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Effects of treatment on soluble tumour necrosis factor receptor type 1 and 2 in chronic periodontitis

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Abstract

Aim: We reported that soluble tumour necrosis factor receptor type 2 (sTNFR2)/type 1 (sTNFR1) ratios in gingival crevicular fluid (GCF) decreased as the severity of chronic periodontitis (CP) increased. This study investigated the effects of the periodontal treatment on TNF-α, sTNFR1 and R2 in GCF and serum of CP patients.

Material and Methods: Thirty-five serum and 90 GCF samples were obtained from 35 CP patients (23 non-smokers and 12 smokers) at baseline and after treatment. The levels of TNF-α, sTNFR1 and R2 in serum and GCF were quantified by enzyme-linked immunosorbant assay.

Results: No significant differences were found in the serum levels of TNF-α, sTNFR1 and R2 and the ratio of sTNFR2/R1 between baseline and after treatment. After treatment, sTNFR1 and R2 levels in GCF of non-smokers and smokers were significantly decreased compared with baseline. However, the sTNFR2/R1 ratio was significantly increased (non-smoker: 0.56 ± 0.03–0.84 ± 0.03, $p < 0.0001$; smoker: 0.59 ± 0.06–0.85 ± 0.04, $p = 0.0019$). There were no significant differences between non-smoking and smoking CP groups in serum and GCF.

Conclusion: The ratio of sTNFR2/R1 in GCF significantly increased after treatment, and could be related to the clinical state of CP.

Key words: chronic periodontitis; gingival crevicular fluid; periodontal treatment; serum; soluble tumour necrosis factor receptor; tumour necrosis factor α

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Tumour necrosis factor α (TNF-α) is a proinflammatory cytokine produced by a wide spectrum of cells. TNF-α was detected in gingival crevicular fluid (GCF) from sites affected by periodontal disease, and it was suggested that it could be found before clinically observable disease (Rossomando et al. 1990). It has also been detected in gingival tissues of periodontitis patients, and known to induce tissue destruction and bone resorption (Wilton et al. 1992, Page et al. 1997).

TNF-α exerts its diverse biologic effects by binding to two high-affinity cell surface receptors, TNF receptor type 1 (TNFR1) and TNF receptor type 2 (TNFR2). Both the receptors are expressed by almost all cell types, including macrophages, lymphocytes, neutrophils and fibroblasts (Aderka 1996). TNFR1 and R2 have differences in both their extracellular and cytoplasmic domains and thus are functionally distinct. TNF signalling is largely mediated by TNFR1, while the main function of TNFR2 is thought to be that of enhancing this activity by binding to TNF and then passing it on to the TNFR1, i.e. ligand passing. Thus, TNFR2 increases the sensitivity to TNF stimulation and enhances the response mediated by TNFR1.

Soluble forms of TNF receptors are generated by proteolytic cleavage of the extracellular domain of TNFR1 and R2. This soluble TNFR1 and R2 (sTNFR1 and R2) can compete with cell surface receptors for TNF binding, and block TNF-α activity (Aderka 1996). Therefore, the role of TNFR1 and R2 is not limited to signal transduction but

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also includes extracellular regulatory functions affecting TNF bioavailability. The potential biological and clinical significance of these receptors has been reported in inflammatory, infectious, malignant and autoimmune disorders such as rheumatoid arthritis (RA) (Cope et al. 1992).

With regard to periodontal disease, it has been reported that sulcular epithelial cells, monocyte/macrophage-like cells, fibroblasts and endothelial cells express TNFR1 and R2 as well as TNF-α (Tervahartiala et al. 2001). Graves and co-workers (1998, Assuma et al. 1998, Delima et al. 2001) reported that sTNFR2 significantly reduced the loss of connective tissue attachment and the loss of alveolar bone in experimental periodontitis. In addition, Ohe et al. (2000) reported that both TNF-α and interleukin-1β (IL-1β) upregulated the gene expression of TNFR2 and did not affect that of TNFR1. These cytokines upregulated the release of sTNFR2 from human gingival fibroblasts, but did not increased that of sTNFR1. These findings suggest that TNFR2 and its soluble products may modulate TNF-α-mediated inflammatory responses in periodontal disease.

The GCF is regarded as a promising source of biomarkers for periodontal disease activity and wound healing (Champagne et al. 2003). We have reported the levels of TNF-α, sTNFR1 and R2 in GCF and serum of healthy subjects and patients with chronic periodontitis (CP). Our study revealed that TNF-α, sTNFR1 and R2 in GCF were significantly increased and the sTNFR2/R1 ratio decreased with increasing probing pocket depth (PPD) values in CP. The imbalance between sTNFR1 and R2 levels in GCF could be related to the severity of CP (Ikezawa et al. 2005).

Smoking is a major risk factor for the development and progression of CP (Haber & Kent 1992). Direct effects of smoking on periodontal tissue are such as fibrotic gingiva, diminished bleeding on probing (BOP), and decreased the GCF (American Academy of Periodontology 1996). Several clinical and epidemiological studies indicate that cigarette smoking has harmful effects on the response to a variety of non-surgical treatment (Preber & Bergström 1986).

Longitudinal studies have evaluated the effects of initial periodontal treatment on the levels of cellular products associated with periodontal disease in the GCF (Buchmann et al. 2002, Tüter et al. 2002). These studies demonstrate that GCF components may be monitored longitudinally, in conjunction with clinical measurements, to assess the inflammatory status and treatment outcomes.

Therefore, in this study, we examined the levels of TNF-α, sTNFR1 and R2 in GCF and serum of CP patients before and after treatment. We also compared these levels between non-smoking CP and smoking CP patients.

Material and Methods

Subjects

In total, 23 non-smoking CP patients and 12 smoking CP patients were recruited for this study. They had been referred to the Periodontal Clinic of the Niigata University Medical and Dental Hospital, and agreed to participate in this research. Subjects with more than 20 teeth present were selected from both genders, and those with relevant systemic illness, pregnancy or recent medication were excluded. We made sure that all CP patients had not used mouthrinses or pulsed oral irrigators on a regular basis, and had not received any periodontal therapy within the preceding 3 years. The study was approved by the Institutional Review Board of the Niigata University, Faculty of Dentistry, and in accordance with the Helsinki declaration, a written informed consent was obtained from all participants before inclusion in the study.

Patients were classified as either smoker (current and former), i.e. regular daily smokers ($n = 8$) and previous regular smokers who had ceased the habit >5 years ago ($n = 4$), or non-smoker, i.e. who had never smoked tobacco (23 patients). The mean pack year of current smokers was 28.2 ± 7.6 packs/year. All the smokers were cigarette smokers. The age differences among the smoking groups were not statistically significant ($p > 0.05$) (Table 1).

CP patients received two or three visits of toothbrushing instructions (TBIs), which according to individual needs included the use of inter-proximal cleaning aids such as fross and interdental brushes. After TBI and scaling, all the patients underwent scaling and root planing under local anaesthesia on a quadrant-by-quadrant basis within 2 months. Scaling and root planing were performed by sharp sickles, gracey and universal curettes, as well as with ultrasonic instruments. Mechanical periodontal therapy was not accompanied by any medications such as antibiotics or non-steroidal anti-inflammatory drugs.

Clinical assessments

All the subjects were evaluated clinically and radiographically at the first visit to assess the following periodontal measurements: number of teeth, PPD, clinical attachment level (CAL), BOP, plaque index and gingival index. Six sites were examined on each tooth: mesio-buccal, buccal, disto-buccal, disto-lingual, lingual and mesio-lingual.

Table 1. Clinical parameters and serum levels in CP patients before and after treatment

	CP patients non-smokers ($n = 23$)		CP patients smokers ($n = 12$)	
	baseline	after treatment	baseline	after treatment
Age (years)	56.9 ± 1.6		51.8 ± 2.2	
Male/female	1/22		8/4	
Clinical periodontal parameters of patients				
Mean PPD (mm)	$3.6 \pm 0.1^{*}$	$2.7 \pm 0.1^{\#\#}$	$4.1 \pm 0.2^{*}$	$3.0 \pm 0.1^{\#\#}$
Mean CAL (mm)	$4.1 \pm 0.2^{*}$	$3.7 \pm 0.2^{\#\#}$	$4.7 \pm 0.3^{*}$	$4.0 \pm 0.2^{\#\#}$
Mean PlI	0.6 ± 0.1	$0.2 \pm 0.0^{\#\#}$	0.9 ± 0.2	$0.3 \pm 0.0^{\#\#}$
Mean GI	0.9 ± 0.1	$0.2 \pm 0.0^{\#\#}$	1.1 ± 0.1	$0.3 \pm 0.1^{\#\#}$
Serum levels				
TNF-α (pg/ml)	1.7 ± 0.2	2.0 ± 0.1	2.0 ± 0.1	1.9 ± 0.1
sTNFR1 (pg/ml)	971.7 ± 38.8	906.4 ± 28.1	1014.8 ± 50.6	960.8 ± 68.9
sTNFR2 (pg/ml)	1726.6 ± 127.7	1706.3 ± 85.7	1794.6 ± 127.0	1828.2 ± 99.3
sTNFR2/R1 ratio	1.8 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	1.9 ± 0.1

Values represent the mean ± standard error.

$^{*}p < 0.05$ (compared non-smokers with smokers).

$^{\#\#}p < 0.01$ (compared baseline with after treatment).

PPD, probing pocket depth; CAL, clinical attachment loss; PlI, plaque index; GI; gingival index; TNF-α, tumor necrosis factor-α; sTNFR1, soluble tumor necrosis factor receptor 1; sTNFR2, soluble tumor necrosis factor receptor 2; CP, chronic periodontitis.

One calibrated examiner took all the measurements and recorded the results.

CP patients were evaluated clinically after 1 month of periodontal treatment to assess the periodontal measurements mentioned above.

Collection of serum and GCF

Serum and GCF sampling was done before and after initial periodontal treatment. Serum was obtained from clotted venous blood samples, and centrifuged at 2000 *g* for 20 min. and then stored at − 80°C until use.

A total of 90 sites, consisting of 60 sites from 23 non-smokers of CP patients and 30 sites from 12 smokers of CP patients, were selected on the basis of clinical examination. The sampling sites were grouped into PPD ⩽3, = 4–6 and ⩾7 mm. One representative site per group was selected from each patient. After treatment, we collected GCF samples from the sites of non-smokers and smokers which were PPD ⩾4 mm at baseline. After isolating the tooth with a cotton roll, supragingival plaque was removed with curettes, without touching the marginal gingiva. The crevicular site was then gently dried with an air syringe and the GCF was collected with filter paper strips (Periopaper®, Proflow® Incorporated, Amityville, NY, USA). The strips were placed into the pocket until mild resistance was sensed and left in place for 30 s. Samples visually contaminated with blood or diluted by saliva during sampling time were discarded and, after 30 s, a new strip was inserted until no contamination was visible. The procedure was repeated four times in the same site. The volume of GCF was measured by means of a pre-calibrated Periotoron 6000® (Harco Electronics Ltd., Winnipeg, Canada). After recording the measurements, they were converted into actual GCF volumes using a calibration graph. The GCF samples were placed in tubes with a transport medium containing 200 μl of phosphate-buffered saline without calcium chloride and magnesium chloride with 0.5% bovine serum albumin. The tubes were vortexed for 10 min. and then centrifuged for 10 min. at 10,000 *g*. Samples were stored at − 80°C.

Measurement of TNF-α, sTNFR1 and R2 in GCF and serum

Levels of TNF-α, sTNFR1 and R2 in serum and GCF samples were determined by using a commercially available enzyme-linked immunosorbant assay (ELISA) kit (R&D Systems Inc., Minneapolis, MN, USA). These are standard ''sandwich'' ELISAs, and were performed using human recombinant standards according to the manufacturer's instructions. These assays have a lower limit of detection for TNF-α (0.5 pg/ml), and of sTNFR1 and R2 (80 pg/ml).

Concentrations of the cytokine and its soluble receptors in 200 μl sample were determined by generation of a standard curve for comparison. We reported results of GCF data as total amount of cytokine and concentrations in picogram per site.

Statistical analysis

All data analyses were performed using a statistical package (Stat View J-4.5 application program, SAS Institute Inc., Cary, NC, USA).

Differences in clinical parameters of each subject, concentrations and ratios of TNF-α, sTNFR1 and R2 in serum between non-smoking and smoking CP patients were compared by using Mann–Whitney *U*-test.

Spearman's rank correlation analysis was used to analyse correlations among the total amount of TNF-α, sTNFR1 and R2 in GCF and PPD of sampling sites, as well as those among sTNFR2/R1 ratio and PPD sampling sites at baseline.

The statistical significance of the difference in TNF-α, sTNFR1 and R2 levels, and clinical parameters between before and after treatment was analysed using the Wilcoxon signed-rank test.

Comparisons of GCF sTNFR2/R1 ratio between BOP (−) and BOP (+) groups in disease sites at baseline and after treatment were also conducted. Statistical analysis was performed using the Mann–Whitney *U*-test.

Results

Clinical characteristics of CP patients

Clinical parameters of non-smokers and smokers with CP are presented in Table 1. Thirty-five CP patients (23 non-smokers and 12 smokers) were re-evaluated 1 month following the completion of initial periodontal treatment. All the clinical parameters significantly improved after treatment in both non-smoking and smoking CP groups.

Serum levels of TNF-α, sTNFR1 and R2

Table 1 shows levels and ratios of TNF-α, sTNFR1 and R2 in serum of CP patients at baseline and after treatment. Significant differences were not found in the serum levels of TNF-α, sTNFR1 and R2 between baseline and after treatment. Even though the serum levels of sTNFR1 and R2 of smoking CP patients were higher than those of non-smoking CP patients at baseline and after treatment, the difference did not reach statistical significance.

We also calculated the sTNFR2/R1 ratio, because the biological activity of TNF-α appears to be related to the ratio of its soluble receptors in vivo (Rooney et al. 2000). We had already reported that the sTNFR2/R1 ratio showed a trend towards smaller values in serum of CP patients in comparison with healthy subjects (Ikezawa et al. 2005). The sTNFR2/R1 ratio was slightly elevated after treatment, but without significant difference.

Clinical parameters and GCF volume in sampling disease sites of non-smokers and smokers before and after treatment

In total, 60 sites were included in this study. Clinical parameters and GCF volume of disease sites of CP patients (non-smokers and smokers) at baseline and after treatment are outlined in Table 2. GCF volume is expressed as the total volume collected for 30 s × 4 times. The GCF volumes and clinical parameters were significantly decreased after treatment of both non-smokers and smokers.

Comparison of GCF levels of TNF-α, sTNFR1 and R2 between non-smokers and smokers CP at baseline

We analysed the relationship between sTNFR1 and R2, and PPD of non-smokers and smokers at baseline (Fig. 1a–c). In both groups, the levels of sTNFR1 and R2 progressively diverged as the PPD increased, with sTNFR2 levels comparatively lower than sTNFR1 ones. The imbalance between the two sTNFRs in non-smokers and smokers was therefore clearly demonstrated in this study. We then calculated the correlation between sTNFR2/R1 ratio and PPD (Fig. 1d). The sTNFR2/R1 ratio significantly decreased as PPD values increased (Spearman's rank order: non-smokers $r_s = -0.62$, $p < 0.0001$; smokers $r_s = -0.51$, $p < 0.0001$).

Comparison of GCF levels of TNF-α, sTNFR1 and R2 between baselines and after treatment

Figure 2 shows GCF levels and ratios of TNF-α, sTNFR1 and R2 of disease sites at baseline and after treatment. The levels of TNF-α in GCF of smoking CP patients increased slightly, with those of non-smoking patients decreasing after treatment; however, differences between baseline and after treatment were non-significant. The GCF levels of sTNFR1 and R2 significantly decreased after treatment (Wilcoxon signed-rank test: sTNFR1 in non-smoking CP, $p<0.0001$; sTNFR1 in smoking CP, $p=0.001$; sTNFR2 in non-smoking CP, $p=0.0005$; sTNFR2 in smoking CP, $p=0.01$). The sTNFR2/R1 ratio was significantly increased (Wilcoxon signed-rank test: the sTNFR2/R1 ratio in non-smoking CP $p<0.0001$, the sTNFR2/R1 ratio in smoking CP $p=0.002$). Again, differences between non-smoking CP and smoking CP groups after treatment did not reach significance.

Table 2. Clinical parameters and GCF volume in sampling disease sites of CP patients before and after treatment

	GCF sampling site non-smokers ($n=40$)		GCF sampling site smokers ($n=20$)	
	baseline	after treatment	baseline	after treatment
PPD (mm)	6.3 ± 0.3	4.2 ± 0.3**	6.5 ± 0.3	3.8 ± 0.3**
CAL (mm)	7.1 ± 0.3	5.4 ± 0.4**	6.9 ± 0.4	4.9 ± 0.5**
PlI	1.0 ± 0.1	0.3 ± 0.1**	0.9 ± 0.2	0.3 ± 0.1**
GI	1.3 ± 0.1	0.6 ± 0.1**	1.3 ± 0.2	0.6 ± 0.1**
BOP	25/40	15/40	15/20	3/20
GCF volume (μl)	2.6 ± 0.2	1.6 ± 0.1**	2.4 ± 0.3	1.3 ± 0.2**

Values represent the mean ± standard error.
**$p<0.01$ (compared baseline with after treatment).
PPD, probing pocket depth, CAL, clinical attachment loss, PlI, plaque index, GI, gingival index, BOP, bleeding on probing, GCF, gingival crevicular fluid; CP, chronic periodontitis.

Comparisons of GCF sTNFR2/R1 ratio between BOP (−) and BOP (+) groups in disease sites at baseline and after treatment

We analysed the relationship between BOP and sTNFR2/R1 ratio in GCF

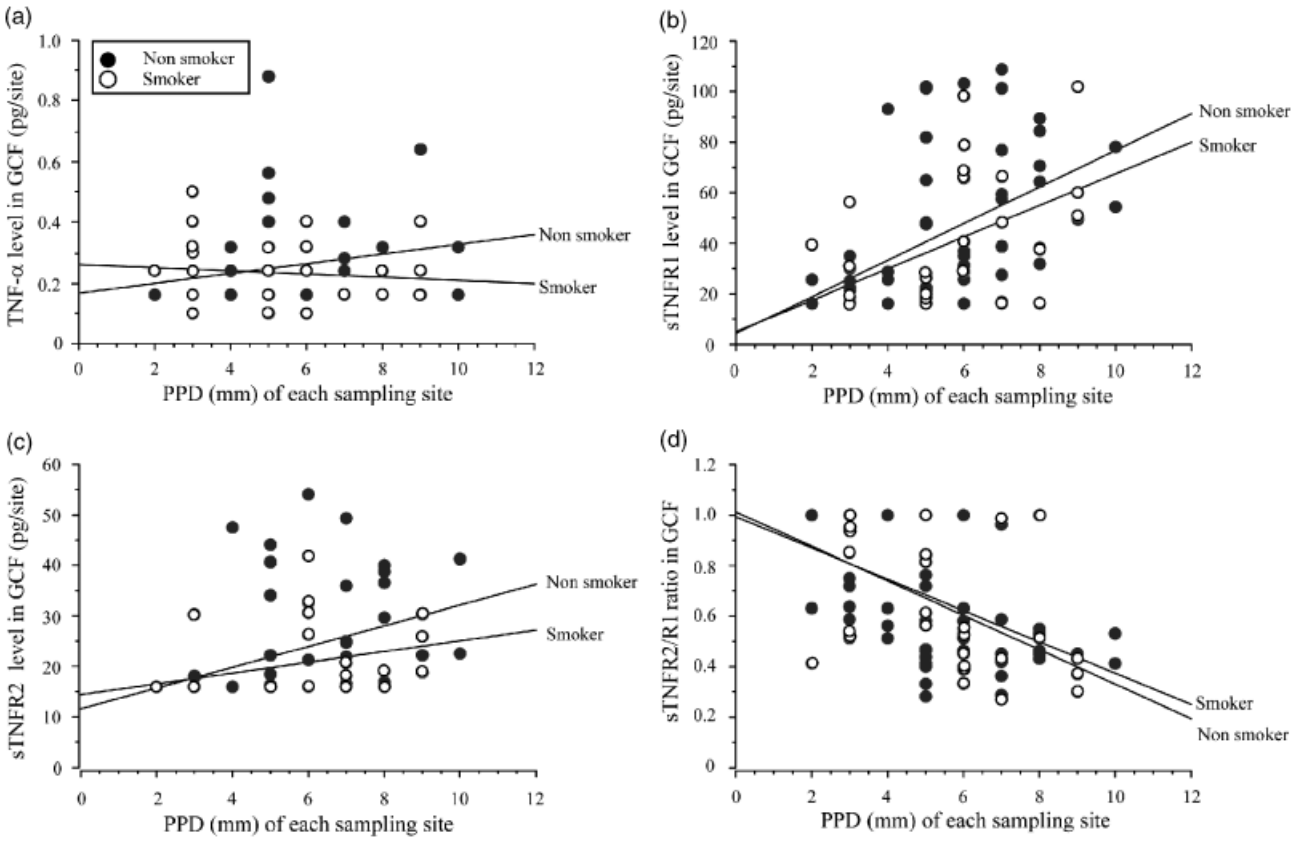

Fig. 1. (a–d) Correlation between probing pocket depth (PPD) (mm) of each sampling site and the levels of tumour necrosis factor α (TNF-α), soluble tumour necrosis factor receptor type 1 (sTNFR1), soluble tumour necrosis factor receptor type2 (sTNFR2) and sTNFR2/R1 ratio in gingival crevicular fluid (GCF) from chronic periodontitis non-smokers and smokers at baseline. Statistical analysis was performed using Spearman's rank order (TNF-α – non-smokers: $r_s=0.30$, $p=0.022$ and smokers: not significant; sTNFR1 – non-smokers: $r_s=0.64$, $p<0.0001$ and smokers: $r_s=0.50$, $p=0.007$; sTNFR2 – non-smokers: $r_s=0.59$, $p<0.0001$ and smokers: $r_s=0.54$, $p=0.004$; sTNFR2/R1 ratio – non-smokers: $r_s=-0.62$, $p<0.0001$ and smokers: $r_s=-0.51$, $p=0.006$).

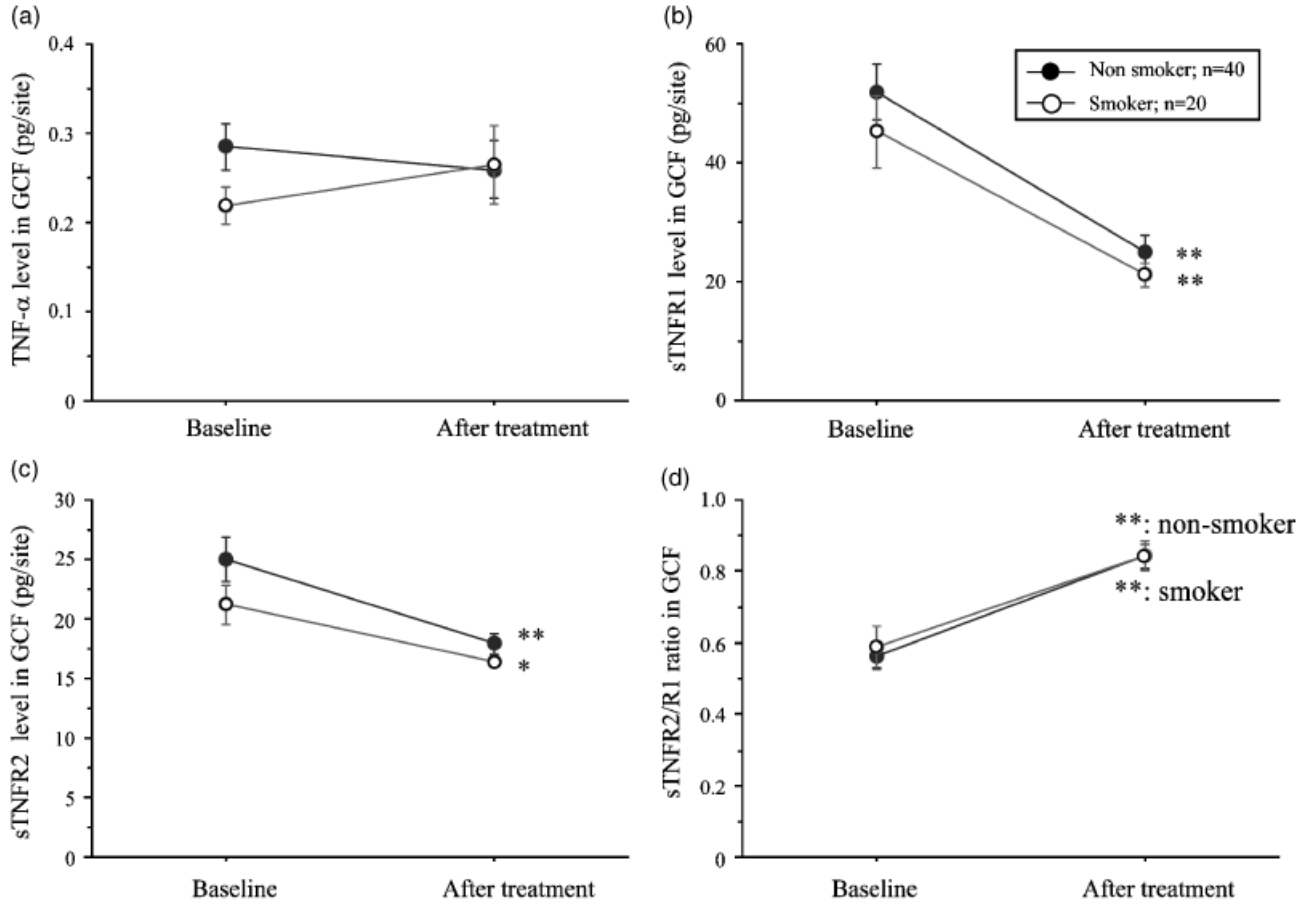

Fig. 2. (a–d) Levels of tumour necrosis factor α (TNF-α), soluble tumour necrosis factor receptor type 1 (sTNFR1), soluble tumour necrosis factor receptor type 2 (sTNFR2) and sTNFR2/R1 ratio in gingival crevicular fluid (GCF) at baseline and after treatment. Line graphs depict the change in them for chronic periodontitis non-smokers and smokers at baseline and following periodontal treatment for disease sites (TNF-α – not significant; sTNFR1 – non-smokers: $p < 0.0001$, smokers: $p = 0.001$; sTNFR2 – non-smokers: $p = 0.0005$, smokers: $p = 0.014$; sTNFR2/R1 ratio – non-smokers: $p < 0.0001$, smoker: $p = 0.0019$). $^{*}p < 0.05$, $^{**}p < 0.01$.

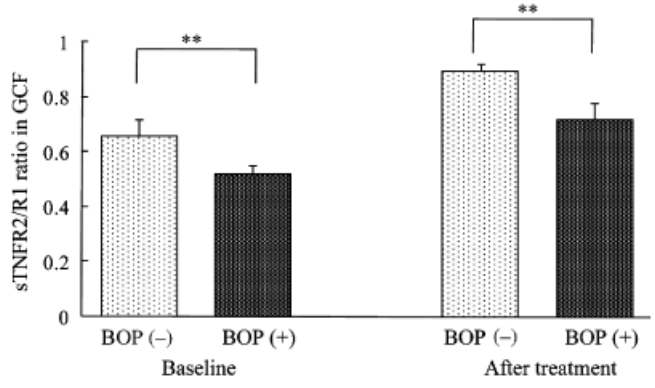

Fig. 3. Comparisons of gingival crevicular fluid (GCF) soluble tumour necrosis factor receptor type 1/type 2 (sTNFR2/R1) ratio between bleeding on probing (BOP) (−) and BOP (+) groups in disease sites at baseline and after treatment. Statistical analysis was performed using the Mann–Whitney *U*-test (baseline; $p = 0.046$, after treatment; $p = 0.024$). $^{**}p < 0.01$.

(Fig. 3). The ratios of sTNFR2/R1 in BOP (+) groups were significantly lower than BOP (−) groups at baseline and after treatment (Mann–Whitney *U*-test: baseline, $p = 0.046$; after treatment, $p = 0.024$).

Discussion

In this study, we have investigated the effects of initial periodontal treatment on serum and GCF levels of TNF-α, sTNFR1 and R2 in smokers and non-smokers of CP patients. Our previous study revealed that TNF-α, sTNFR1 and R2 in GCF were significantly increased and the sTNFR2/R1 ratio decreased with increasing PPD values in CP patients. Interestingly, the ratio of sTNFR2/R1 in GCF is also related to the severity of CP (Ikezawa et al. 2005).

Cseh et al. (2000) reported that the sTNFR2/R1 ratio seemed to be a good correlate of TNF-α action in serum of type II diabetes mellitus (DM-2) subjects with insulin resistance. Fernandez-Real et al. (2002) have shown that changes in plasma sTNFR2/R1 ratio run parallel with blood pressure variations in DM-2 and possibly with insulin sensitivity. Furthermore, Petelin et al. (2004) analysed the change in sTNFR2/R1 ratio to assess the net balance between sTNFR1 and R2 in a mouse model inflammatory lung tissue. The ratio of sTNFR2/R1 in serum of these mice was significantly enhanced 2 h after *Porphiromonas gingivalis* infec-

tion. Although a number of studies have presented conflicting results regarding the relationship between periodontitis and RA, there have been recent reports suggesting a significant association between these two common chronic inflammatory conditions (Mercado et al 2001). Increased synovial fluid levels of TNF-α and sTNFRs are also found in RA and seronegative arthropathies (Cope et al. 1992). The shedding of sTNFRs is believed to be related to TNF-α production, and increased levels of sTNFRs in biological fluids appear to reflect the activation state of the TNF-α/TNFR system (Diez-Ruiz et al. 1995). It has been proposed that the measurement of sTNFRs in body fluids, especially that of sTNFR2, is useful for the quantification of the immune response (Diez-Ruiz et al. 1995, Aderka 1996). The ratio of sTNFR2/R1 could possibly predict the susceptibility to, and progression of, any inflammatory reaction.

We raised the possibility that measurement of sTNFR1 and R2 instead of TNF-α in GCF may be a useful marker for diagnosis and monitoring periodontal disease activity (Ikezawa et al. 2005). In this study, GCF levels of sTNFR1 and R2 decreased significantly in non-smokers and smokers of CP patients, and the sTNFR2/R1 ratio significantly increased after treatment. The sTNFR2/R1 ratio in GCF was 0.96 ± 0.03 for healthy subjects ($n = 16$) and, after treatment, the ratio in GCF of CP patients is shown to be close to that of healthy subjects. In addition, our study has revealed that sTNFR2/R1 ratio in GCF could relate to BOP. We suggest that sTNFR2/R1 ratio in GCF is a useful marker of periodontal activity.

In RA, it is known that the state of disease is modulated by IL-1, TNF-α, and that TNF-α antagonists (e.g. sTNFR1 and R2) can arrest the disease in both animal and human studies (Moreland 1998). Etanercept, a recombinant sTNFR2:Fc fusion protein, has demonstrated excellent safety and efficacy in large-scale, randomized, double-blind, placebo-controlled trials of patients with RA and juvenile RA (Garrison & McDonnell 1999). Therefore, it is suggested that a recombinant sTNFR2 or etanercept could one day be applied to treat periodontal diseases. However, the method, timing and duration of administration suitable for periodontal therapy and potential untoward side effects would need to be investigated in order to make use of such cytokine antagonists practical.

The toxic components in cigarette smoke such as nicotine and reactive oxygen species alter the immune cell functions in heavy smokers (Ginns et al. 1982). The consequence of such changes may include effects on cytokine secretion, because the production of TNF-α by peripheral blood mononuclear cells is elevated in smokers (Lei et al. 2002). Lei et al. (2002) further showed that nicotine was capable of inducing increased TNF-α release. We have shown that the serum levels of TNF-α, sTNFR1 and R2 for smoking CP patients were higher than those for non-smoking CP subjects at baseline and after treatment. Significant difference was not reached.

Boström et al. (1998, 1999) have observed significantly increased levels of TNF-α in GCF of current smokers, treated and untreated for periodontal disease by gingival crevice lavage. On the other hand, Erdemir et al. (2004) reported that cigarette smoking did not influence GCF content of TNF-α by using paper strips. These conflicting results may be due to different sampling methods. By using paper strips, we found that the amount of GCF and the GCF levels of TNF-α, sTNFR1 and R2 in smokers have a tendency very similar to that of non-smokers. Erdemir et al. (2004) also reported that, after treatment, the total amount of TNF-α in GCF was decreased in the smoking group with CP. However, there were no significant differences in comparison with the TNF-α levels from the non-smoker group. Our data indicate that the GCF levels of TNF-α of smoking CP patients were increasing slightly and those of non-smoking decrease after treatment; however, significant differences between baseline and after treatment were not achieved. In this study, we detected GCF levels of TNF-α using high-sensitive ELISA with the minimum detectable by dose set at 0.5 pg/ml. The data of total amount was influenced by GCF volume, and indicated smaller levels in smokers after treatment.

Recently, the TNFR has been drawing the attention of dental researchers all over the world. Pericoronitis is an inflammation of the soft and hard tissues surrounding the crown of an erupting or impacted tooth. The clinical condition and the progression mechanism of pericoronitis are similar to those of periodontitis. Beklen et al. (2005) reported that the transmembrane TNFR1 and R2 were found in macrophage- and fibroblast-like cells, vascular endothelial cells in post-capillary venules and basal epithelial cells in pericoronitis, but were only weakly expressed in controls. They concluded that TNF-α and its receptors may play an important and active role in pericoronitis. Andrade et al. (2007) suggested that the TNFR1 plays a significant role in orthodontic tooth movement, one that might be associated with changes in chemokine (CCL5) levels. Spears et al. (2003) reported that gene microarray analysis evidenced that gene levels, notably those of TNF-α, and TNFR1, but not TNFR2, were significantly elevated in complete Freund's adjuvant (CFA)-treated temporomandibular joint (TMJ) tissue of adult male rats. However, protein levels of TNF-α, TNFR1 and R2 were all significantly increased in CFA-treated TMJ tissues. We support the possibility that measurement of TNF-α and its receptors may be useful makers for diagnosis and monitoring periodontal disease.

In conclusion, our study has revealed that TNF-α, sTNFR1 and R2 in GCF are significantly decreased, and that the sTNFR2/R1 ratio increased in non-smokers and smokers of CP after treatment. We suggest that the sTNFR2/R1 ratio may be a valuable maker for periodontal disease. A large prospective study would be needed to establish the relationship among TNF-α, sTNFR1 and R2, and periodontal disease activity.

References

Aderka, D. (1996) The potential biological and clinical significance of the soluble tumor necrosis factor receptors. *Cytokine and Growth Factor Reviews* **7**, 231–240.

American Academy of Periodontology. (1996) Tobacco use and the periodontal patient (position paper). *Journal of Periodontology* **67**, 51–56.

Andrade, I. Jr., Silva, T. A., Silva, G. A., Teixeira, A. L. & Teixeira, M. M. (2007) The role of tumor necrosis factor receptor type 1 in orthodontic tooth movement. *Journal of Dental Research* **86**, 1089–1094.

Assuma, R., Oates, T., Cochran, D., Amar, S. & Graves, D. T. (1998) IL-1 and TNF antagonists inhibit the inflammatory response and bone loss in experimental periodontitis. *Journal of Immunology* **160**, 403–409.

Beklen, A., Laine, M., Ventä, I., Hyrkäs, T. & Konttinen, Y. T. (2005) Role of TNF-alpha

and its receptors in pericoronitis. *Journal of Dental Research* **84**, 1178–1182.

Boström, L., Linder, L. E. & Bergström, J. (1998) Clinical expression of TNF-α in smoking-associated periodontal disease. *Journal of Clinical Periodontology* **25**, 767–773.

Boström, L., Linder, L. E. & Bergström, J. (1999) Smoking and crevicular fluid levels of IL-6 and TNF-α in periodontal disease. *Journal of Clinical Periodontology* **26**, 352–357.

Buchmann, R., Hasilik, A., Van Dyke, T. E. & Lange, D. E. (2002) Resolution of crevicular fluid leukocyte activity in patients treated for aggressive periodontal disease. *Journal of Periodontology* **73**, 995–1002.

Champagne, C. M. E., Buchanan, W., Reddy, M. S., Preisser, J. S., Beck, J. D. & Offenbacher, S. (2003) Potential for gingival crevice fluid measures as predictors of risk for periodontal diseases. *Periodontology 2000* **31**, 167–180.

Cope, A. P., Aderka, D., Doherty, M., Engelmann, H., Gibbons, D., Jones, A. C., Brennan, F. M., Maini, R. N., Wallach, D. & Feldmann, M. (1992) Increased levels of soluble tumor necrosis factor receptors in the sera and synovial fluid of patients with rheumatic diseases. *Arthritis and Rheumatism* **35**, 1160–1169.

Cseh, K., Winkler, G., Melczer, Z. & Baranyi, E. (2000) The role of tumor necrosis factor (TNF)-α resistance in obesity, and insulin resistance. *Diabetologia* **43**, 525.

Delima, A. J., Oates, T., Assuma, R., Schwartz, Z., Cochran, D., Amar, S. & Graves, D. T. (2001) Soluble antagonists to interleukin-1 (IL-1) and tumor necrosis factor (TNF) inhibits loss of tissue attachment in experimental periodontitis. *Journal of Clinical Periodontology* **28**, 233–240.

Diez-Ruiz, A., Tilz, G. P., Zangerle, R., Baier-Bitterlich, G., Wachter, H. & Fuchs, D. (1995) Soluble receptors for tumor necrosis factor in clinical laboratory diagnosis. *European Journal of Haematology* **54**, 1–8.

Erdemir, E. O., Duran, I. & Haliloglu, S. (2004) Effects of smoking on clinical parameters and the gingival crevicular fluid levels of IL-6 and TNF-α in patients with chronic periodontitis. *Journal of Clinical Periodontology* **31**, 99–104.

Fernandez-Real, J. M., Lainez, B., Vendrell, J., Rigla, M., Castro, A., Peñarroja, G., Broch, M., Pérez, A., Richert, C., Engel, P. & Ricart, W. (2002) Shedding of TNF-α receptors, blood pressure, and insulin sensitivity in type 2 diabetes mellitus. *American Journal of Physiology – Endocrinology and Metabolism* **282**, E952–E959.

Garrison, L. & McDonnell, N. D. (1999) Etanercept: therapeutic use in patients with rheumatoid arthritis. *Annals of the Rheumatic Diseases* **58**, 165–169.

Ginns, L. C., Goldenheim, P. D., Miller, L. G., Burton, R. C., Gillick, L., Colvin, R. B., Goldstein, G., Kung, P. C., Hurwitz, C. & Kazemi, H. (1982) T-lymphocyte subsets in smoking and lung cancer: analysis of monoclonal antibodies and flow cytometry. *The American Review of Respiratory Disease* **126**, 265–269.

Graves, D. T., Delima, A. J., Assuma, R., Amar, S., Oates, T. & Cochran, D. (1998) Interleukin-1 and tumor necrosis factor antagonists inhibit the progression of inflammatory cell infiltration toward alveolar bone in experimental periodontitis. *Journal of Periodontology* **69**, 1419–1425.

Haber, J. & Kent, R. L. (1992) Cigarette smoking in periodontal practice. *Journal of Periodontology* **63**, 100–106.

Ikezawa, I., Tai, H., Shimada, Y., Komatsu, Y., Galicia, J. C. & Yoshie, H. (2005) Imbalance between soluble tumor necrosis factor receptors type 1 and 2 in chronic periodontitis. *Journal of Clinical Periodontology* **32**, 1047–1054.

Lei, G. H., Li, K. H. & Zhou, J. N. (2002) Effect of nicotine on the secretion of TNF of human peripheral blood mononuclear cells in vitro. *Bulletin of Hunan Medical University* **27**, 285–287.

Mercado, F. B., Marshall, R. I., Klestov, A. C. & Bartold, P. M. (2001) Relationship between rheumatoid arthritis and periodontitis. *Journal of Periodontology* **72**, 779–787.

Moreland, L. W. (1998) Soluble tumor necrosis factor receptor (p75) fusion protein (ENBREL) as a therapy for rheumatoid arthritis. *Rheumatic Diseases Clinics of North America* **24**, 579–591.

Ohe, H., Takashiba, S., Naruishi, K., Chou, H. H., Yamada, H., Nishimura, F., Arai, H. & Murayama, Y. (2000) Tumor necrosis factor-α (TNF-α)-induced and interleukin-1β (IL-1β)-induced shedding of TNF receptors from gingival fibroblasts. *Journal of Interferon and Cytokine Research* **20**, 1077–1082.

Page, R. C., Offenbacher, S., Schroeder, H. E., Seymour, G. J. & Kornman, K. S. (1997) Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. *Periodontology 2000* **14**, 216–248.

Petelin, M., Naruishi, K., Shiomi, N., Mineshiba, J., Arai, H., Nishimura, F., Takashiba, S. & Murayama, Y. (2004) Systemic upregulation of sTNFR2 and IL-6 in *Porphyromonas gingivalis* pneumonia in mice. *Experimental and Molecular Pathology* **76**, 76–81.

Preber, H. & Bergstoröm, J. (1986) The effect of non-surgical treatment on periodontal pockets in smokers and non-smokers. *Journal of Clinical Periodontology* **13**, 319–323.

Rooney, M., Varsani, H., Martin, K., Lombard, P. R., Dayer, J. M. & Woo, P. (2000) Tumor necrosis factor alpha and its soluble receptors in juvenile chronic arthritis. *Rheumatology (Oxford, England)* **39**, 432–438.

Rossomando, E. F., Kennedy, J. E. & Hadjimichael, J. (1990) Tumor necrosis factor alpha in gingival crevicular fluid as a possible indicator of periodontal disease in humans. *Archives of Oral Biology* **35**, 431–434.

Spears, R., Oakes, R., Moore, C., Bellinger, L. L. & Hutchins, B. (2003) A determination of tumor necrosis factor expression in TMJ inflammation with the use of microarray analysis. *Journal of Dental Research* **82**, 807–813.

Tervahartiala, T., Koski, H., Xu, J. W., Häyrinen-Immonen, R., Hietanen, J., Sorsa, T. & Konttinen, Y. T. (2001) Tumor necrosis factor-α and its receptors, p55 and p75, in gingiva of adult periodontitis. *Journal of Dental Research* **80**, 1535–1539.

Tüter, G., Kurtiş, B. & Serdar, M. (2002) Effects of phase I treatment on gingival crevicular fluid matrix metalloprotease-1 and tissue inhibitor of metalloprotease-1. *Periodontology 2000* **73**, 487–493.

Wilton, J. M., Bampton, J. L., Griffiths, G. S., Curtis, M. A., Life, J. S., Johnson, N. W., Powell, J. R., Harrap, G. J. & Critchley, P. (1992) Interleukin-1 beta (IL-1β) levels in gingival crevicular fluid from adults with previous evidence of destructive periodontitis. *Journal of Clinical Periodontology* **19**, 53–57.

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Clinical Relevance

Scientific rationale for the study: sTNFR1 and R2 modulate the TNF-α-mediated inflammatory responses in CP. This study aimed to investigate the changes in the ratio of sTNFR2/R1 after periodontal treatment.

Principal findings: The ratio of sTNFR2/R1 in GCF significantly increased after treatment, indicating that the imbalance between these receptors could recover periodontal health through periodontal treatment.

Practical implications: Soluble TNF receptors, particularly sTNFR2/R1 ratio, may be new candidates as markers of periodontal disease activity.