Journal of
PERIODONTAL RESEARCH

J Periodont Res 2013; 48: 44–51

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2012.01500.x

Comparison of CCL28, interleukin-8, interleukin-1β and tumor necrosis factor-alpha in subjects with gingivitis, chronic periodontitis and generalized aggressive periodontitis

A. S. Ertugrul, H. Sahin, A. Dikilitas, N. Alpaslan, A. Bozoglan
Department of Periodontology, Faculty of Dentistry, Yuzuncu Yil University, Van, Turkey

Ertugrul AS, Sahin H, Dikilitas A, Alpaslan N, Bozoglan A. Comparison of CCL28, interleukin-8, interleukin-1β and tumor necrosis factor-alpha in subjects with gingivitis, chronic periodontitis and generalized aggressive periodontitis. J Periodont Res 2013; 48: 44–51. © 2012 John Wiley & Sons A/S

Background and Objective: Cytokines produced by various cells are strong local mediators of inflammation. Mucosa-associated epithelial chemokine (CCL28), interleukin-8 (IL-8), interleukin-1beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) are major cytokines that play important roles in the periodontal inflammatory process. In this study we aimed to compare the levels of CCL28, IL-8, IL-1β and TNF-α in the gingival crevicular fluid of both periodontally healthy subjects and in subjects diagnosed with gingivitis, chronic periodontitis and generalized aggressive periodontitis.

Material and Methods: A total of 84 subjects participated in the study: 21 subjects had gingivitis, 21 subjects had chronic periodontitis, 21 subjects had generalized aggressive periodontitis and 21 were periodontally healthy. The levels of CCL28, IL-8, IL-1β and TNF-α were analyzed using enzyme-linked immune sorbent assay (ELISA).

Results: The total levels of CCL28 and IL-8 in the gingival crevicular fluid of the generalized aggressive periodontitis group (324.74 ± 42.62 pg/30 s, 487.62 ± 49.21 pg/30 s) were significantly higher than those of the chronic periodontitis group (268.81 ± 28.64 pg/30 s, 423.65 ± 35.24 pg/30 s), the gingivitis group (146.35 ± 17.46 pg/30 s, 310.24 ± 48.20 pg/30 s) and the periodontally healthy group (92.46 ± 22.04 pg/30 s, 148.41 ± 24.64 pg/30 s). Similarly, the total levels of IL-1β and TNF-α in the generalized aggressive periodontitis group (110.23 ± 9.20 pg/30 s, 1284.46 ± 86.32 pg/30 s) were significantly higher than those in the chronic periodontitis group (423.65 ± 35.24 pg/30 s, 82.64 ± 9.12 pg/30 s), the gingivitis group (52.10 ± 7.15 pg/30 s, 824.24 ± 44.68 pg/30 s) and the periodontally healthy group (36.44 ± 8.86 pg/30 s, 628.26 ± 34.61 pg/30 s).

Abdullah Seckin Ertugrul, Department of Periodontology, Faculty of Dentistry, Yuzuncu Yil University, 65080 Van, Turkey
Tel: +90 432 2231744
Fax: +90 432 2231747
e-mails: ertugrulseckin@yahoo.com, ertugrul@yyu.edu.tr

Key words: aggressive periodontitis; chemokines; chronic periodontitis; cytokines; gingival crevice fluid

Accepted for publication May 18, 2012

Conclusion: CCL28, IL-8, IL-1β and TNF-α may play key roles in the host response to inflammation in periodontal diseases. As the severity of periodontal diseases increases, destruction of periodontal tissues also increases. Inflammation is one among many factors that trigger periodontal tissue destruction. Identification of the mediators that influence the development and progression of inflammation in periodontal diseases may be very important in understanding the prognoses of periodontal diseases.

Chemokines are molecules of 6–14 kDa in size that contain stretches of cationic amino acids (1). The chemokine superfamily consists of four families grouped according to the pattern of cysteines in the N-terminal region: CXC (alpha), CC (beta), C (gamma) and CX3C (delta). Usually, a different chemokine member attack different leukocytes. CC chemokines attack mononuclear cells, eosinophils or some or most of the basophils, CXC chemokines attack neutrophils and lymphocytes, C chemokines attack T cells and CX3C chemokines attack natural killer cells (2–5).

CC chemokines are secreted by T lymphocytes and are always present in combination with sulfate proteoglycans on endothelial cell surfaces. CC chemokines play important roles in processes occurring between immune cells during the change from acute inflammation to chronic inflammation (5). CC chemokines play several roles in the defense against invasion with microorganisms, homeostasis, cell growth, leukocyte functions, angiogenesis and inflammation (6). Human CCL28 is the most recently identified CC chemokine and is known to have antimicrobial activity against *Candida albicans* and both gram-negative and gram-positive bacteria. It was demonstrated that, like many peptides, CCL28 quickly increases membrane permeability in target microorganisms and shows its antimicrobial action by conferring a positive charge to the bacterial cell membrane, which is negatively loaded (7–10). Recent studies showed that CCL28 levels were higher in samples from a group of subjects with inflammation than in samples from a healthy control group (11–14).

Human interleukin (IL)-8 is also a member of the chemokine family and is primarily released from monocytes, macrophages, fibroblasts, keratinocytes and endothelial cells. IL-8 participates in the activation and degranulation of neutrophils. It is known that IL-8 plays a role in angiogenesis by stimulating the formation of new blood vessels through inducing the proliferation of endothelial cells (15,16). Microorganisms and toxins in periodontal tissues stimulate the formation of IL-8, which induces a signal for the collection of neutrophils in local sites (17). IL-8 is identified to be a key molecule in the localization, collection and activation of neutrophils (18).

Microbial toxins are known to stimulate connective tissue epithelial cells to release various inflammatory mediators, including IL-1β or tumor necrosis factor-alpha (TNF-α). All of these mediators can pass through the connective tissue epithelium and reach the gingival crevicular fluid. The normal flora of the body may play an active role as a shield against infections by preventing the growth of pathogenic microorganisms (19). Proinflammatory cytokines, such as IL-1β and TNF-α, also play major roles in the induction, regulation and elongation of tissue (20). These cytokines induce vascular alterations and the migration of cells, such as neutrophils, to the periodontium (21). IL-1 and TNF-α are primary mediators of chronic inflammatory diseases and have the potential to induce tissue destruction and bone loss in periodontal diseases (22,23).

There are no reports in the literature regarding the relationships between CCL28, IL-8, IL-1β and TNF-α in oral tissue and periodontal diseases, and some questions remain unanswered. The aim of this study was to determine and compare the levels of CCL28, IL-8, IL-1β and TNF-α in the gingival crevicular fluid of periodontally healthy subjects and in subjects with gingivitis, chronic periodontitis and generalized aggressive periodontitis, using ELISA.

Material and methods

The 84 subjects who participated in this trial were randomly selected from subjects who consulted the Faculty of Dentistry, Yuzuncu Yil University, for the treatment of periodontal diseases between July 2011 and October 2011 (Fig. 1). The study groups were as follows: periodontally healthy ($n = 21$), gingivitis ($n = 21$), chronic periodontitis ($n = 21$) and generalized aggressive periodontitis ($n = 21$). The patients were selected according to the clinical and radiographic criteria proposed by the 1999 International Workshop for a Classification of Periodontal Diseases and Conditions (24).

Chronic periodontitis group. At the time of the oral examination, there should be inflammation in the gingiva, microbial dental-plaque formation, vertical and horizontal bone loss in the radiographic examinations, a probing depth of ≥ 5 mm in at least six sites of at least four teeth with one root and clinical attachment loss (CAL) of ≥ 4 mm (25,26).

Gingivitis group. At the time of the oral examination, there should be bleeding on probing (BOP) in at least 50% of the total gingival and no vertical or horizontal bone loss in the

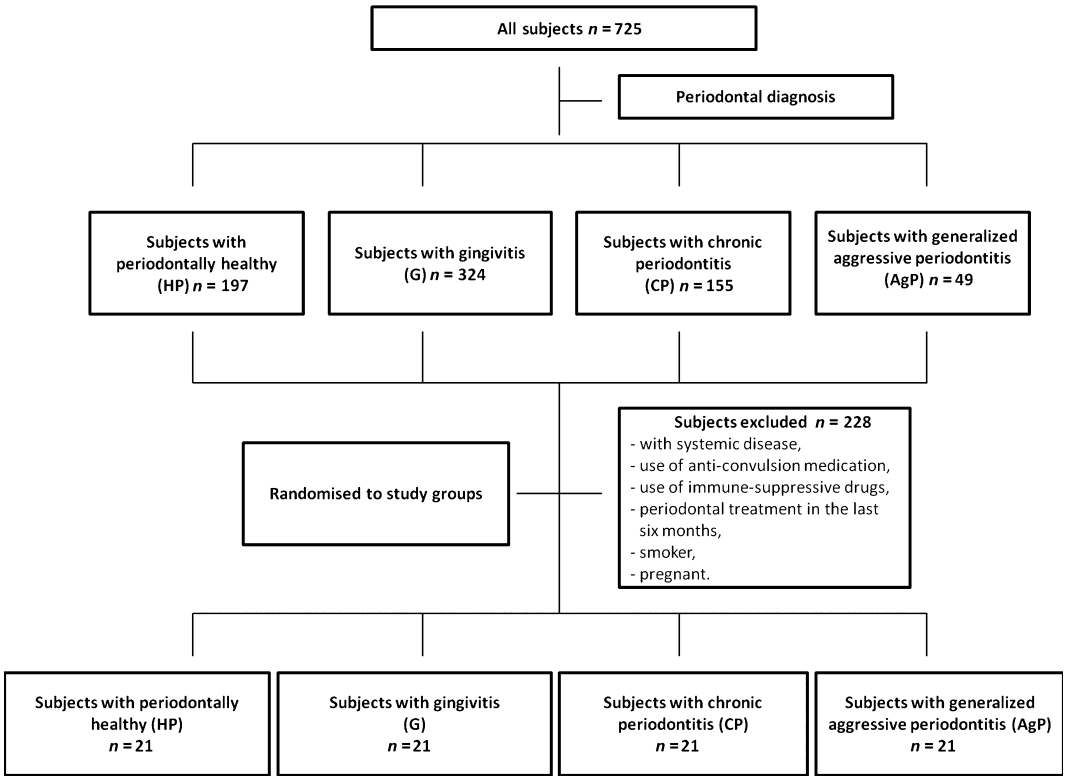

Fig. 1. Consort flow chart of the progress of subjects through the phases of the study.

radiographic examinations (bone crest at > 95% of the proximal tooth sites and < 3 mm between the cemento–enamel junction) (26).

Periodontally healthy group. At the time of the oral examination, there should be no CAL of > 2 mm or probing depth > 3 mm, BOP at < 15% of sites, no horizontal or vertical bone loss in the radiographic examinations (bone crest at > 95% of the proximal tooth sites and < 3 mm between the cemento–enamel junction) (26,27).

Generalized aggressive periodontitis group. At the time of the oral examination, patients should be between 16 and 30 years of age and have at least 20 natural teeth, a minimum of six incisors and/or first molars with at least one site with probing depth and CAL of > 5 mm, as well as a minimum of six teeth other than first molars and incisors also presenting at least one site with a probing depth and CAL of > 5 mm (28,29).

The Ethics Committee for Clinical Research at Yuzuncu Yil University approved the study protocols (YYU-040711), including those for the periodontal examination and for taking gingival crevicular fluid samples. All subjects read and signed the informed consent form and read the Helsinki Declaration before participating in the study. Exclusion criteria were the presence of any systemic disease, the use of any anticonvulsant medication or immunosuppressive drugs, periodontal treatment in the last 6 months, any drug addiction, smoking and pregnancy. All measurements were performed by a calibrated examiner (A.S.E.). Periodontal status was evaluated by measuring the plaque index (PI) (30), gingival index (GI) (31), probing depth, BOP and CAL (32). Clinical periodontal measurements (PI, GI, probing depth and CAL) were obtained from four points of each tooth: mesial, distal, lingual (palatinal) and labial (buccal). Orthopantomographic radiograms were taken of all subjects.

Site selection and gingival crevicular fluid sampling

Gingival crevicular fluid samples were taken from four Ramfjord sample teeth in the periodontally healthy and gingivitis groups (33) and from four teeth showing the deepest pocket formation in the chronic periodontitis and generalized aggressive periodontitis groups. Gingival crevicular fluid samples were taken from teeth that were positive for BOP in both gingivitis and periodontally healthy groups. Each tooth zone was dried by air-spraying to prevent irritation and isolated carefully with cotton tampons. To avoid contamination of samples with saliva, saliva absorbents were used. Standard paper strips (Periopaper®; Oraflow, Plainview, NY, USA) were placed in the mesial and distal parts of the sulcus by pushing until resistance was felt. Each paper strip was kept in the sulcus for 30 s, and the gingival crevicular fluid samples were immediately transferred to the Periotron device (Periotron 8000®, OraFlow, PlainView, NY, USA) to determine the gingival crevicular fluid volume. Two previously numbered Eppendorf tubes containing 500 μL of phosphate-buffered saline were used for each patient. Samples were kept at −80°C until analysis.

ELISA analyses of CCL28, IL-8, IL-1β and TNF-α

The levels of CCL28, IL-8, IL-1β and TNF-α were analyzed in gingival crevicular fluid using commercial ELISA kits [CCL28 ELISA Kit (Cat#: ELH-CCL28-001), IL-8 ELISA Kit (Cat#: ELH-IL8-001), IL-1β ELISA Kit (Cat#:ELH-IL1beta-001) and TNF-α ELISA Kit (Cat#:ELH-TNF alpha-001); RayBiotech, Inc., Norcross, GA, USA]. Standards in the commercial kit were diluted according to the manufacturer's directions, and serum samples were added to wells coated with CCL28-, IL-8-, IL-1β- and TNF-α-specific antibodies. All assay procedures were performed according to the manufacturer's instructions. Stop solution was added to each well, and the absorbance values were determined by a spectrophotometric ELISA-Reader (Microplate Reader; Biotek, Winooski, VT, USA). The total amounts of CCL28, IL-8, IL-1β and TNF-α collected in 30 s (pg/30 s) were determined.

Statistical analysis

Data were analyzed using SPSS for Windows 17.0 (SPSS Inc., Chicago, IL, USA) and expressed as the mean and standard deviation. The Kolmogorov–Smirnov normality test was applied to show normality. Data for CCL28, IL-8, IL-1β, TNF-α and clinical periodontal parameters were analyzed using nonparametric tests (the Kruskal–Wallis test and the Mann–Whitney *U*-test with Bonferroni correction). Spearman's correlation test was used to analyze the relationship between the levels of CCL28, IL-8, IL-1β and TNF-α and clinical periodontal parameters. The level of significance was set at $p < 0.05$ with a 90% confidence interval.

Results

Periodontal clinical parameters

There were no significant differences in age and gender among the different study groups ($p > 0.05$). The mean age ± standard deviation of the 21 subjects with generalized aggressive periodontitis (31.92 ± 4.53 years), 21 subjects with chronic periodontitis (35.01 ± 7.36 years), 21 subjects with gingivitis (33.62 ± 3.23 years) and 21 periodontally healthy subjects (32.88 ± 4.16 years) are shown in Table 1.

Clinical periodontal parameters (PI, GI, probing depth, BOP and CAL) are shown in Table 2. The periodontally healthy subjects had significantly lower scores for the clinical periodontal parameters (PI, GI, probing depth, BOP and CAL) than the subjects with gingivitis, chronic periodontitis and generalized aggressive periodontitis ($p < 0.05$). The subjects with generalized aggressive periodontitis had significantly higher scores for the clinical periodontal parameters (PI, GI, probing depth, BOP and CAL) than the subjects with chronic periodontitis and gingivitis ($p < 0.05$, Table 1).

Gingival crevicular fluid markers

The total CCL28, IL-8, IL-1β and TNF-α levels in gingival crevicular fluid are shown in Figure 2. The total CCL28 and IL-8 levels and the volume of gingival crevicular fluid were significantly lower in periodontally healthy subjects than in subjects of the other groups ($p < 0.05$). The total CCL28 and IL-8 levels in the gingival crevicular fluid of the subjects with generalized aggressive periodontitis were significantly higher than those of the chronic periodontitis, gingivitis and periodontally healthy groups ($p < 0.05$). The CCL28 and IL-8 levels in the gingivitis group were significantly higher than those in the periodontally healthy group ($p < 0.05$, Fig. 2). The total IL-1β and TNF-α levels were significantly lower in the gingival crevicular fluid of periodontally healthy subjects than in the gingival crevicular fluid of the other groups ($p < 0.05$). The total IL-1β and TNF-α levels in the generalized aggressive periodontitis group were significantly higher than in the chronic periodontitis andgingivitis groups ($p < 0.05$, Fig. 2).

Correlations of clinical periodontal parameters and CCL28, IL-8, IL-1β and TNF-α levels

The correlations between clinical periodontal parameters and total gingival crevicular fluid CCL28, IL-8, IL-1β and TNF-α levels and gingival

Table 1. Demographic characteristics and clinical periodontal parameters of the study population

	Periodontally healthy ($n = 21$)	Gingivitis ($n = 21$)	Chronic periodontitis ($n = 21$)	Generalized aggressive periodontitis ($n = 21$)
Age (years)	32.88 ± 4.16	33.62 ± 3.23	35.01 ± 7.36	31.92 ± 4.53
Gender (f/m)	11/10	10/11	11/10	10/11
PI	1.28 ± 0.12[bcd]	1.68 ± 0.24[acd]	2.16 ± 0.28[abd]	2.42 ± 0.35[abc]
GI	1.25 ± 0.11[bcd]	1.62 ± 0.28[acd]	2.18 ± 0.34[abd]	2.31 ± 0.44[abc]
Probing depth (mm)	1.64 ± 0.42[bcd]	2.36 ± 0.23[acd]	3.45 ± 0.46[abd]	3.83 ± 0.54[abc]
CAL (mm)	1.74 ± 0.42[bcd]	2.32 ± 0.18[acd]	3.62 ± 0.34[abd]	3.93 ± 0.27[abc]
BOP (%)	5.80 ± 3.50[bcd]	71.24 ± 12.40[acd]	81.24 ± 14.20[abd]	86.41 ± 10.20[abc]
Gingival crevicular fluid (μL)	0.44 ± 0.08[bcd]	0.74 ± 0.12[acd]	1.20 ± 0.24[abd]	1.34 ± 0.32[abc]

Values are given as mean ± standard deviation or as female/male ratio.
[a]Significantly different from periodontally healthy, $p < 0.05$.
[b]Significantly different from gingivitis, $p < 0.05$.
[c]Significantly different from chronic periodontitis, $p < 0.05$.
[d]Significantly different from generalized aggressive periodontitis, $p < 0.05$.
BOP, bleeding on probing; CAL, clinical attachment loss; GI, gingival index; PI, plaque index.

Table 2. Correlations between total gingival crevicular fluid CCL28, interleukin (IL)-8, IL-1β and TNF-α levels and clinical periodontal parameters of the study groups

Parameter	CCL28	IL-8	IL-1β	TNF-α
BOP (*p*)	0.768 (0.000)	0.779 (0.000)	0.779 (0.000)	0.711 (0.000)
PI (*p*)	0.747 (0.000)	0.780 (0.000)	0.747 (0.000)	0.762 (0.000)
GI (*p*)	0.773 (0.000)	0.792 (0.000)	0.763 (0.000)	0.787 (0.000)
Probing depth (*p*)	0.883 (0.000)	0.838 (0.000)	0.901 (0.000)	0.901 (0.000)
CAL (*p*)	0.860 (0.000)	0.832 (0.000)	0.880 (0.000)	0.889 (0.000)

CAL, clinical attachment loss; GI, gingival index; PI, plaque index; BOP, bleeding on probing. Correlation was significant at $p < 0.05$.

crevicular fluid volume are shown in Table 2. Positive correlations were observed between the clinical periodontal parameters (GI, PI, BOP, probing depth and CAL) and the total amounts of CCL28, IL-8, IL-1β and TNF-α in the gingival crevicular fluid, as well as the gingival crevicular fluid volume.

Discussion

Complements of gingival crevicular fluid, saliva and serum are components of the host defense mechanism (34–36). Defining markes in Gingival crevicular fluid is easily diagnostic method for periodontal diseases (36–38). Increased production of inflammatory cytokines (IL-1β and TNF-α), chemotactic cytokines (IL-8) and tissue-destructive enzymes occurs owing to the immunoinflammatory response that develops in the periodontium after encountering pathogenic microorganisms. The imbalance between these proinflammatory mediators and enzymes and their inhibitors are responsible for most of the tissue destruction observed in periodontal diseases (37–40).

This is the first study to compare clinical periodontal parameters and total levels of CCL28, IL-8, IL-1β and TNF-α in the gingival crevicular fluid of periodontally healthy subjects and those with gingivitis, generalized aggressive periodontitis and chronic periodontitis. In this study, the lowest levels of CCL28, IL-8, IL-1β and TNF-α were found in periodontally healthy

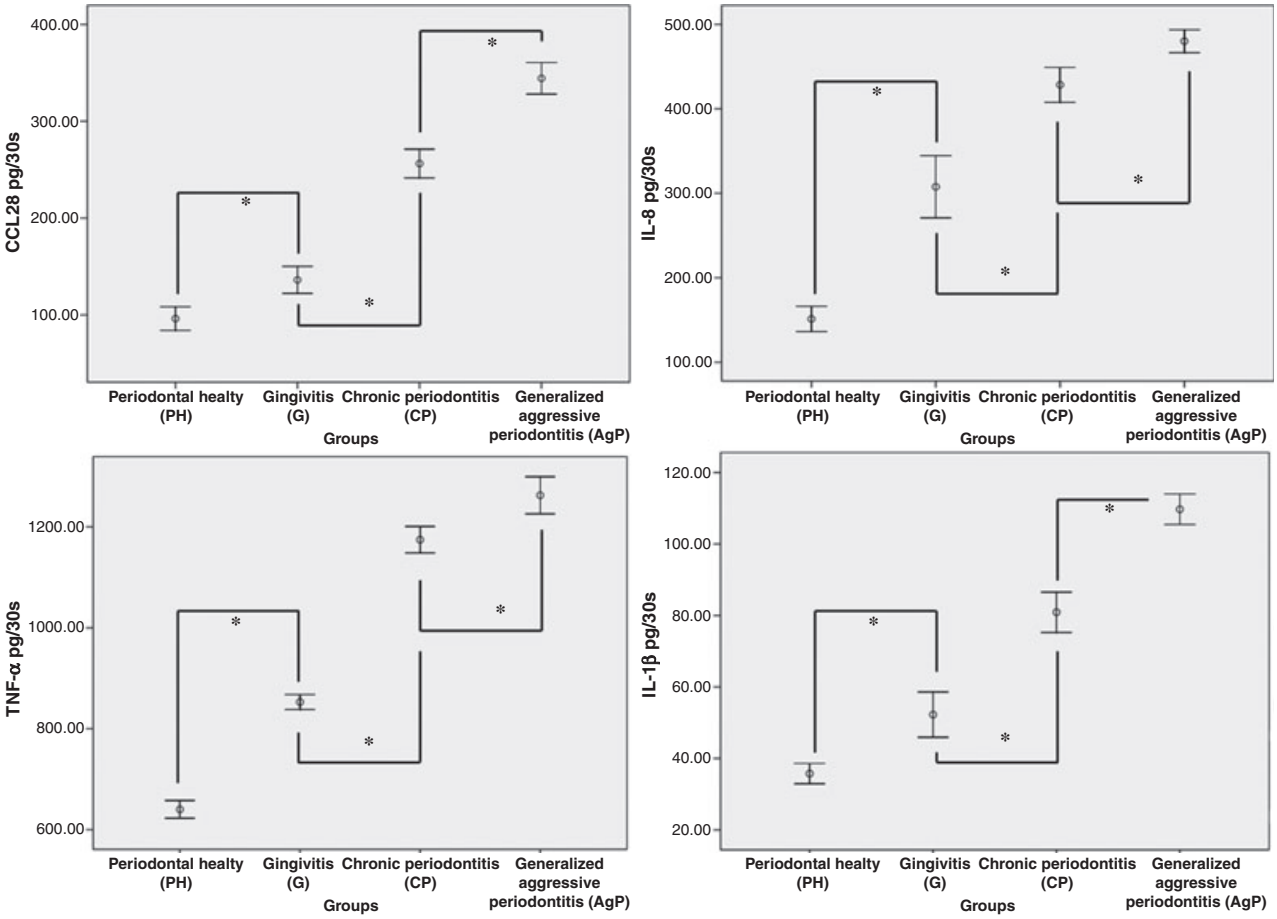

Fig. 2. Comparison of the total levels of CCL28, interleukin (IL)-8, IL-1β and tumor necrosis factor-alpha (TNF-α) in the gingival crevicular fluid of periodontally healthy subjects and in subjects diagnosed with, gingivitis, chronic periodontitis and generalized aggressive periodontitis. The total levels of CCL28, IL-8, IL-1β and TNF-α in the gingical crevicular fluid were evaluated using ELISA. *Groups statistically different from each other, $p < 0.05$.

subjects, whereas the highest levels of these factors were found in subjects with generalized aggressive periodontitis.

CCL28 is a member of the chemokine family (41,42) that has also been shown to have antimicrobial activity against both gram-negative and gram-positive bacteria, and fungi (43). Expression of this antimicrobial chemokine has been shown to be up-regulated during epithelial inflammation (44,45). CCL28 levels were found to be higher in subjects with chronic inflammatory diseases than in healthy subjects (11–14). It is known that the production of the proinflammatory cytokines IL-1β and TNF-α is increased following contact with a microbial stimulus, which, in turn, significantly increases the expression of CCL28. John *et al*. (46) investigated the levels of CCL28 in mouse lung biopsy using ELISA and reported that CCL28 levels were highest in mice with inflamed lungs compared with healthy albino mice in the control group. Hieshima *et al*. (14) evaluated the CCL28 levels in human milk and saliva samples of different groups and found increased levels of CCL28 in groups with inflammation predominantly caused by gram-negative bacteria. Ogawa *et al*. (47) demonstrated that IL-1β, induced by microbial stimuli, up-regulated CCL28 in human colon epithelial cell lines. CCL28 was significantly up-regulated in inflamed colon epithelium, and its production was up-regulated in human colon epithelial cells by proinflammatory stimuli. Kagami *et al*. (8) investigated CCL28 levels in patients diagnosed with psoriasis vulgaris and bullous pemphigus and found these levels to be higher than those of healthy control subjects. English *et al*. (48) examined the relationship between CCL28 levels and respiratory tract inflammation in asthma patients and healthy subjects. They found that CCL28 expression was low in the airways of nonasthma models.

Watkins *et al*. (49) determined CCL28 levels in serum and saliva and reported that CCL28 had a killing activity against periodontal pathogens. They estimated that the 50% effective concentration of CCL28 was ~ 0.7 μM for *Porphyromonas gingivalis* and ~ 2.0 μM for *Aggregatibacter actinomycetemcomitans*.

In our study, a positive correlation was observed between clinical periodontal parameters and total CCL28 levels. Both clinical periodontal parameters and CCL28 levels were found to be higher in subjects with generalized aggressive periodontitis (in whom a high level of inflammation was detected) compared with other groups. Given these data, a positive correlation was observed between CCL28 levels and inflammation. It is believed that CCL28 and CCL28 receptors participate in many important biological processes, such as leukocyte-mediated inflammation, homeostasis and regulation of cellular movements. Considering the increased secretion of CCL28 observed in subjects with inflammatory diseases compared with healthy subjects, the higher levels of CCL28 observed in this study are likely to be a consequence of the tissue response to the inflammation present in the chronic periodontitis group. Periodontal tissues were examined in healthy subjects in this study, but no inflammation was observed. As it is known that CCL28 levels are higher in inflamed tissues than in healthy tissues, the low CCL28 levels seen in the periodontally healthy group were thought to be a result of the absence of inflammation in tissues.

IL-8 is induced and secreted by monocytes, lymphocytes, fibroblasts, epithelial cells, tumor cells, lipopolysaccharides and proinflammatory cytokines. IL-1β and TNF-α are known to be two major cytokines with important roles in alveolar bone destruction (50). IL-1β and TNF-α induce to migration of effector cells (neutrophils) to the periodontium. Therefore periodontal pathogens are eliminated. However, the combination of the chronic and persistent nature of subgingival plaque and inappropriate cytokine responses may result in inflammation in addition to tissue destruction (22,50). Induction of primary mediators, such as IL-1β and TNF-α, stimulates the secretion of secondary mediators, such as cyclooxygenase, which, in turn, induce chemokines to act like chemotactic cytokines or prostaglandins. This condition maintains the inflammatory response in two ways: by releasing the enzymes causing connective tissue destruction; and via osteoclastic bone resorption (50).

A significant increase was observed in the levels of IL-8 in the gingival crevicular fluid of adult subjects with chronic periodontitis relative to healthy control subjects and these levels were found to decrease significantly after periodontal treatment (51). Mathur *et al*. (20) and Tsai *et al*. (52) also reported that the IL-8 levels were higher in subjects with chronic periodontitis. However, Chung *et al*. (53) and Özmeriç *et al*. (54) found higher IL-8 levels in their periodontally healthy control group. Chung *et al*. (53) demonstrated that IL-8 levels in subjects with chronic periodontitis were low before periodontal treatment and decreased further after such treatment. Studies on proinflammatory cytokines showed that the levels of IL-1β or TNF-α in the gingival crevicular fluid of subjects with chronic periodontitis were significantly higher compared with those in the gingivitis and periodontally healthy groups (51,55–57).

In our study, PI values were higher in subjects with chronic periodontitis and generalized aggressive periodontitis than in subjects with gingivitis and in periodontally healthy subjects. In subjects with chronic periodontitis, continuous migration of neutrophils towards localized tissue damage developed after plaque accumulation, and was probably caused by chemotactic factors such as IL-8 and other chemokines (CCL28). It is believed that such factors in subjects with generalized aggressive periodontitis may stimulate greater neutrophil migration from periodontal tissues to the gingival crevicular fluid as a result of increased levels of plaque, increased periodontal tissue inflammation and increased numbers of microorganisms or their products. Such neutrophil migration to gingival crevicular fluid may be lower in subjects with gingivitis and in periodontally healthy subjects with lower degrees of inflammation and tissue destruction.

In conclusion, cytokines are mediators that regulate local, systemic

and inflammatory responses and that participate in many biological events, such as wound healing and inflammation, by regulating the duration and severity of the inflammatory response. Cytokines (IL-8, CCL28, IL-1β and TNF-α) may induce periodontal tissue destruction by increasing the synthesis of some mediators from periodontal ligament fibroblasts and other cells, such that both the severity of generalized aggressive periodontitis and cytokine release increase as tissue destruction increases. The levels of IL-8, CCL28, IL-1β and TNF-α in gingival crevicular fluid could be used as inflammatory activity markers in periodontal tissues. Furthermore, determination of the roles of IL-8, CCL28, IL-1β and TNF-α in understanding the prognoses of periodontal diseases may be very important. Despite the limitations of the present study, the data suggest that the levels of CCL28, IL-8, IL-1β and TNF-α increased in parallel with the severity of the periodontal disease and inflammation. The levels of CCL28, IL-8, IL-1β and TNF-α in gingival crevicular fluid may have potential as indicators of periodontal inflammation. Further studies are needed to understand the full effects of IL-8, CCL28, IL-1β and TNF-α in periodontal diseases.

Acknowledgements

The authors thank the School of Dentistry at Yuzuncu Yil University for its support. The authors declare that they have no conflicts of interest.

Source of funding

This study was self-funded by the authors and by The University of Yuzuncu Yil University (Committee of Research 2010-DF-B010).

References

1. Goebeler M, Trautmann A, Voss A, Brocker EV, Toksoy A, Gillitzer R. Differential and sequential expression of multiple chemokines during elicitation of allergic contact hypersensitivity. *Am J Pathol* 2001;**158:**431–440.
2. Elsner J, Mack M, Brühl H *et al.* Differential activation of CC chemokine receptors by AOP-RANTES. *J Biol Chem* 2000;**275:**7787–7794.
3. Politz O, Kodelja V, Guillot P, Orfanos CE, Goerdt S. Pseudoexons and regulatory elements in the genomic sequence of the beta-chemokine, alternative macrophage activation-associated CC-chemokine (AMAC)-1. *Cytokine* 2000;**12:**120–126.
4. Kunkel EJ, Boisvert J, Murphy K *et al.* Expression of the chemokine receptors CCR4, CCR5, and CXCR3 by human tissue-infiltrating lymphocytes. *Am J Pathol* 2002;**160:**347–355.
5. Kim CH, Rott L, Kunkel EJ *et al.* Rules of chemokine receptor association with T cell polarization in vivo. *J Clin Invest* 2001;**108:**1331–1339.
6. Loetscher P, Moser B, Baggiolini M. Chemokines and their receptors in lymphocyte traffic and HIV infection. *Adv Immunol* 2000;**74:**127–128.
7. Ezzat MH, Sallam MA, Shaheen KY. Serum mucosa-associated epithelial chemokine (MEC/CCL28) in atopic dermatitis: a specific marker for severity. *Int J Dermatol* 2009;**48:**822–829.
8. Kagami S, Kakinuma T, Saeki H *et al.* Increased serum CCL28 levels in patients with atopic dermatitis, psoriasis vulgaris and bullous pemphigoid. *J Invest Dermatol* 2005;**124:**1088–1090.
9. Teran LM. CCL chemokines and asthma. *Immunol Today* 2000;**21:**235–241.
10. Horuk R, Ng HP. Chemokine receptor antagonists. *Med Res Rev* 2000;**20:**155–168.
11. Wang W, Soto H, Oldham ER *et al.* Identification of a novel chemokine (CCL28), which binds CCR10 (GPR2). *J Biol Chem* 2000;**275:**22313–22323.
12. Feng N, Jaimes MC, Lazarus NH *et al.* Redundant role of chemokines CCL25/TECK and CCL28/MEC in IgA+ plasmablast recruitment to the intestinal lamina propria after rotavirus infection. *J Immunol* 2006;**176:**5749–5759.
13. Eksteen B, Miles A, Curbishley SM *et al.* Epithelial inflammation is associated with CCL28 production and the recruitment of regulatory T cells expressing CCR10. *J Immunol* 2006;**177:**593–603.
14. Hieshima K, Ohtani H, Shibano M *et al.* CCL28 has dual roles in mucosal immunity as a chemokine with broad-spectrum antimicrobial activity. *J Immunol* 2003;**170:**1452–1461.
15. Chen RH, Chen WC, Wang TY, Tsai CH. Lack of association between pro-inflammatory cytokine (IL6-8, TNF-alpha) gene polymorphism and graves disease. *Int J Immunogenet* 2005;**32:**343–347.
16. Senturk T, Kozaci LD, Kok F, Kadikoylu G, Bolaman Z. Proinflammatory cytokine levels in hyperthyroidism. *Clin Invest Med* 2003;**26:**58–63.
17. Haake SK, Nisengard RJ, Newman MG, Miyasaki KT. Microbial interactions with the host in periodontal diseases. In: Newman MG, Takei HH, Carranza FA, eds. *Carranza*'s Clinical Periodontology. 9th edition. Philadelphia, PA: WB Saunders Company, 2002:132–152.
18. Wilson ME, Zambon JJ, Suzuki JB, Genco RJ. Generalized juvenile periodontitis, defective neutrophil chemotaxis and bacteriodes gingivalis in a 13-year-old female. A case report. *J Periodontol* 1985;**56:**457–462.
19. Kinane DF, Podmore M, Ebersole J. Etiopathogenesis of periodontitis in children and adolescents. *Periodontol 2000* 2001;**26:**54–91.
20. Mathur A, Yang C, Wolff L. Cytokines in gingival crevicular fluid of periodontally disseased and healthy sites. *J Periodontal Res* 1996;**31:**489–495.
21. Okada H, Murakami S. Cytokine expression in periodontal health and disease. *Crit Rev Oral Biol Med* 1998;**9:**248–266.
22. Meikle MC, Atkinson SJ, Ward RV, Murphy G, Reynolds JJ. Gingival fibroblasts degrade type I collagen films when stimulated with tumor necrosis factor and interleukin 1: evidence that breakdown is mediated by metalloproteinases. *J Periodontal Res* 1989;**24:**207–213.
23. Birkedal-Hansen H. Role of cytokines and inflammatory mediators in tissue destruction. *J Periodontal Res* 1993;**28:**500–510.
24. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* 1999;**4:**1–6.
25. Öztürk A, Yildız L. Expression of transient receptor potential vanilloid receptor 1 and toll-like receptor 4 in aggressive periodontitis and in chronic periodontitis. *J Periodontal Res* 2011;**46:**475–482.
26. Pradeep AR, Raghavendra NM, Prasad MV, Kathariya R, Patel SP, Sharma A. Gingival crevicular fluid and serum visfatin concentration: their relationship in periodontal health and disease. *J Periodontol* 2011;**82:**1314–1319.
27. Xie YF, Shu R, Jiang SY, Liu DL, Zhang XL. Comparison of microRNA profiles of human periodontal diseased and healthy gingival tissues. *Int J Oral Sci* 2011;**3:**125–134.
28. Oliveira AP, Faveri MD, Gursky LC *et al.* Effects of periodontal therapy on GCF cytokines in generalized aggressive periodontitis subjects. *J Clin Periodontol* 2012;**39:**295–302.
29. Griffiths GS, Ayob R, Guerrero A *et al.* Amoxicillin and metronidazole as an adjunctive treatment in generalized aggressive periodontitis at initial therapy or re-treatment: a randomized controlled

clinical trial. *J Clin Periodontol* 2011;**38:** 43–49.
30. Silness P, Löe H. Periodontal disease in pregnancy. *Acta Odontol Scand* 1964; **22:**121.
31. Löe H. The gingival index, the plaque index and the retantion index systems. *J Clin Periodontol* 1967;**38:**61–70.
32. Glavind L, Löe H. Errors in the clinical assessment of periodontal destruction. *J Periodontal Res* 1967;**2:**180–184.
33. Megson E, Fitzsimmons T, Dharmapatni K, Bartold P. C-reactive protein in gingival crevicular fluid may be indicative of systemic inflammation. *J Clin Periodontol* 2010;**37:**797–804.
34. Lamster IB, Novak MJ. Host mediators in gingival crevicular fluid: implications for the pathogenesis of periodontal disease. *Crit Rev Oral Biol Med* 1992;**3:** 31–60.
35. Kinane DF, Lappin DF. Clinical, pathological and immunological aspects of periodontal disease. *Acta Odontol Scand* 2001;**59:**154–160.
36. Sorsa T, Tervahartiala T, Leppilahti J *et al*. Collagenase-2 (MMP-8) as a point-of-care biomarker in periodontitis and cardiovascular diseases. therapeutic response to non-antimicrobial properties of tetracyclines. *Pharmacol Res* 2011;**63:**108–113.
37. Mäntylä P, Stenman M, Kinane DF *et al*. Gingival crevicular fluid collagenase-2 (MMP-8) test stick for chair-side monitoring of periodontitis. *J Periodontal Res* 2003;**38:**436–439.
38. Sorsa T, Mäntylä P, Tervahartiala T, Pussinen PJ, Gamonal J, Hernandez M. MMP activation in diagnostics of periodontitis and systemic inflammation. *J Clin Periodontol* 2011;**38:**817–819.
39. Preshaw PM. Host response modulation in periodontics. *Periodontol 2000* 2008;**48:** 92–110.
40. Van Dyke TE, Serhan CN. Resolution of inflammation: a new paradigm for the pathogenesis of periodontal diseases. *J Periodontal Res* 2003;**82:**82–90.
41. Hansson M, Hermansson M, Svensson H *et al*. CCL28 is increased in human *Helicobacter pylori*-induced gastritis and mediates recruitment of gastric immunoglobulin A-secreting cells. *Infect Immun* 2008;**76:**3304–3311.
42. Allen SJ, Crown SE, Handel TM. Chemokine: receptor structure, interactions, and antagonism. *Annu Rev Immunol* 2007;**25:**787–820.
43. Liu B, Wilson E. The antimicrobial activity of CCL28 is dependent on C-terminal positively-charged amino acids. *Eur J Immunol* 2010;**40:**186–196.
44. Luster AD. Chemokines-chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998;**338:**436–445.
45. Ying S, Meng Q, Zeibecoglou K *et al*. Eosinophil chemotactic chemokines (eotaxin, eotaxin-2, RANTES, monocyte chemoattractant protein-3 (MCP-3), and MCP-4), and C-C chemokine receptor 3 expression in bronchial biopsies from atopic and nonatopic (Intrinsic) asthmatics. *J Immunol* 1999;**163:**6321–6329.
46. John AE, Thomas MS, Berlin AA, Lukacs NW. Temporal production of CCL28 corresponds to eosinophil accumulation and airway hyperreactivity in allergic airway inflammation. *Int J Immunogenet* 2005;**166:**345–353.
47. Ogawa H, Iimura M, Eckmann L, Kagnoff MF. Regulated production of the chemokine CCL28 in human colon epithelium. *Am J Physiol Gastrointest Liver Physiol* 2004;**287:**G1062–G1069.
48. English K, Brady C, Corcoran P, Cassidy JP, Mahon BP. Inflammation of the respiratory tract is associated with CCL28 and CCR10 expression in a murine model of allergic asthma. *Immunol Lett* 2006;**103:** 92–100.
49. Watkins HR, Lapp CA, Hanes PJ *et al*. CCL28 effects on periodontal pathogens. *J Periodontol* 2007;**78:**2356–2363.
50. Graves DT, Cochran D. The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. *J Periodontol* 2003;**74:**391–401.
51. Gamonal J, Acevedo A, Bascones A, Jorge O, Silva A. Levels of interleukin-1β, -8 and -10 and RANTES in gingival crevicular fluid and cell populations in adult periodontitis patients and the effect of periodontal treatment. *J Periodontol* 2000;**71:**1535–1545.
52. Tsai C, Ho Y, Chen C. Levels of interleukin-1β and interleukin-8 in gingival crevicular fluids in adult periodontitis. *J Periodontol* 1995;**66:**852–859.
53. Chung RM, Grbic JT, Lamster IB. Interleukin-8 and β-glucuronidase in gingival crevicular fluid. *J Clin Periodontol* 1997;**24:**146–152.
54. Özmeriç N, Bal B, Baloş K, Berker E, Bulut S. The correlation of gingival crevicular fluid interleukin-8 levels and periodontal status in localized juvenile periodontitis. *J Periodontol* 1998;**69:**1299–1304.
55. 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.
56. Preiss DS, Meyle J. Interleukin-1 beta concentration of gingival crevicular fluid. *J Periodontol* 1994;**65:**423–428.
57. Zhong Y, Slade GD, Beck JD, Offenbacher S. Gingival crevicular fluid interleukin-1beta, prostaglandin E2 and periodontal status in a community population. *J Periodontol* 2007;**34:**285–293.