J Clin Periodontol 2011; 38: 687–693 doi: 10.1111/j.1600-051X.2011.01731.x

Journal of Clinical Periodontology

Serum interleukin-6 may modulate periodontal inflammation in type 1 diabetic subjects

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Passoja A, Knuuttila M, Hiltunen L, Karttunen R, Niemelä O, Raunio T, Vainio O, Hedberg P, Tervonen T. Serum IL-6 may modulate periodontal inflammation in type 1 diabetic subjects. J Clin Periodontol 2011; 38: 687–693. doi: 10.1111/j.1600-051X.2011.01731.x.

Abstract

Aims: To evaluate the associations between serum inflammatory biomarkers and periodontal inflammation in subjects with type 1 diabetes mellitus (T1DM). Our hypothesis was that local host responses may be modulated by the serum inflammatory mediators.

Material and methods: Plaque, bleeding on probing and probing pocket depth (PD) were examined in 80 T1DM subjects at the baseline and in 58 subjects 8 weeks after periodontal therapy. The levels of glycosylated haemoglobin, serum interleukin (IL)-6, ultrasensitive C-reactive protein and the lipid profile were measured at the baseline and after therapy. Stratification of the sample separately by smoking and body mass index (BMI) was performed. Adjusted associations between the levels of systemic biomarkers and periodontal parameters were studied using multiple regression models.

Results: The level of serum IL-6 was associated with the extent of bleeding and PD $\geqslant$ 4 mm at the baseline in non-smokers and in subjects with BMI $\leqslant$ 26 kg/m^2. These associations were also evident after periodontal therapy. Subjects with a high after-therapy IL-6 level presented poorer periodontal healing than those with a low level.

Conclusions: The observed associations may be considered to be suggestive of a modulatory effect of IL-6 on host responses in T1DM subjects.

Key words: bleeding on probing; diabetes mellitus; inflammation; interleukin (IL)-6; periodontitis; serum

Accepted for publication 20 March 2011

Hyperglycaemia is known to induce a pro-inflammatory state characterized by increased concentrations of systemic inflammatory biomarkers such as cell adhesion molecules, C-reactive protein (CRP) and pro-inflammatory cytokines, including interleukin (IL)-6 and tumour necrosis factor (TNF)-α (Devaraj et al. 2007, Kaul et al. 2010). While it is generally accepted that the major causative factor behind diabetic complications is hyperglycaemia, growing evidence exists that the development and progression of diabetic micro- and macro-vascular complications are also linked with systemic inflammation (Saraheimo et al. 2003, Devaraj et al. 2007). Both gingivitis and periodontitis have been considered as hyperglycaemia-related diabetic complications (Taylor 2001, Lalla et al. 2007a). Since the very first reports by Gislen et al. (1980) and by our own group (Ervasti et al. 1985, Tervonen & Knuuttila 1986), the role of the metabolic control of diabetes mellitus as the main determinant of increased periodontal inflammation has been confirmed in a large number of studies (Taylor 2001, Lalla et al. 2007a). Importantly, both micro-vascular complications and periodontal inflammation seem to share at least some potential hyperglycaemia-related pathways in their development, including hyperglycaemia-enhanced formation of advanced glycation end products, protein kinase C activation, increased oxidative stress and apoptosis (Graves et al. 2006, Devaraj et al. 2007, Nassar et al. 2007, van den Oever et al. 2010).

It is nowadays generally agreed that chronic periodontitis is associated with low-grade systemic inflammation both in non-diabetic (Loos 2005, Nibali et al.

Conflict of interest and source of funding statement

The authors declare that they have no conflicts of interests.
The study was self-funded by the authors and their institutions.

2007, Saxlin et al. 2009) and in diabetic (Engebretson et al. 2007, Lim et al. 2007, Chen et al. 2010) individuals. On the contrary, less is known about the contribution of elevated levels of serum inflammatory mediators to periodontal inflammation. This biological pathway was studied recently by Andriankaja et al. (2009), who observed a significant association between serum IL-6 and gingivitis in type 2 diabetic subjects with no deepened periodontal pockets ($\geqslant$3 mm) and bleeding on probing (BOP) $\geqslant$10%. One of the conclusions drawn by the authors was that serum IL-6 might have a specific role in modulating the local inflammatory reaction in individuals with type 2 diabetes mellitus .

We determined the periodontal health status and systemic inflammation in a group of type 1 diabetes mellitus (T1DM) subjects before and after periodontal therapy to explore the possible bidirectional nature of the association between periodontal health and systemic inflammatory status. Moreover, our aim was to study whether the possible link between periodontal and systemic inflammation is analogous to the link between systemic inflammation and the development and progression of micro-vascular complications of diabetes mellitus.

Material and Methods

The study protocol was approved by the Ethical Committee of Oulu University Hospital, Oulu, Finland, and an informed consent form was signed by the subjects.

Subjects

Originally, a total of 80 patients with T1DM were recruited on a volunteer basis and examined clinically by a periodontal specialist (T. R.) at the Specialist Dental Health Care Unit, City of Oulu, Finland (Tervonen et al. 2009). Subjects requiring prophylactic antibiotic medication in association with periodontal probing and those with immunosuppressive medication or antibiotics during the past 4 months were excluded. After anti-infective periodontal therapy, a total of 65 subjects were re-examined. Data on glycosylated haemoglobin (HbA1c), high-density HDL cholesterol, low-density LDL cholesterol, IL-6 and ultrasensitive C-reactive protein (usCRP) were available for all subjects at the baseline and for 59 subjects after therapy. One subject with a severe and acute wound infection and maxillary sinusitis showed a pronounced increase in serum IL-6 (from 3.6 to 8.2 pg/ml) during the study and was excluded, leaving a total of 58 treated subjects for the analyses.

Patient records were used to retrieve data regarding the general health status of the subjects. Four subjects had either chronic maxillary sinusitis, skin infection or symptoms of a urinary tract infection during the study (indicated in Fig. 1a). Smoking data were obtained by an interview and the subjects were categorized as smokers and non-smokers. A total of 56 subjects of those examined at the baseline and 44 out of the treated subjects reported to be non-smokers. The subjects' body mass index (BMI kg/m^2) and age were also recorded. A total of 58 subjects out of those studied at the baseline and 44 of the treated subjects had BMI $\leqslant$ 26 kg/m^2 (Table 1) (according to the WHO, the lower limit for overweight is 25 kg/m^2).

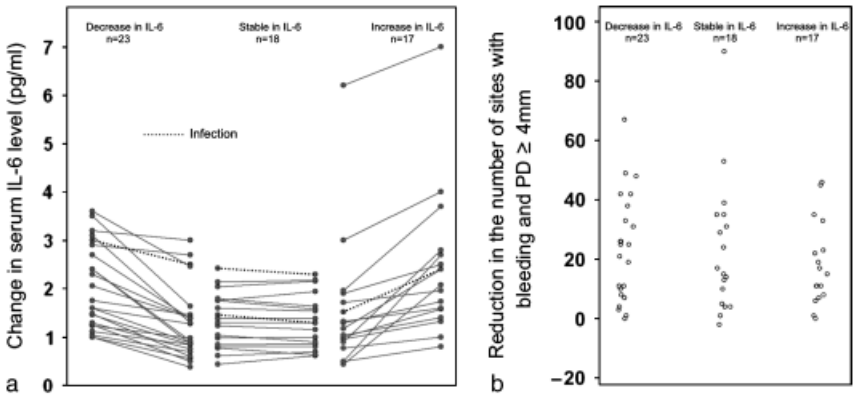

Fig. 1. (a) Subject-level changes in serum IL-6 level. A decrease in serum IL-6 level ($\geq$ 0.2 pg/ml) between the baseline and the follow-up examination was observed in 23 subjects (lines on the left) and an increase ($\geq$ 0.2 pg/ml) in 17 subjects (lines on the right). The lines in the middle present subjects with a stable IL-6 level (change $<$0.2 pg/ml). Subjects with chronic infections, other than gingivitis or periodontitis, are indicated using dashed lines. (b) Healing of periodontal inflammation indicated as the reduction in the number of sites with bleeding and PD $\geq$ 4 mm in subjects presenting increased, stable or decreased serum IL-6 level between the baseline and the follow-up examination (see Fig. 1a).

Table 1. Subject characteristics presented as mean values/subject ($\pm$ SD) and numbers of subjects

	Baseline		After therapy
	All subjects (n = 80)	Treated subjects (n = 58)	(n = 58)
Age (years)	38.6 $\pm$ 12.3	39.5 $\pm$ 12.6	39.5 $\pm$ 12.6
Gender (female/male)	46/34	34/24	34/24
Smoking (non-smoker/smoker)	56/24	44/14	44/14
BMI $\leqslant$ 26 (kg/m^2)	58/22	44/14	44/14
Duration of DM, years	19.9 $\pm$ 11.9	19.7 $\pm$ 12.4	19.7 $\pm$ 12.4
Number of			
Teeth	25.7 $\pm$ 4.1	26.2 $\pm$ 3.2	26.0 $\pm$ 3.3
Sites	102.9 $\pm$ 16.5	105.0 $\pm$ 12.8	103.8 $\pm$ 13.1
Number of sites with			
Plaque	30.7 $\pm$ 22.3	30.1 $\pm$ 21.8	3.4 $\pm$ 8.2
BOP	69.3 $\pm$ 18.1	70.9 $\pm$ 15.1	14.9 $\pm$ 12.9
Bleeding and PD $\geqslant$ 4 mm	23.1 $\pm$ 18.5	23.0 $\pm$ 19.0	1.0 $\pm$ 2.1
IL-6 (pg/ml)	1.7 $\pm$ 1.0	1.7 $\pm$ 1.0	1.6 $\pm$ 1.1
HbA1c (%)	8.5 $\pm$ 1.4	8.5 $\pm$ 1.4	8.4 $\pm$ 1.4
HDL (mmol/l)	1.6 $\pm$ 0.4	1.5 $\pm$ 0.4	1.5 $\pm$ 0.3
LDL (mmol/l)	2.3 $\pm$ 0.8	2.4 $\pm$ 0.7	2.4 $\pm$ 0.7
usCRP (mg/l)	2.3 $\pm$ 3.9	2.3 $\pm$ 3.9	2.9 $\pm$ 4.8
BMI (kg/m^2)	24.7 $\pm$ 3.3	24.5 $\pm$ 3.1	24.5 $\pm$ 3.1

BOP, bleeding on probing; bleeding and PD $\geqslant$ 4 mm, bleeding pocket $\geqslant$ 4 mm in depth; BMI, body mass index; HbA1c, glycosylated haemoglobin; HDL, high-density lipoprotein; IL-6, interleukin; LDL, low-density lipoprotein; usCRP, ultrasensitive C-reactive protein.

Clinical periodontal examination and periodontal therapy

Periodontal variables were recorded on the mesiobuccal, midbuccal, distobuccal

and midlingual surfaces of each tooth as described earlier (Tervonen et al. 2009). Briefly, the presence of visible plaque according to the criteria of the scores 2 and 3 of the plaque index (Silness & Löe 1964), probing depth (PD) and BOP were registered. Anti-infective periodontal therapy including oral hygiene education, scaling and root planing were performed. As the goal was to minimize periodontal inflammation, these treatments were repeated and periodontal surgery was performed whenever indicated. In the follow-up examination approximately 8 weeks after the completion of the treatment, one to nine bleeding sites were found in 14 subjects, 10–19 sites in 19 and >20 sites in 16 subjects. Moreover, bleeding and PD≥4 mm were detected at one to two sites in 14 subjects and at 3–10 sites in seven subjects.

Table 2. Unadjusted associations, based on Spearman's rank correlation analysis, between the numbers of sites presenting bleeding on probing (BOP) and bleeding and PD≥4 mm, serum inflammatory markers and body mass index (BMI) at the baseline

	BOP	Bleeding PD≥4 mm	HbA1c	IL-6	HDL	LDL	usCRP
Bleeding PD≥4 mm	**0.715**						
	<0.001						
HbA1c	**0.332**	**0.258**					
	0.003	**0.021**					
IL-6	0.137	**0.234**	0.071				
	0.226	**0.037**	0.534				
HDL	−0.128	−0.219	0.025	−0.127			
	0.257	0.051	0.823	0.260			
LDL	0.142	0.049	0.136	0.148	**0.322**		
	0.210	0.669	0.231	0.191	**0.004**		
usCRP	0.156	0.140	**0.348**	**0.427**	**0.223**	**0.279**	
	0.167	0.215	**0.002**	**<0.001**	**0.047**	**0.012**	
BMI	0.045	0.028	-0.045	0.187	-0.031	**0.293**	**0.272**
	0.692	0.808	0.694	0.097	0.787	**0.008**	**0.015**

Correlation coefficients, *p*-values; statistically significant values are presented in boldface.
Bleeding and PD≥4 mm, bleeding pocket≥4 mm in depth; BMI, body mass index; HbA1c, glycosylated haemoglobin; HDL, high-density lipoprotein; IL-6, interleukin; LDL, low-density lipoprotein; usCRP, ultrasensitive C-reactive protein.

Laboratory assays

Venous blood samples were drawn at the baseline and in the follow-up examination. Glycosylated haemoglobin (%) was analysed using a latex immunoturbidimetric method (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA). Serum samples were stored at −70°C until assayed. Serum IL-6 level (pg/ml) was measured using a sensitive ELISA Quantikine HS Immunoassay kit (R&D Systems GmbH, Wiesbaden-Nordenstadt, Germany). The usCRP (mg/l) analyses were performed using an Innotrac Aio! analyser (Innotrac Diagnostics Oy, Turku, Finland), which utilizes a unique all-in-one (Aio!) dry-reagent concept and time-resolved fluorometric detection (Hedberg et al. 2004). Low-density LDL cholesterol (mmol/l) and high-density HDL cholesterol (mmol/l) were measured using direct enzymatic methods implemented in the Advia 2400 Chemistry systems (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA).

An ''increase'' in the IL-6 level between the baseline and the follow-up examination was recorded if the level was elevated by ≥0.2 pg/ml, a ''decrease'' if the level declined by ≥0.2 pg/ml and a lack of either of the previous was considered a ''stable'' level (Fig. 1a).

Statistical methods

Statistical analyses were performed using statistical software (SPSS Statistical Package for Social Sciences, version 16.0 for Windows; SPSS Inc., Chicago, IL, USA) and the level of statistical significance was set at <5% ($p<0.05$). All analyses were based on subject-level data. The extent of periodontal inflammation was measured as the number of affected sites per subject. The mean serum IL-6 levels before and after therapy were compared using the Wilcoxon signed ranks test. Spearman's rank correlation analysis was used to study unadjusted associations between periodontal parameters and serum inflammatory markers at the baseline (Table 2). Depending on the distribution of the outcome, variable-adjusted associations between periodontal inflammation and serum inflammatory markers were studied using either linear regression or binomial regression analyses. In the binomial regression models, the *ln* of the number of measured sites was used as an offset variable. In order to control the confounding/modifying effect of smoking and BMI on the association between serum IL-6 and periodontal inflammation, we used the interaction terms BMI*IL-6 and smoking*IL-6 in the models. We also stratified the sample by BMI (≤26 kg/m^2/>26 kg/m^2) and smoking (non-smokers/smokers) to study the associations in the stratified samples. The association between periodontal healing and serum IL-6 level was studied using linear regression analysis, with the reduction in the number of sites with bleeding and PD≥4 mm (number of sites after therapy deducted from the number of sites at the baseline, range −2 to 90) as the outcome.

Results

The subject characteristics are presented in Table 1. The group of treated subjects was comparable with the entire study group in terms of age, gender, smoking habits, BMI, duration of DM and number of teeth. Regardless of a favourable response to periodontal therapy, no essential changes in the mean levels of serum inflammatory markers from the baseline to the follow-up examination could be observed.

Based on the unadjusted associations between serum inflammatory markers and the periodontal parameters at the baseline (Table 2), we studied the adjusted associations between the number of sites with BOP and bleeding and PD≥4 mm (the outcome variables) and serum IL-6 and HbA1c in further analyses using multiple regression models. The regression analyses of all subjects both at the baseline and after periodontal therapy (non-stratified samples) revealed statistically significant associations between serum IL-6 and periodontal inflammation. We then controlled for possible interaction between serum IL-6 and smoking and serum IL-6 and BMI by adding the interaction term smoking*IL-6 and BMI*IL-6 in the model and found that the interaction terms were not statistically significant in the models. Neither was there any statistically significant association between serum IL-6 level and periodontal inflammation in the new model (data not shown). Therefore, further analyses were performed in samples stratified separately by smoking

and BMI. While the serum HbA1c level was associated significantly with BOP and bleeding and PD ⩾ 4 mm both in subjects with BMI ⩽ 26 kg/m^2 and in non-smokers at the baseline, the serum IL-6 level turned out to be a statistically significant determinant of the number of sites with bleeding and PD ⩾ 4 mm in the same subjects (Table 3, Models I and II). At the baseline, the adjusted associations between serum IL-6 level and the number of sites with BOP and bleeding and PD ⩾ 4 mm were not statistically significant in subjects with BMI > 26 kg/m^2 ($n = 22$) and in smokers ($n = 24$) (data not shown).

After periodontal therapy, a statistically significant association was found between the level of serum IL-6 and the number of sites with bleeding and PD ⩾ 4 mm in subjects with BMI ⩽ 26 kg/m^2 ($p = 0.043$) (Table 4, Model III). In non-smokers, this association was of borderline significance ($p = 0.064$) (Table 4, Model IV). The number of sites with bleeding and PD ⩾ 4 mm after therapy was not associated with smoking. The actual serum levels of IL-6 in subjects with BMI ⩽ 26 kg/m^2 and in non-smokers according to the number of residual sites with bleeding and PD ⩾ 4 mm after periodontal therapy are presented in Table 5. These results also indicate that the level of serum IL-6 is higher in subjects with higher numbers of residual sites with inflammation. The adjusted associations between serum IL-6 level and the number of sites with BOP and bleeding and PD ⩾ 4 mm were not statistically significant in subjects with BMI > 26 kg/m^2 ($n = 14$) and in smokers ($n = 14$) after therapy (data not shown).

A decrease (⩾ 0.2 pg/ml) in the serum IL-6 level was observed in 23 (39.7%) subjects between the baseline and the follow-up examination. The level remained stable in 18 (31.0%) subjects and an increase (⩾ 0.2 pg/ml) was observed in 17 (29.3%) subjects (Fig. 1a). Subjects with chronic infections were evenly distributed into the previous groups (Fig. 1a). The reduction in the number of sites with bleeding and PD ⩾ 4 mm was similar in subjects with a decreased, a stable or an increased serum IL-6 level (Fig. 1b). After controlling for confounding factors, a statistically significant association ($p = 0.003$) was found between periodontal healing (the outcome variable) and the after-therapy serum IL-6 level, indicating poorer periodontal healing in subjects with a high IL-6 level (Table 6). In a second model (data not shown) including the interaction terms BMI*IL-6 and smoking*IL-6, the above association turned out to be weaker ($p = 0.022$). The interaction terms were, however, not statistically significant.

Table 3. Adjusted associations between the number of bleeding sites (BOP) and sites with bleeding and PD ⩾ 4 mm (the outcome variables) and serum IL-6 level at the baseline in subjects with BMI ⩽ 26 kg/m^2 and in non-smokers

	B	95% CI	*p*-value
Model I; BMI ⩽ 26 kg/m^2 ($n = 58$)			
BOP*			
IL-6	3.029	− 0.553 to 6.612	0.096
HbA1c	**4.032**	**1.515** to **6.549**	**0.002**
Smoking†	− 6.383	− 14.429 to 1.662	0.117
Bleeding and PD ⩾ 4 mm‡			
IL-6	**0.327**	**0.075** to **0.578**	**0.011**
HbA1c	**0.274**	**0.087** to **0.460**	**0.004**
Smoking†	**− 0.653**	**− 1.245** to **− 0.061**	**0.031**
Model II; Non-smokers ($n = 56$)			
BOP*			
IL-6	1.021	− 2.686 to 4.728	0.582
HbA1c	**3.998**	**1.181** to **6.815**	**0.006**
Bleeding and PD ⩾ 4 mm‡			
IL-6	**0.288**	**0.040** to **0.536**	**0.023**
HbA1c	**0.218**	**0.011** to **0.425**	**0.039**

*Linear regression.

†Smoking as a categorized variable; non-smokers *versus* smokers (reference). Boldface denotes statistical significance.

‡Negative binomial regression models: Model I; adjusted for age, gender, smoking, HbA1c, serum HDL, plaque and number of sites. Model II; adjusted for age, gender, HbA1c, BMI, serum HDL, plaque and number of sites.

Bleeding and PD ⩾ 4 mm, bleeding pocket ⩾ 4 mm in depth; BMI, body mass index; HbA1c, glycosylated haemoglobin; IL-6, interleukin.

Table 4. Adjusted associations between the number of bleeding sites (BOP) and sites with bleeding and PD ⩾ 4 mm (the outcome variables) and serum IL-6 level after therapy in subjects with BMI ⩽ 26 kg/m^2 and in non-smokers

	B	95% CI	*p*-value
Model III; BMI ⩽ 26 kg/m^2 ($n = 44$)			
BOP			
IL-6	0.287	− 0.069 to 0.644	0.114
HbA1c	0.078	− 0.159 to 0.315	0.518
Smoking*	− 0.210	− 0.968 to 0.548	0.588
Bleeding and PD ⩾ 4 mm			
IL-6	**0.550**	**0.016** to **1.084**	**0.043**
HbA1c	− 0.052	− 0.454 to 0.350	0.800
Smoking*	0.308	− 0.911 to 1.527	0.621
Model IV; Non-smokers ($n = 44$)			
BOP			
IL-6	0.142	− 0.146 to 0.429	0.335
HbA1c	0.015	− 0.214 to 0.244	0.896
Bleeding and PD ⩾ 4 mm			
IL-6	0.402	− 0.023 to 0.827	0.064
HbA1c	0.040	− 0.320 to 0.401	0.827

*Smoking as a categorized variable; non-smokers *versus* smokers (reference). Boldface denotes statistical significance.

Negative binomial regression models: Model III, adjusted for age, gender, smoking, HbA1c, serum HDL, plaque and number of sites. Model IV, adjusted for age, gender, HbA1c, BMI, serum HDL, plaque and number of sites.

Bleeding and PD ⩾ 4 mm, bleeding pocket ⩾ 4 mm in depth; BMI, body mass index; HbA1c, glycosylated haemoglobin; IL-6, interleukin.

Discussion

In line with earlier studies (Loos et al. 2000, Buhlin et al. 2003) reporting higher serum IL-6 levels in patients with chronic periodontitis than in healthy control subjects, we found a statistically

Table 5. Mean serum IL-6 levels (pg/ml) according to the number of sites with bleeding and PD⩾4 mm after periodontal therapy

	BMI⩽26 kg/m^2		Non-smokers	
	N	IL-6 (pg/ml)	*N*	IL-6 (pg/ml)
All subjects	44	1.6 ± 1.1	44	1.6 ± 1.1
Subjects with				
No sites with bleeding and PD⩾4 mm	29	1.2 ± 0.6	27	1.3 ± 0.6
⩾1 sites with bleeding and PD⩾4 mm	15	2.2 ± 1.6	17	2.0 ± 1.5
⩾2 sites with bleeding and PD⩾4 mm	8	2.9 ± 1.9	10	2.3 ± 1.8
⩾3 sites with bleeding and PD⩾4 mm	5	3.2 ± 2.3	5	2.8 ± 2.4

Bleeding and PD⩾4 mm, bleeding pocket⩾4 mm in depth; BMI, body mass index; IL-6, interleukin.

Table 6. Adjusted associations between periodontal healing (the outcome variable) and serum IL-6 level (pg/ml) after therapy using linear regression analysis

	B	95 % CI for B	*p*-value
IL-6 after therapy (pg/ml)	**− 0.973**	**− 1.599 to − 0.348**	**0.003**
IL-6 at the baseline (pg/ml)	0.381	− 0.288 to 1.050	0.258
Number of sites with bleeding and PD⩾4 mm at the baseline	**0.951**	**0.922 to 0.980**	**<0.001**
Reduction in the number of sites with plaque	**0.028**	**0.002** to **0.055**	**0.039**
Smoking	− 0.800	− 1.920 to 0.320	0.158
BMI	0.014	− 0.141 to 0.168	0.860

The outcome variable: the number of sites with bleeding and PD⩾4 mm after therapy deducted from the number of the sites at the baseline (range − 2 to 90).
Smoking as a categorized variable; non-smokers *versus* smokers (reference).
Bleeding and PD⩾4 mm, bleeding pocket⩾4 mm in depth; BMI, body mass index; IL-6, interleukin. Boldface denotes statistical significance.

significant association between the level of serum IL-6 and the number of sites with bleeding and PD ≥ 4 mm at the baseline. A new finding was that this association was also evident after periodontal therapy. We also observed that periodontal therapy was not consistently followed by a decrease in the serum IL-6 level and that periodontal healing turned out to be poorer in subjects with a high after-therapy IL-6 level than in those with a low level.

When interpreting the above observations, we started with the premise that the causal pathway between serum IL-6 and periodontal inflammation may be bidirectional. Increased serum levels of inflammatory markers, such as IL-6, in subjects with chronic periodontitis have been explained by dumping of locally concentrated IL-6 from the inflamed areas into the circulation, its production by distant immune cells as a response to bacterial irritation from the site of infection or both. In light of the above, a high serum IL-6 level observed at the baseline of this study may be interpreted as reflecting a high extent of periodontal inflammation.

The association between serum IL-6 level and the number of sites with bleeding and PD⩾4 mm after therapy on the one hand and the reduction in the number of sites with bleeding and PD⩾4 mm on the other was interpreted to indicate poorer periodontal healing in subjects with higher serum IL-6 levels and to be suggestive of a causal pathway, where a high serum IL-6 level acts as a susceptibility factor for periodontal inflammation. IL-6 is known for its pro-inflammatory properties (Kaplanski et al. 2003, Gabay 2006, Kishimoto 2006) and it also accelerates bone resorption (Tamura et al. 1993). Therefore, a high serum IL-6 level may boost inflammation and modulate tissue responses locally in the periodontal area. Although we consider the above direction of the causal pathway more likely, one could also speculate that the resolution of inflammation contributed to the decreased serum IL-6 levels; the higher the number of healed sites, the less IL-6 could be detected in serum after therapy. Supporting this, slightly decreased levels of serum inflammatory markers after anti-infective therapies have been reported (D'Aiuto et al. 2004, 2005, Kardeşler et al. 2010). However, in our subjects, the decrease in the mean serum IL-6 level from the baseline to the follow-up examination (0.1 pg/ml) was not statistically significant. In addition, similar periodontal healing was followed by a decrease in the IL-6 level in 40%, a stable level in 31% and an increase in 29% of the subjects (Fig. 1a,b). These results further support our interpretation that serum IL-6 acts as a susceptibility factor for periodontal inflammation. We cannot fully exclude that there were also other mechanisms/conditions, such as sub-clinical or unreported infections, which may have contributed to the serum IL-6 levels. Also, including a non-diabetic group with a comparable periodontal health status would have made it possible to assess the influence of diabetes mellitus itself on the systemic IL-6.

One of the methodological challenges in studies of the periodontal–systemic relationship is to adequately control the confounding/modifying effects of smoking and BMI. Smoking is known to deteriorate periodontal health (Bergström 2006) and evidently increase the serum IL-6 level (Tappia et al. 1995). On the other hand, adipose tissue is considered an important source of IL-6 (Mohamed-Ali et al. 1997, Fried et al. 1998), and obesity/BMI has been associated with chronic periodontitis (Ylöstalo et al. 2008). We took into consideration the above both by using respective interaction terms in the multivariate models and by stratifying the samples according to smoking and BMI. Because of the small sample size, stratification according to smoking and BMI at the same time was not possible. As regards a more complete control of the confounding/modifying effect of smoking and BMI, a need for verification of our result in larger samples remains. Moreover, a more definite measure of smoking, such as pack-years, should be used.

As the goal of periodontal therapy was to minimize periodontal inflammation, anti-infective treatments were continued until this goal was reached. The lack of a statistically significant association between smoking and periodontal inflammation after therapy can be explained by the setting of the study, where additional treatments were delivered to poorly responding subjects such as smokers. Considering the treatment protocol used and the suggested poorer healing response of subjects with higher IL-6 levels, we speculate that the strength of the association between serum IL-6 and the number of inflamed sites after therapy was underestimated.

The shortcomings of this study include the fairly small sample size and the lack of power analysis for the sample size. Moreover, this study allows us to suggest a bidirectional relationship between serum IL-6 level and periodontal inflammation exclusively in T1DM subjects. Regarding the association observed between serum IL-6 level and periodontal inflammation, the likelihood of a spurious association must also be considered; in that case, there are one or more common background factors contributing to both the increased serum level and the higher extent of periodontal inflammation.

In summary, while the serum IL-6 level was associated significantly with periodontal inflammation at the baseline, resolution of inflammation was not followed by a decrease in the serum IL-6 level in all subjects. The response to anti-infective periodontal therapy was poorer in subjects with a high after-therapy level of serum IL-6 than in those with a low level. Thus, a high serum IL-6 level may modulate inflammatory responses in the periodontal area as it modulates the development and progression of diabetic micro- and macro-vascular complications. The evident bidirectional causal pathway between serum IL-6 and periodontal inflammation is essential in elucidating both the pathogenesis of periodontitis and periodontal–systemic relationships. Therefore, future studies should be focused on confirming the observed associations also in non-diabetic individuals.

References

Andriankaja, O. M., Barros, S. P., Moss, K., Panagakos, F. S., DeVizio, W., Beck, J. & Offenbacher, S. (2009) Levels of serum interleukin (IL)-6 and gingival crevicular fluid of IL-1beta and prostaglandin E(2) among non-smoking subjects with gingivitis and type 2 diabetes. *Journal of Periodontology* **80**, 307–316.

Bergström, J. (2006) Periodontitis and smoking. *Journal of Evidence Based Dental Practice* **6**, 33–41.

Buhlin, K., Gustafsson, A., Pockley, A. G., Frostegård, J. & Klinge, B. (2003) Risk factors for cardiovascular disease in patients with periodontitis. *European Heart Journal* **24**, 2099–2107.

Chen, L., Wei, B., Li, J., Liu, F., Xuan, D., Xie, B. & Zhang, J. (2010) Association of periodontal parameters with metabolic level and systemic inflammatory markers in patients with type 2 diabetes. *Journal of Periodontology* **81**, 364–371.

D'Aiuto, F., Nibali, L., Parkar, M., Suvan, J. & Tonetti, M. S. (2005) Short-term effects of intensive periodontal therapy on serum inflammatory markers and cholesterol. *Journal of Dental Research* **84**, 269–273.

D'Aiuto, F., Parkar, M., Andreaou, G., Brett, P. M., Ready, D. & Tonetti, M. S. (2004) Periodontitis and atherogenesis: causal association or simple coincidence? *Journal of Clinical Periodontology* **31**, 402–411.

Devaraj, S., Cheung, A. T., Jialal, I., Griffen, S. C., Nguyen, D., Glaser, N. & Aoki, T. (2007) Evidence of increased inflammation and microcirculatory abnormalities in patients with type 1 diabetes and their role in microvascular complications. *Diabetes* **56**, 2790–2796.

Engebretson, S., Chertog, R., Nichols, A., Hey-Hadavi, J., Celenti, R. & Grbic, J. (2007) Plasma levels of tumour necrosis factor-α in patients with chronic periodontitis and type 2 diabetes. *Journal of Clinical Periodontology* **34**, 18–24.

Ervasti, T., Pohjamo, L., Haukipuro, K. & Knuuttila, M. (1985) Relation between control of diabetes and gingival bleeding. *Journal of Periodontology* **56**, 154–157.

Fried, S. K., Bunkin, D. A. & Greenberg, A. S. (1998) Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *Journal of Clinical Endocrinology and Metabolism* **83**, 847–850.

Gabay, C. (2006) Interleukin-6 and chronic inflammation. *Arthritis Research & Therapy* **8** (Suppl. 2), S3.

Gislen, G., Nilsson, K. & Mattson, L. (1980) Gingival inflammation in diabetic children related to degree of metabolic control. *Acta Odontologica Scandinavica* **38**, 241–246.

Graves, D. T., Liu, R., Alikhani, M., Al-Mashat, H. & Trackman, P. C. (2006) Diabetes-enhanced inflammation and apoptosis – impact on periodontal pathology. *Journal of Dental Research* **85**, 15–21.

Hedberg, P., Ylitalo, K. & Puukka, M. (2004) Highly sensitive determination of C-reactive protein on the Innotrac Aio! immunoanalyzer. *Scandinavian Journal of Clinical and Laboratory Investigation* **64**, 677–686.

Kaplanski, G., Marin, V., Montero-Julian, F., Mantovani, A. & Farnarier, C. (2003) IL-6: a regulator of the transition from neutrophil to monocyte recruitment during inflammation. *Trends in Immunology* **24**, 25–29.

Kardeşler, L., Buduneli, N., Cetinkalp, S. & Kinane, D. F. (2010) Adipokines and inflammatory mediators after initial periodontal treatment in patients with type 2 diabetes and chronic periodontitis. *Journal of Periodontology* **81**, 24–33.

Kaul, K., Hodkinson, A., Tarr, J., Koehner, E. M. & Chibber, R. (2010) Is inflammation a common retinal-renal-nerve pathogenic link in diabetes? *Current Diabetes Reviews* **6**, 294–303.

Kishimoto, T. (2006) Interleukin-6: discovery of a pleiotypic cytokine. *Arthritis Research & Therapy* **8** (Suppl. 2), S2.

Lalla, E., Cheng, B., Lal, S., Kaplan, S., Softness, B., Greenberg, E., Goland, R. S. & Lamster, I. B. (2007a) Diabetes mellitus promotes periodontal destruction in children. *Journal of Clinical Periodontology* **34**, 294–298.

Lim, L. P., Tay, F. B. K., Sum, C. F. & Thai, A. C. (2007) Relationship between markers of metabolic control and inflammation on severity of periodontal disease in patients with diabetes mellitus. *Journal of Clinical Periodontology* **43**, 118–123.

Loos, B. G. (2005) Systemic markers of inflammation in periodontitis. *Journal of Periodontology* **76**, 2106–2115.

Loos, B. G., Craandijk, J., Hoek, F. J., Wertheim-van Dillen, P. M. E. & van der Velden, U. (2000) Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. *Journal of Periodontology* **71**, 1528–1534.

Mohamed-Ali, V., Goodrick, S., Rawesh, A., Katz, D. R., Miles, J. M., Yudkin, J. S., Klein, S. & Coppack, S. W. (1997) Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis-α, in vivo. *Journal of Clinical Endocrinology and Metabolism* **82**, 4196–4200.

Nassar, H., Kantarci, A. & VanDyke, T. (2007) Diabetic periodontitis: a model for activated innate immunity and impaired resolution of inflammation. *Periodontology 2000* **43**, 233–244.

Nibali, L., D'Aiuto, F., Griffiths, G., Patel, K., Suvan, J. & Tonetti, M. S. (2007) Severe periodontitis is associated with systemic inflammation and a dysmetabolic status: a case-control study. *Journal of Clinical Periodontology* **34**, 931–937.

Saraheimo, M., Teppo, A. M., Forsblom, C., Fagerudd, J. & Groop, P. H. (2003) Diabetic nephropathy is associated with low-grade inflammation in Type 1 diabetics. *Diabetologia* **46**, 1402–1407.

Saxlin, T., Suominen-Taipale, L., Leiviskä, J., Jula, A., Knuuttila, M. & Ylöstalo, P. (2009) Role of serum cytokines tumour necrosis factor-alpha and interleukin-6 in the association between body weight and periodontal infection. *Journal of Clinical Periodontology* **36**, 100–105.

Silness, J. & Löe, H. (1964) Periodontal disease in pregnancy (II). Correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavica* **24**, 747–759.

Tamura, T., Udagawa, N., Takahashi, N., Miyaura, C., Tanaka, S., Yamada, Y., Koishihara, Y., Ohsugi, Y., Kumaki, K., Taga, T., Kishimoto, T. & Suda, T. (1993) Soluble interleukin-6 reseptor triggers osteoclast formation by interleukin-6. *Proceedings of the National Academy of Science of the USA* **90**, 11924–11928.

Tappia, P. S., Troughton, K. L., Langley-Evans, S. C. & Grimble, R. F. (1995) Cigarette smoking influences cytokine production and antioxidant defences. *Clinical Science* **88**, 485–489.

Taylor, G. W. (2001) Bidirectional interrelationships between diabetes and periodontal diseases: an epidemiologic perspective. *Annals of Periodontology* **6**, 99–112.

Tervonen, T. & Knuuttila, M. (1986) Relation of diabetes control to periodontal pocketing and alveoral bone level. *Oral Surgery Oral Medicine Oral Pathology* **61**, 346–349.

Tervonen, T., Lamminsalo, S., Hiltunen, L., Raunio, T. & Knuuttila, M. (2009) Resolution of periodontal inflammation does not guarantee improved glycemic control in type 1 diabetic subjects. *Journal of Clinical Periodontology* **36**, 51–57.

van den Oever, I. A. M., Raterman, H. G., Nurmohamed, M. T. & Simsek, S. (2010) Endothelial dysfunction, inflammation, and apoptosis in diabetes mellitus. *Mediators of Inflammation*, doi:10.1155/2010/792393.

Ylöstalo, P., Suominen-Taipale, L., Reunanen, A. & Knuuttila, M. (2008) Association between body weight and periodontal infection. *Journal of Clinical Periodontology* **35**, 297–304.

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Clinical Relevance

Scientific rationale for the study: While some studies indicate a causative role of periodontal infection on systemic inflammatory responses, less is known of the influence of high serum levels of pro-inflammatory mediators on local host responses.

Principal findings: The level of serum IL-6 was associated with the extent of bleeding and PD $\geqslant$ 4 mm at the baseline in non-smokers and in subjects with BMI $\leqslant$ 26 kg/m^2. These associations were also evident after periodontal therapy. Subjects with a high IL-6 level presented poorer periodontal healing than those with a low level.

Practical implications: Serum IL-6 may modulate inflammatory responses in the periodontal area and contribute to both the progression and the healing of periodontal inflammation as it contributes to the initiation and progression of other inflammation-associated diabetic complications.