *et al.*, 2009)
	n=35 (only graph, no numbers)	CP n=35 (only graph, no numbers)	no difference between periodontitis and health (Mirrielees *et al.*, 2010)
	n=18 (healthy) 9.8 (0 to 1,788.3) pg/ml (median + range) n= 23 (gingivitis) 0.0 (0 to 3,720.5) pg/ml (median + range)	n=28 (mild CP) 8.1 (0 to 4,370.2) pg/ml (median + range) n=21 (moderate-severe PD) 0.0 (0 to 8,212.7) pg/ml (median + range)	no difference between periodontitis and health (Ramseier *et al.*, 2009)

CRP			
	n=14 (no ELISA, radial-immuno diffusion) 90.20±79.67 (no unit given)	CP n=10 (no ELISA, radial-immuno diffusion) 27.45±29.59* (no unit given) AgP n=9 (no ELISA, radial-immuno diffusion) 102.11±79.02 (no unit given)	significantly decreased concentrations in chronic periodontitis, no difference to aggressive periodontitis (Aurer *et al.*, 2005)
	n=15 (no ELISA, ETC lab-on-a-chip system) 92 pg/ml	CP n=15 (no ELISA, LOC lab-on-a-chip system) 2001 pg/ml*	significantly increased concentrations in periodontitis (Christodoulides *et al.*, 2005)
	n=29 (not detected in ELISA, LOC lab-on-a-chip system) (only graph, no numbers)	CP n=28 (not detected in ELISA, LOC lab-on-a-chip system (only graph, no numbers) significantly higher*	significantly increased concentrations in periodontitis (Christodoulides *et al.*, 2007)
	n=5 6.7±6.5 pg/ml	mild-moderate CP n=18 43.4±49.6 pg/ml* moderate-severe CP n=8 80.4±104.3 pg/ml*	significantly increased concentrations in periodontitis (Pederson *et al.*, 1995)
Chemokines			
IL-8 (CXCL8)	n=303 457±668 pg/ml	mild CP n=89 575±532 pg/ml severe CP n= 49	no difference between periodontitis and health, significant

		664±1112 pg/ml	positive correlation with BOP (Rathnayake *et al.*, 2013b)
	n=44 1945±181 (SEM) pg/ml	CP n=74 2268±111 (SEM) pg/ml	no difference between periodontitis and health, significant positive correlation with BOP (Teles *et al.*, 2009)
MIP1-α (CCL3)	n=40 (only graph, no numbers)	CP n=40 (only graph, no numbers) significantly higher*	significantly increased concentrations in periodontitis; significant positive correlation with BOP and with CAL (Al-Sabbagh *et al.*, 2012)
MCP-1 (CCL2)			
	n=15 (only graph, no numbers)	CP n=30 (only graph, no numbers) significantly higher*	significantly increased concentrations in periodontitis; significant positive correlation with CAL (Gupta *et al.*, 2013)
Adipokines			

Chemerin	n=23 0.042 (0.023-0.063) ng/ml (median + 25-75%) gingivitis n=24 0.042 (0.042-0.063) ng/ml (median + 25-75%)	CP n=25 0.084 (0.063-0.105) ng/ml (median + 25-75%)*	significantly increased concentrations in periodontitis; significant positive correlation with CAL (Ozcan *et al.*, 2014)
Visfatin	n=20 23.38±7.58 ng/ml	CP n=20 33.43±15.72 ng/ml*	significantly increased concentrations in periodontitis; significant positive correlation with CAL (Tabari *et al.*, 2014)
	n=23 267 (125-616) ng/ml (median + 25-75%) gingivitis n=24 791 (267-1127) ng/ml (median + 25-75%)*	CP n=25 589 (302-1195) ng/ml (median + 25-75%)*	significantly increased concentrations in both gingivitis and periodontitis; no significant correlation with CAL (Ozcan *et al.*, 2014)
Growth factors			
GM-CSF	n=44 (only graph, no numbers)	CP n=74 (only graph, no numbers)	no difference between periodontitis and health, no significant correlation with BOP (Teles *et al.*, 2009)
HGF	n=12 443.82±295.14 pg/ml	moderate CP n=12 1878.99±1713.54 pg/ml*	significantly increased concentrations in periodontitis; significant positive correlation with BOP,

		severe CP n=12 3430.8±1640.2 pg/ml*	no significant correlation with CAL (Rudrakshi *et al.*, 2011)
	n=20 0.68 (0-7.33) median (range) ng/ml	CP n=26 1.87±1.32 ng/ml* 0.06-5.38 ng/ml (range)	significantly increased concentrations in periodontitis; significant positive correlation with BOP, no significant correlation with CAL (Wilczynska-Borawska *et al.*, 2006)

Table 2: Longitudinal analysis of salivary cytokine concentrations in systemically healthy subjects before and after periodontal treatment

Cytokine	Concentration pre-treatment	Concentration post-treatment	Main findings
IL-1 family			
IL-1β	CP, n=24 (only graph, no numbers)	12 months later (only graph, no numbers)* significantly lower	significantly decreased concentration after periodontal treatment (Kinney *et al.*, 2011)
	CP, n=34 (only graph, no	7 months later (only graph, no	significantly decreased

	numbers)	numbers)* significantly lower	concentration after periodontal treatment (Sexton *et al.*, 2011)
	CP n=28 1,312.75 ± 691.22 pg/ml	1 month later 674.34±480.89 pg/ml*	significantly decreased concentration after periodontal treatment (Kaushik *et al.*, 2011)
Other Interleukins			
IL-2	CP n=24 (only graph, no numbers)	12 months later no difference (only graph, no numbers)	concentrations are not effected by periodontal treatment (Kinney *et al.*, 2011)
IL-4	CP n=24 (only graph, no numbers)	12 months later no difference (only graph, no numbers)	concentrations are not effected by periodontal treatment (Kinney *et al.*, 2011)
	CP n= 20 (only graph, no numbers)	6 weeks later significantly lower* (only graph, no numbers)	significantly decreased concentration after periodontal treatment (Prakasam and Srinivasan, 2013)
IL-5	CP n=24 (only graph, no numbers)	12 months later no difference (only graph, no numbers)	concentrations are not effected by periodontal treatment (Kinney *et al.*, 2011)
IL-6	CP n=24 (only graph, no	12 months later	concentrations are not effected by periodontal

	numbers)	no difference (only graph, no numbers)	treatment (Kinney *et al.*, 2011)
	CP n= 20 (only graph, no numbers)	6 weeks later (only graph, no numbers) no difference	concentrations are not effected by periodontal treatment (Prakasam and Srinivasan, 2013)
IL-10	CP n=24 (only graph, no numbers)	12 months later no difference (only graph, no numbers)	concentrations are not effected by periodontal treatment (Kinney *et al.*, 2011)
	CP n= 20 (only graph, no numbers)	6 weeks later (only graph, no numbers) no difference	concentrations are not effected by periodontal treatment (Prakasam and Srinivasan, 2013)
IL-17	CP n= 20 (only graph, no numbers)	6 weeks later (only graph, no numbers) no difference	concentrations are not effected by periodontal treatment (Prakasam and Srinivasan, 2013)
IFN-γ	CP n=24 (only graph, no numbers)	12 months later no difference (only graph, no numbers)	concentrations are not effected by periodontal treatment (Kinney *et al.*, 2011)
TNF-α	CP n=24 (only graph, no numbers)	12 months later no difference (only graph, no numbers)	concentrations are not effected by periodontal treatment (Kinney *et al.*, 2011)

	CP, n=34 (only graph, no numbers)	7 months later (only graph, no numbers)* significantly lower	significantly decreased concentration after periodontal treatment (Sexton *et al.*, 2011)
OPG			
OPG	CP, n=24 (only graph, no numbers)	12 months later (only graph, no numbers)* significantly lower	significantly decreased concentration after periodontal treatment (Kinney *et al.*, 2011)
	CP, n=34 (only graph, no numbers)	7 months later (only graph, no numbers)* significantly lower	significantly decreased concentration after periodontal treatment (Sexton *et al.*, 2011)
Chemokines			
IL-8 (CXCL8)	CP, n=34 (only graph, no numbers)	7 months later (only graph, no numbers) no difference	concentrations are not effected by periodontal treatment (Sexton *et al.*, 2011)
MIP1-α (CCL3)	CP, n=34 (only graph, no numbers)	7 months later (only graph, no numbers) no difference	concentrations are not effected by periodontal treatment (Sexton *et al.*, 2011)
MCP-1 (CCL2)	CP n=30 (only graph, no numbers)	1/1/2 months later (only graph, no numbers) significantly lower*	significantly decreased concentration after periodontal treatment (Gupta *et*

			al., 2013)