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Interleukin-17 and interleukin-18 levels in saliva and plasma of patients with chronic periodontitis

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Background and Objective: This study was planned to investigate whether patients with chronic periodontitis exhibit different salivary and/or plasma concentrations of interleukin (IL)-17 and IL-18 compared with clinically healthy subjects.

Material and Methods: Whole saliva and blood samples, together with full-mouth clinical periodontal recordings, were obtained from 22 otherwise healthy untreated nonsmokers with chronic periodontitis and from 21 systemically and periodontally healthy control subjects. The concentrations of IL-17 and IL-18 in saliva and plasma were determined using ELISAs.

Results: The healthy control group exhibited significantly lower values in all clinical periodontal measurements ($p < 0.001$). The salivary concentration of IL-17 was significantly lower, and that of IL-18 significantly higher, in patients from the chronic periodontitis group compared with healthy control subjects ($p = 0.025$ and $p = 0.009$, respectively). Plasma IL-17 and IL-18 concentrations were similar in the two study groups ($p > 0.05$).

Conclusion: Within the limits of the present study, it may be suggested that an elevated salivary IL-18 level in untreated nonsmoker chronic periodontitis patients has the potential to be a biomarker for periodontal tissue destruction.

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Chronic periodontitis is a bacterially induced inflammatory disease, and immune response plays a major role in alveolar bone destruction observed in this disease entity. It has been postulated that 'appropriate' cytokine production results in protective immunity, while 'inappropriate' cytokine production leads to tissue destruction and disease progression (1). Furthermore, it is largely accepted that the control of the T helper 1 (Th1)/T helper 2 (Th2) cell balance is central to the immunoregulation of periodontal disease (1,2).

Interleukin (IL)-17 is a T-cell-derived cytokine that is also produced by macrophages, dendritic cells, mast cells and natural killer cells (3). The IL-17 family includes IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25) and IL-17F. IL-17-producing $CD4^+$ T cells (Th17 cells) are a cell population distinct from Th1 and Th2 cells. IL-17 synergizes with other cytokines, including IL-1β, tumor necrosis factor-α (TNF-α), oncostatin M and interferon-γ, resulting in profound biologic effects *in vivo* (4). IL-17 increases RANKL expression and concomitantly decreases osteoprotegerin expression in osteoblastic cells *in vitro* and *in vivo*, thereby enhancing osteoclast formation and bone erosion in a mouse model of arthritis (5). IL-17 drives bone remodeling, like many inflammatory cytokines, and favors

bone loss (6). On the other hand, IL-17 has been suggested to play a bone-protective role from pathogens such as *Porphyromonas gingivalis* (7). Therefore, the role of IL-17 in periodontal disease is still obscure. IL-17 was detected in biopsy samples of periodontal tissue obtained during periodontal surgery (8). The levels of IL-17 are significantly higher in gingival crevicular fluid samples and culture supernatants of gingival cells in periodontitis patients than in healthy controls (9). IL-17 has been suggested to have pro-inflammatory effects, causing bone resorption and inflammation by inducing the secretion of IL-1β, TNF-α and IL-6, while it also stimulates chemokine release and induces the expression of various MMPs (10,11).

IL-18 is a pro-inflammatory cytokine of the IL-1 superfamily and was originally termed interferon-γ-inducing factor. IL-18 is produced by activated macrophages, keratinocytes, dendritic cells, intestinal epithelial cells, osteoblasts and adrenal cortex cells (12). It plays a pro-inflammatory role alongside IL-1β, and amplifies immune responses by inducing other cytokines (IL-1β, TNF-α and IL-8) (13). IL-18 can drive both Th1 and Th2 responses (12,14). It can stimulate neutrophil migration/activation and osteoclastic activity, and is important in the removal of intracellular pathogens and viruses (15–17). IL-18 has been suggested to be related to periodontal disease because increased levels have been reported in gingival crevicular fluid, serum and gingival tissue samples (18–23).

Saliva has the potential to provide information on systemic, as well as oral health status. Whole saliva contains constituents of exocrine glands in the oral cavity and also gingival crevicular fluid. Saliva is readily available and easily collected without specialized equipment or personnel. In addition, as whole saliva represents a pooled sample with contributions from all periodontal sites, analysis of biomarkers in saliva may provide an overall assessment of disease status as opposed to site-specific gingival crevicular fluid analysis (24). Plasma, on the other hand, can provide information about the inflammatory stimulus and/or response generated in the circulation towards periodontal pathogens that colonize the subgingival area.

As yet, the relationship between chronic periodontitis and the concentrations of salivary and plasma IL-17 and IL-18 has not been clarified. As a result of their pro-inflammatory functions, our hypothesis was that the salivary and/or plasma concentrations of IL-17 and IL-18 may be increased in chronic periodontitis patients and may have potential as biomarkers. Therefore, the aim of this study was to investigate whether patients with chronic periodontitis exhibit different salivary and plasma concentrations of IL-17 and IL-18 than the periodontally healthy control subjects.

Material and Methods

Study population

A total of 43 untreated subjects initially presenting to the School of Dentistry, Ege University, were included in the present study. Twenty-two otherwise healthy, untreated nonsmoker chronic periodontitis patients and 21 nonsmoker systemically and periodontally healthy subjects were recruited between May 2008 and December 2009. The male/female ratios were 16/6 and 11/10, respectively, in the chronic periodontitis and clinically healthy control groups. The study was conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki. The study protocol was explained, and written informed consent was received from each individual before clinical periodontal examination and saliva sampling were carried out. Medical and dental histories were obtained. All patients with chronic periodontitis had at least 20 teeth. Patients with medical disorders such as diabetes mellitus, immunologic disorders and hepatitis, and those who had received antibiotic or periodontal treatment in the previous 6 mo, were excluded from the study.

Chronic periodontitis was diagnosed in accordance with previously reported clinical criteria (25). The group of patients with chronic periodontitis had at least four teeth in each jaw with a probing depth of ≥ 5 mm, a clinical attachment level of ≥ 4 mm and ≥ 50% alveolar bone loss in at least two quadrants. Assessment of the extent and severity of alveolar bone loss was performed radiographically. Bitewing radiographs were evaluated to determine interproximal bone loss from the cemento–enamel junction of the tooth to the bone crest. These patients also had bleeding on probing at > 80% of the proximal sites. Moreover, the clinical attachment level was commensurate with the amount of supragingival plaque. The healthy control group had at least 20 teeth, and ≥ 90% of the measured sites exhibited a probing depth of < 3 mm, a clinical attachment level of ≤ 1 mm, low levels of bleeding on probing and no radiographic sign of alveolar bone loss (i.e. a distance of < 3 mm between the cemento–enamel junction and the bone crest at > 95% of the proximal tooth sites).

Saliva sampling

In the morning following an overnight fast, during which subjects were requested not to drink (except water) or to chew gum, whole saliva samples were obtained by expectorating into polypropylene tubes; clinical periodontal measurements and any necessary periodontal interventions were then carried out. The method described by Navazesh (26) was used for saliva sampling. The saliva samples were clarified by centrifugation (800 *g*) for 10 min at +4°C and aliquoted into 500-μL amounts with H_2O. The samples were immediately frozen and stored at −40°C until the sample collection period was completed; samples were kept frozen until required for assays, when they were thawed immediately before use.

Plasma sampling

The venous blood samples were collected using a standard venipuncture method and the plasma was separated from blood by centrifugation at 1500 *g* for 10 min. The plasma

samples were then stored at −40°C until required for biochemical analyses, when they were thawed immediately before use.

Clinical measurements

Subsequent to saliva sampling, clinical periodontal recordings, including dichotomous plaque index (+/−), probing depth, clinical attachment level and presence of bleeding on probing (+/−), were performed at six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual) on each tooth present, except for the third molars, using a Williams periodontal probe (Hu-Friedy, Chicago, IL, USA). The clinical attachment level was assessed from the cemento–enamel junction to the base of the probable pocket. Bleeding on probing (deemed positive if it occurred within 15 s after periodontal probing) and visible plaque accumulation were recorded dichotomously by visual examination. All measurements were performed by a single calibrated examiner (Ö.Ö.). The intra-examiner reliability was high, as revealed by intraclass correlation coefficients of 0.87 and 0.85 for probing depth and clinical attachment level measurements, respectively.

Measurement of IL-17A and IL-18 in saliva and plasma samples

The ELISA development kits (Bender MedSystems, Vienna, Austria) were used to analyze the concentrations of both IL-17 and IL-18 in the saliva and plasma samples. For both saliva and plasma samples a standard volume of 100 μL was assayed. The saliva samples were assayed neat, whereas the plasma samples were diluted by ½. The manufacturers' guidelines were followed for each assay. The concentrations of IL-17A and IL-18 in the saliva and plasma samples were then determined by comparing the average absorbance readings of each sample with the concentrations in the assay standard curve. The results were expressed as pg/mL and the lower detection thresholds for the IL-17A and IL-18 assays were 0.5 and 9 pg/mL, respectively.

Statistical analysis

A pilot experiment, where the concentration of IL-18 was measured and a 20% difference was obtained, was utilized for statistical power calculations. With a power of 80% and $\alpha = 0.05$, the minimum number of subjects required for the comparisons was 20 for each group.

Both clinical and biochemical parameters were tested for normality using the Shapiro–Wilk test. As they were not normally distributed, the data were analyzed using nonparametric tests. The concentrations of IL-17 and IL-18 were compared between the chronic periodontitis and periodontally healthy groups using the Mann–Whitney *U*-test, separately for plasma and saliva samples. The possible correlations between the biochemical variables and the clinical periodontal measurements were computed using the Spearman rho rank test. All tests were performed at a significance level of $\alpha = 0.05$. All statistical calculations were performed using the statistical software package (SPSS Türkiye, version 17.0; Maltepe, İstanbul, Turkey).

Results

Clinical analyses

Demographic variables, median values and mean values of clinical periodontal measurements are outlined in Table 1. The age range of the healthy control group was 31–65 years, whereas that of the chronic periodontitis group was 35–65 years. The patients in the healthy control group were significantly younger than the patients in the chronic periodontitis group ($p = 0.004$). There was no significant difference in gender distribution between the study groups ($p = 0.21$).

Salivary and plasma concentrations of IL-17 and IL-18

The salivary IL-17 concentrations were significantly lower in the chronic periodontitis group than in the control group ($p = 0.009$). The salivary IL-18 concentrations were significantly higher in the chronic periodontitis group than in the control group ($p = 0.025$). The plasma IL-17 and IL-18 concentrations were similar in both the chronic periodontitis and control groups ($p > 0.05$) (Table 2).

Salivary IL-17 concentrations correlated with plaque index in the chronic periodontitis patients ($r = 0.437$, $p = 0.042$). Correlation analyses between the clinical parameters and analyte concentrations revealed significant, positive correlations between the plasma IL-18 concentrations and probing depth and plaque index in the chronic periodontitis group ($r = 0.599$, $p = 0.003$ and $r = 0.563$, $p = 0.044$, respectively). Salivary IL-18 concentrations correlated with probing depth and bleeding on probing in the chronic periodontitis group ($r = 0.428$, $p = 0.047$ and $r = 0.403$, $p = 0.029$, respectively). No significant correlations were observed between plasma and salivary IL-17 and IL-18 concentrations.

Table 1. Clinical periodontal measurements in patients with chronic periodontitis and in periodontally healthy subjects

	Chronic periodontitis ($n = 22$)		Periodontally healthy ($n = 21$)		
Clinical variable	Median (min.–max.)	Mean (SD)	Median (min.–max.)	Mean (SD)	*p* value
Age (years)	48.00 (35–65)	49.60 (8.27)	41.00 (31–65)	41.90 (8.13)	0.004
PD (mm)	3.91 (3.1–6.6)	4.09 (0.97)	1.60 (1.2–1.9)	1.60 (0.19)	< 0.001
CAL (mm)	5.40 (3.4–9.1)	5.59 (1.4)	0.00 (0–0.6)	0.02 (0.13)	< 0.001
BOP (%)	72.00 (14–100)	68.00 (27.7)	10.00 (0–25)	12.00 (7.88)	< 0.001
PI (%)	95.00 (22–100)	79.70 (27.4)	18.00 (0–31)	16.10 (9.7)	< 0.001

BOP, bleeding on probing; CAL, clinical attachment level; max., maximum; min. minimum; PD, probing depth; PI, plaque index; SD, standard deviation.

Table 2. Salivary and plasma concentrations of interleukin (IL)-17 and IL-18 in the study groups

	Chronic periodontitis (n = 22)		Periodontally healthy (n = 21)		
	Median (min.–max.)	Mean (SD)	Median (min.–max.)	Mean (SD)	p value
Saliva					
IL-17	1.09 (0.94–8.25)	2.22 (1.87)	2.51 (1.83–4.24)	2.9 (0.82)	0.009
IL-18	193.91 (35.78–1386.4)	275. 05 (289.46)	121.73 (35.73–442.06)	143.71 (103.68)	0.025
Plasma					
IL-17	2.38 (2.03–8.01)	3.19 (1.43)	2.54 (2.06–8.56)	3.32 (1.6)	0.961
IL-18	219.72 (79.26–532.73)	238.39 (106.42)	180.97 (79.26–837.53)	273.18 (198.32)	0.865

max., maximum; min. minimum; SD, standard deviation.

Discussion

In the present study, we analyzed the salivary and plasma concentrations of IL-17 and IL-18 in chronic periodontitis patients as well as in healthy subjects, all of whom were nonsmokers. Significantly lower salivary IL-17 concentrations and higher IL-18 concentrations were present in the chronic periodontitis patients than in the patients of the control group. The IL-17 and IL-18 concentrations in plasma were similar in both study groups. It has been reported that host cytokine levels are affected by smoking (27–29). Therefore, only nonsmoker individuals were included in the present study. The subjects in the healthy control group were significantly younger than the patients in the chronic periodontitis group. However, because there was no correlation between age and IL-17 and IL-18 levels, and no publication has suggested that age affects the levels of IL-17 and IL-18, we claim that this age difference had no effect on our present findings.

Several methods of saliva collection are available, including the collection of unstimulated whole saliva, the collection of whole saliva stimulated with, typically, paraffin wax, gum base or citric acid, or the collection of saliva from specific salivary glands. Whole saliva contains gingival crevicular fluid, immune cells and tissue metabolites (26,30) and reflects most closely the predominant intraoral condition (31). Stimulation, on the other hand, may increase the flow of gingival crevicular fluid and this may result in false increases in the concentration of various components in the saliva (32). Thus, we collected expectorated whole saliva, where the degree of stimulation was minimal relative to that obtained when using gum, citric acid or paraffin wax.

Th17 cells have been suggested to play an important role in protective antibacterial host responses in many gram-negative infections (7,33,34). The IL-17 family includes IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25) and IL-17F. IL-17A is the most studied family member and is usually referred to as IL-17. Accordingly, we evaluated the saliva and plasma concentrations of IL-17A in the present study.

Vernal *et al.* (9) reported a higher concentration of IL-17 in the gingival crevicular fluid of periodontitis patients than in the gingival crevicular fluid of healthy controls and suggested a role for IL-17 in the pathogenesis of chronic periodontitis. Lester *et al.* (35) found an increased concentration of IL-17 in gingival tissue supernatants at sites of severe attachment loss where a positive correlation was detected between the tissue level of this cytokine and clinical attachment loss. Yetkin Ay *et al.* (36) suggested that the decreased ratio of IL-11/IL-17 in the gingival crevicular fluid samples of patients with chronic periodontitis compared with clinically healthy subjects confirms that IL-17 may have a role in the pathogenesis of chronic periodontitis. In a recent intervention study (37), the IL-17 concentration in gingival crevicular fluid was reported to increase 4 wk after completion of scaling and root planing, which can be explained by the decrease in gingival crevicular fluid volume sampled after treatment. Johnson *et al.* (38) reported similar gingival tissue concentrations of IL-17 adjacent to pockets of ≥ 6 mm and healthy sulci of ≤ 3 mm. According to the present findings, the plasma levels of IL-17 were similar in the chronic periodontitis patients and healthy control subjects, while salivary IL-17 was lowered in the chronic periodontitis patient group. Increased gingival crevicular fluid levels of IL-17 in the above-mentioned studies may indicate a local event adjacent to areas of bone resorption, with no significant systemic sequelae. Therefore, saliva or plasma samples may not reveal significant effects on IL-17 content and indeed this seems to be the case according to the present findings.

In fact, understanding the role of Th17 cells and IL-17 in periodontal disease is at its very early stages and their specific role in disease pathogenesis and host protection is not known (39). Increased levels of IL-17 in patients with chronic periodontitis have been reported in clinical studies (8,9,40). Furthermore, *IL-17* mRNA was shown to be expressed at higher levels in diseased gingival tissue (41,42). Increased expression of *IL-17* and *RANKL* mRNA were found in chronic periodontitis lesions from progressive periodontal lesions compared with quiescent lesions (43), leading to speculation that Th17 cytokines associated with bone resorption may be markers of, and mediators of, periodontal disease activity (44). The lack of a significant difference between the present study groups in plasma IL-17 levels may be explained by the state of periodontal disease activity, namely periodontal tissue breakdown possibly being in the quiescent period. The rather high variation also suggests that samples from different disease activity characteristics may have been grouped together.

In a recent study, Schenkein *et al.* (44) investigated serum levels of IL-17 in patients with aggressive periodontitis in comparison with healthy controls

and reported that it was barely detectable in healthy individuals (1.9 pg/mL), but was significantly elevated in patients with localized and generalized aggressive periodontitis (7.6 and 17.1 pg/mL, respectively). They speculated that this elevated serum IL-17 level may well indicate an autoimmune response when combined with evidence that aggressive periodontitis is familial and likely to have a significant component of genetic risk (44). Our present findings revealed low plasma levels of IL-17 in periodontally healthy subjects as well as in chronic periodontitis patients (2.54 and 2.38 pg/mL, respectively). These levels are close to those reported in the healthy control group in the study of Schenkein *et al*. (44) and may be regarded as expected because chronic periodontitis differs from aggressive periodontitis in many aspects, such as genetic factors, progression rate and susceptibility factors.

IL-18 contributes to the inflammatory response by working in synergy with other inflammatory cytokines, particularly IL-12 (45). Elevated plasma IL-18 concentrations were found in severe inflammatory and septic conditions, in association with poor clinical outcome, and were proposed as a marker of severe inflammatory conditions (46). Schallhorn *et al*. (47) reported a significant, positive correlation between plasma IL-18 levels and bleeding on probing in patients undergoing cardiac catheterization.

Johnson and Serio (19) reported that the IL-18 concentrations in gingival tissue samples were higher in sites with a probing depth of > 6 mm compared with healthy sites. Moreover, they detected a positive correlation between the concentration of IL-18 and probing depth, suggesting a contribution of IL-18 to a nonresolving hyper-inflammation mediated by a shift towards a Th2 response (19). IL-18 is up-regulated during rheumatoid arthritis (48). Periodontitis and rheumatoid arthritis are chronic inflammatory diseases that share similar biologic mechanisms of tissue destruction. An altered systemic immune response, manifested with increased serum levels of IL-18, was suggested to make patients with juvenile idiopathic arthritis more prone to periodontal disease progression (20). Furthermore, Orozco *et al*. (21) investigated the gingival crevicular fluid levels of various cytokines in gingivitis and periodontitis patients and suggested that there is an association between the severity of periodontal disease and the concentration of IL-18. Figueredo *et al*. (18) also detected an increased concentration of IL-18 in the gingival crevicular fluid of periodontitis patients. Recently, Pradeep *et al*. (23) reported that the IL-18 concentrations in gingival crevicular fluid were higher in periodontitis patients compared with gingivitis patients and healthy controls, and that the IL-18 concentration decreased after initial periodontal therapy. They also confirmed the positive correlation between gingival crevicular fluid IL-18 levels and probing depth and attachment loss. Similarly, we detected positive correlations between salivary IL-18 levels and probing depth and bleeding on probing in chronic periodontitis patients. Our present findings in saliva samples are in agreement with previous reports and suggest that at least some of this cytokine is produced locally in the periodontal tissues, providing further support for the hypothesis that IL-18 may be a biomarker for periodontal tissue destruction (23).

IL-18 is synthesized intracellularly as a biologically inactive precursor (49) and is secreted as an 18-kDa inactive form, requiring caspase-1 to cleave it into the active IL-18 molecule (12). This process could occur after toll-like receptor (TLR)4 is activated by lipopolysaccharide (45). TLR2 and TLR5 in human gingival epithelial cells have been suggested to co-operate with IL-17 (50). In a previous part of this study (51), we detected significantly elevated concentrations of TLR4 in saliva samples of patients with chronic periodontitis. Taken together with our previous findings, the present data now suggest a relationship between elevated salivary TLR4 and IL-18 levels.

Highly specific and sensitive biomarkers for the diagnosis and monitoring of periodontal diseases are still needed for early and better detection of periodontal tissue destruction. The cut-off level for a periodontitis site at risk of disease progression has been described previously for chairside MMP8 point-of-care assays, and for the dentoAnalyzer and dip-stick methods (52,53). Studies on saliva samples and various potential biomarkers in saliva are currently receiving much attention and may eventually lead to the development of such chairside tests for the early diagnosis of periodontal tissue destruction.

In conclusion, within the limits of the present study, it may be suggested that an elevated concentration of salivary IL-18 in untreated nonsmoker chronic periodontitis patients has the potential to be a biomarker for periodontal tissue destruction and gingival inflammation. Larger-scale and intervention studies are required to address this issue in greater detail.

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References

1. Gemmell E, Seymour GJ. Immunoregulatory control of Th1/Th2 cytokine profiles in periodontal disease. *Periodontol 2000* 2004;**35:**21–41.
2. Seymour GJ, Gemmell E. Cytokines in periodontal disease: where to from here? *Acta Odontol Scand* 2001;**59:**167–173.
3. Yao Z, Painter SL, Fanslow WC *et al*. Human IL-17: a novel cytokine derived from T cells. *J Immunol* 1995;**155:**5483–5486.
4. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 cells. *Annu Rev Immunol* 2009;**27:**485–517.
5. Lubberts E, van den Bersselaar L, Oppers-Walgreen B *et al*. IL-17 promotes bone erosion in murine collagen-induced arthritis through loss of the receptor activator of NF-kappa B ligand/osteoprotegerin balance. *J Immunol* 2003;**170:**2655–2662.
6. Kramer JM, Gaffen SL. Interleukin-17: a new paradigm in inflammation, autoimmunity, and therapy. *J Periodontol* 2007; **78:**1083–1093.

7. Yu JJ, Ruddy MJ, Wong GC *et al*. An essential role for IL-17 in preventing pathogen-initiated bone destruction: recruitment of neutrophils to inflamed bone requires IL-17 receptor-dependent signals. *Blood* 2007;**109:**3794–3802.
8. Takahashi K, Azuma T, Motohira H, Kinane DF, Kitetsu S. The potential of interleukin-17 in the immunopathology of periodontal disease. *J Clin Periodontol* 2005;**32:**369–374.
9. Vernal R, Dutzan N, Chaparro A, Puente J, Valenzuela MA, Gammonal J. Levels of interleukin-17 in gingival crevicular fluid and in supernatants of cellular cultures of gingival tissue from patients with chronic periodontitis. *J Clin Periodontol* 2005;**32:** 383–389.
10. Beklen A, Ainola M, Hukkanen M, Gürgan C, Sorsa T, Konttinen YT. MMPs, IL-1, and TNF are regulated by IL-17 in periodontitis. *J Dent Res* 2007;**86:** 347–351.
11. Onishi RM, Gaffen SL. Interleukin-17 and its target genes: mechanisms of interleukin-17 function in disease. *Immunology* 2010;**129:**311–321.
12. Dinarello CA. Interleukin-18. *Methods* 1999;**19:**121–132.
13. Gracie JA, Robertson SE, McInnes IB. Interleukin-18. *J Leukoc Biol* 2003;**73:** 213–224.
14. Dinarello CA. Interleukin-18, a proinflammatory cytokine. *Eur Cytokine Netw* 2000;**11:**483–486.
15. Arend WP, Palmer G, Gabay C. IL-1, IL-18, and IL-33 families of cytokines. *Immunol Rev* 2008;**223:**20–38.
16. Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol* 2009;**27:**519–550.
17. Puren AJ, Razeghi P, Fantuzzi G, Dinarello CA. Interleukin-18 enhances lipopolysaccharide-induced interferon-gamma production in human whole blood cultures. *J Infect Dis* 1998;**178:**1830–1834.
18. Figueredo CM, Rescala B, Teles RP *et al*. Increased interleukin-18 in gingival crevicular fluid from periodontitis patients. *Oral Microbiol Immunol* 2008;**23:**173–176.
19. Johnson RB, Serio FG. Interleukin-18 concentrations and the pathogenesis of periodontal disease. *J Periodontol* 2005;**76:** 785–790.
20. Miranda LA, Fischer RG, Sztajnbok FR, Johansson A, Figueredo CM, Gustafsson A. Increased interleukin-18 in patients with juvenile idiopathic arthritis and early attachment loss. *J Periodontol* 2005;**76:**75–82.
21. Orozco A, Gemmell E, Bickel M, Seymour GJ. Interleukin-18 and periodontal disease. *J Dent Res* 2007;**86:**586–593.
22. Orozco A, Gemmell E, Bickel M, Seymour GJ. Interleukin-1beta, interleukin-12 and interleukin-18 levels in gingival fluid and serum of patients with gingivitis and periodontitis. *Oral Microbiol Immunol* 2006;**21:**256–260.
23. Pradeep AR, Happy D, Hadge P, Garg G, Thorat M. Correlation of gingival crevicular fluid interleukin-18 and monocyte chemoattractant protein-1 levels in periodontal health and disease. *J Periodontol* 2009;**80:**1454–1461.
24. Miller CS, King CP Jr, Langub MC, Kryscio RJ, Thomas MV. Salivary biomarkers of existing periodontal disease: a cross-sectional study. *J Am Dent Assoc* 2006;**137:**322–329.
25. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* 1999;**4:**1–6.
26. Navazesh M. Methods for collecting saliva. *Ann N Y Acad Sci* 1993;**694:**72–77.
27. Boström L, Linder LE, Bergström J. Smoking and crevicular fluid levels of IL-6 and TNF-alpha in periodontal disease. *J Clin Periodontol* 1999;**26:**352–357.
28. Buduneli N, Bıyıkoğlu B, Sherrabeh S, Lappin DF. Saliva concentrations of RANKL and osteoprotegerin in smoker versus non-smoker chronic periodontitis patients. *J Clin Periodontol* 2008;**35:**846–852.
29. Özçaka Ö, Bıçakcı N, Pussinen P, Sorsa T, Köse T, Buduneli N. Smoking and matrix metalloproteinases, neutrophil elastase and myeloperoxidase in chronic periodontitis. *Oral Dis* 2011;**17:**68–76.
30. Kaufman E, Lamster I. Analysis of saliva for periodontal diagnosis. *J Clin Periodontol* 2000;**27:**453–465.
31. Edgar WM. Saliva: its secretion, composition and functions. *Br Dent J* 1992;**172:** 305–312.
32. Chapple IL, Mason GI, Garner I *et al*. Enhanced chemiluminescent assay for measuring the total antioxidant capacity of serum, saliva and crevicular fluid. *Ann Clin Biochem* 1997;**34:**412–421.
33. Tesmer LA, Lundy SK, Sarkar S, Fox DA. Th17 cells in human disease. *Immunol Rev* 2008;**223:**87–113.
34. Yu JJ, Gaffen SL. Interleukin-17: a novel inflammatory cytokine that bridges innate and adaptive immunity. *Front Biosci* 2008;**13:**170–177.
35. Lester SR, Bain JL, Johnson RB, Serio FG. Gingival concentrations of interleukin-23 and -17 at healthy sites and at sites of clinical attachment loss. *J Periodontol* 2007;**78:**1545–1550.
36. Yetkin Ay Z, Sütçü R, Uskun E, Bozkurt FY, Berker E. The impact of the IL-11: IL-17 ratio on the chronic periodontitis pathogenesis: a preliminary report. *Oral Dis* 2009;**15:**93–98.
37. Buduneli N, Buduneli E, Kütükçüler N. Interleukin-17, RANKL, and osteoprotegerin levels in gingival crevicular fluid from smoking and non-smoking patients with chronic periodontitis during initial periodontal treatment. *J Periodontol* 2009; **80:**1274–1280.
38. Johnson RB, Wood N, Serio FG. Interleukin-11 and IL-17 and the pathogenesis of periodontal disease. *J Periodontol* 2004; **75:**37–43.
39. Gaffen SL, Hajishengallis G. A new inflammatory cytokine on the block: re-thinking periodontal disease and the Th1/Th2 paradigm in the context of Th17 cells and IL-17. *J Dent Res* 2008;**87:**817–828.
40. Ohyama H, Kato-Kogoe N, Kuhara A *et al*. The involvement of IL-23 and the Th17 pathway in periodontitis. *J Dent Res* 2009;**88:**633–638.
41. Honda T, Aoki Y, Takahashi N *et al*. Elevated expression of IL-17 and IL-12 genes in chronic inflammatory periodontal disease. *Clin Chim Acta* 2008;**395:**137–141.
42. Cardoso CR, Garlet GP, Crippa GE *et al*. Evidence of the presence of T helper type 17 cells in chronic lesions of human periodontal disease. *Oral Microbiol Immunol* 2009;**24:**1–6.
43. Dutzan N, Gamonal J, Silva A, Sanz M, Vernal R. Over-expression of forkhead box P3 and its association with receptor activator of nuclear factor-kappa B ligand, interleukin (IL)-17, IL-10 and transforming growth factor-beta during the progression of chronic periodontitis. *J Clin Periodontol* 2009;**36:**396–403.
44. Schenkein HA, Koertge TE, Brooks CN, Sabatini R, Purkall DE, Tew JG. IL-17 in sera from patients with aggressive periodontitis. *J Dent Res* 2010;**89:**943–947.
45. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 regulates both Th1 and Th2 responses. *Annu Rev Immunol* 2001;**19:**423–474.
46. Tschoeke SK, Oberholzer A, Moldawer LL. Interleukin-18: a novel prognostic cytokine in bacteria-induced sepsis. *Crit Care Med* 2006;**34:**1225–1233.
47. Schallhorn RA, Patel DN, Chandrasekar B, Mealey BL. Periodontal disease in association with systemic levels of interleukin-18 and CXC ligand 16 in patients undergoing cardiac catheterization. *J Periodontol* 2010; **81:**1180–1186.
48. Yamamura M, Kawashima M, Taniai M *et al*. Interferon-gamma-inducing activity of interleukin-18 in the joint with rheumatoid arthritis. *Arthritis Rheum* 2001;**44:** 275–285.
49. Ushio S, Namba M, Okura T *et al*. Cloning of the cDNA for human IFN-gamma-inducing factor, expression in Escherichia coli, and studies on the biological activities of the protein. *J Immunol* 1996;**156:**4274–4279.

50. Beklen A, Sorsa T, Konttinen YT. Toll-like receptors 2 and 5 in human gingival epithelial cells co-operate with T-cell cytokine interleukin-17. *Oral Microbiol Immunol* 2009;**24:**38–42.
51. Buduneli N, Özçaka Ö, Nalbantsoy A. Salivary and plasma levels of Toll-like receptor 2 and Toll-like receptor 4 in chronic periodontitis. *J Periodontol* 2011;**82**. (in press) doi**:** 10.1902/jop.2010.100467.
52. Sorsa T, Hernandez M, Leppilahti J, Munjal S, Netuschil L, Mantyla P. Detection of gingival crevicular fluid MMP-8 levels with different laboratory and chair-side methods. *Oral Dis* 2010;**16:** 39–45.
53. Leppilahti JM, Ahonen M-M, Hernandez M *et al*. Oral rinse MMP-8 point-of-care immune test identifies patients with strong periodontal inflammatory burden. *Oral Dis* 2011;**17:**115–122.