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Clinical and microbiological effect of scaling and root planing in smoker and non-smoker chronic and aggressive periodontitis patients

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Abstract

Objectives: To compare the effects of scaling and root planing (SRP) on clinical and microbiological parameters at selected sites in smoker and non-smoker chronic and generalized aggressive periodontitis patients.

Materials and Methods: Clinical parameters including probing depth (PD), relative attachment level (RAL), and bleeding upon probing (BOP), and subgingival plaque samples were taken from four sites in 28 chronic periodontitis (CP) and 17 generalized aggressive periodontitis (GAgP) patients before and after SRP. Polymerase chain reaction assays were used to determine the presence of *A. actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythensis*, *Prevotella intermedia* and *Treponema denticola*.

Results: Both CP and GAgP non-smokers had significantly greater reduction in pocket depth (1.0 ± 1.3 mm in CP smokers *versus* 1.7 ± 1.4 mm in non-smokers, $p = 0.007$ and 1.3 ± 1.0 in GAgP smokers *versus* 2.4 ± 1.2 mm in GAgP non-smokers, $p < 0.001$) than respective non-smokers, with a significant decrease in *Tannerella forsythensis* in CP sites (smokers 25% increase and non-smokers 36.3% decrease, $p < 0.001$) and *Prevotella intermedia* at GAgP sites (smokers 25% reduction *versus* 46.9% in non-smokers, $p = 0.028$).

Conclusion: SRP was effective in reducing clinical parameters in both groups. The inferior improvement in PD following therapy for smokers may reflect the systemic effects of smoking on the host response and the healing process. The lesser reduction in microflora and greater post-therapy prevalence of organisms may reflect the deeper pockets seen in smokers and poorer clearance of the organisms. These detrimental consequences for smokers appear consistent in both aggressive and CP.

Key words: chronic periodontitis; generalized aggressive periodontitis; microflora; polymerase chain reaction; scaling and root planing; smoking

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Smoking is increasingly accepted as a risk factor for periodontitis and has been shown to affect various aspects of the host immune response (Barbour et al. 1997, Kinane & Chestnutt 2000). Epidemiological evidence indicates that cigarette smoking is a stronger risk indicator for the presence of periodontitis than the presence of certain suspected periodontal pathogens (Stoltenberg et al. 1993). However, few studies have examined the oral microflora of smokers and non-smokers and the changes in response to treatment in these subjects. No significant difference in the prevalence of various plaque bacteria was found between dental plaque of smokers and non-smokers utilizing culture techniques (Colman et al. 1976, Preber et al. 1992), or when using immunoassay for detecting bac-

teria (Stoltenberg et al. 1993). Recently Zambon (1996) found that smokers harboured significantly higher levels of *Tannerella forsythensis*, *A. actinomycetemcomitans* and *Porphyromonas gingivalis* and there were significantly higher levels of *Capnocytophaga* species and *Eubacterium Saburrem* in non-smokers using immunoflourescence. Darby et al. (2000) found little difference in the microflora of smoker and non-smoker chronic periodontitis (CP) and generalized aggressive periodontitis (GAgP) subjects using polymerase chain reaction (PCR).

Given the effect of smoking on the prevalence and severity of periodontal disease, and its multitude of effects on the host, it is not surprising that smoking has an effect on periodontal therapy (Kinane & Chestnutt 2000). Studies have consistently shown that smokers respond less well to scaling and root planing (SRP) (Preber & Bergstrom, 1986b, Ah et al. 1994, Preber et al. 1995, Kaldahl et al. 1996b, Kinane & Radvar 1997, Haffajee et al. 1997, Machtei et al. 1998, Grossi et al. 1997), with as much as 50% greater improvement in probing depth (PD) reduction in non-smokers (Machtei et al. 1998). Similarly tobacco users have a poorer response to surgical pocket therapy (Preber & Bergstrom 1990, Ah et al. 1994, Kaldahl et al. 1996b). Preber & Bergstrom (1990) reported that during maintenance post-surgery smokers experienced a greater loss of horizontal attachment level, and patients with a higher incidence of breakdown tended to be smokers at the initial examination (Kaldahl et al. 1996b).

PCR is a rapid, accurate and sensitive technique for the detection of bacterial DNA sequences. The sensitivity of PCR allows detection of periodontal pathogens below the normal level of detection by culture methods, immunofluorescence, enzyme-based tests and DNA probes (Ashimoto et al. 1996, Riggio et al. 1996). Riggio et al. (1996) concluded that PCR is much more sensitive than conventional culture methods for the identification of periodontal pathogens.

The aim of the present study was to compare the effect of smoking on SRP in CP and GAgP patients, both clinically and microbiologically, using PCR to determine the prevalence of the accepted periodontal pathogens, *A. actinomycetemcomitans*, *Porphyromonas gingivalis, Tannerella forsythensis*, *Prevotella intermedia*, and *Treponema denticola,* before and after therapy.

Materials and Methods

Patient selection

Fifty-seven patients with untreated periodontal disease, who met specific clinical criteria for inclusion in this study, were recruited consecutively from new referrals to Glasgow Dental Hospital and School between January 1997 and July 1998. Thirty-three patients were aged 35 and over, and were designated as severe CP patients, based on pocket depths greater than 6 mm in all sextants. Twenty-four patients aged between 24 and 35 at the time of referral were designated as GAgP patients. GAgP patients were categorized following the diagnostic criteria of Hart et al. (1991). Cigarette consumption was determined by verbal questioning. The mean age and standard deviation of the CP group was 47 ± 7 years and the GAgP group 33 ± 3 years, as shown in Table 1. The study protocol demanded that each patient had at least two non-adjacent sites per quadrant with pocket depth of at least 5 mm, with no history of systemic disease or antibiotic therapy within the last 3 months. All patients gave informed consent.

Following initial examination and sampling, each patient subsequently underwent quadrant scaling and root planning under local anaesthesia over a 4-week period. Following the last visit of SRP, patients were reviewed 8 weeks later and re-sampled. The same clinician performed the initial examination, sampling, treatment and re-sampling (I. B. D. for CP and P. J. H. for AgP).

Twenty-eight CP and 17 GAgP patients completed SRP. Twelve patients were dropped from the study because of antibiotic usage for non-dental reasons or systemic illness. The mean time between sampling for CP patients was 17.8 ($\pm$ 6.6) weeks and 16.2 ($\pm$ 5.2) weeks for GAgP patients. Only the results from the patients that completed SRP have been analysed and presented in this paper.

Of the patients that completed initial therapy, in the CP group there were 10 smokers and two former smokers, who had given up well over 5 years previously. The smoker subjects smoked between five and 30 cigarettes a day. There were nine AgP smoker subjects, and two former smokers, both of whom had given up 2 or more years ago. The number of cigarettes smoked was in the same range as CP smokers.

Study outline

After an initial screening visit for recruitment, baseline measurements were recorded and samples taken. Subsequently, quadrant SRP, with local anaesthetic, was performed on each patient by experienced periodontists. The patients were then reassessed 6–8 weeks after completion of therapy and post-treatment clinical measurements recorded and samples were taken from the same sites.

Clinical measurements and sampling

In each patient, four sites with pocket depths of 5 mm or greater were selected, where possible one in each quadrant, and with no furcation involvement. At each site the modified gingival index (MGI) (Lobene et al. 1986), plaque index (PLI) (Silness and Löe 1964), bleeding on probing (BOP), suppuration (Supp), PD and relative attachment level (RAL) were recorded. Each tooth was air-dried, MGI was assessed, and a pocket-charting probe was used to determine PLI. PD and relative attachment level were measured at each site using the Florida probe, using the pocket depth and disc probes, respectively, and each site measured twice to assess the variability of the probing measurements. BOP and Suppuration were recorded between pocket depth measurements. The Florida probes were wiped with 70% Isopropyl alcohol wipes between measurements to reduce bacterial contamination of the sites. After the clinical measurements were recorded, a subgingival plaque sample was taken from each site using separate sterile cuvettes and a single vertical stroke.

Table 1. Demographic details of the CP and GAgP patient groups

Disease	No of patients		Mean age ($\pm$ SD)	Male	Female	Smokers	Mean time between samples (weeks)
	baseline	post-SRP					
CP	33	28	47 ($\pm$ 7)	13	20	10	17.8 ($\pm$ 6.6)
GAP	27	17	33 ($\pm$ 3)	7	10	9	16.2 ($\pm$ 5.2)

CP, chronic periodontitis; GAgP, generalized aggressive periodontitis; SRP, scaling and root planing.

Each sample was immediately placed in a sterile microcentrifuge tube containing 0.5 ml TE Buffer (10 mM Tris HCl (pH 7.6), 1 mM EDTA (pH 8.0)). Samples were stored on ice before being taken to the laboratory where they were vortexed and stored at -20°C until analysed.

Polymerase chain reaction

For PCR analysis, 90 µl of vortex-mixed subgingival plaque was added to 10 µl of 10 × lysis buffer (100 mM Tris-HCl pH 8.0, 10 mM ethylenediamine tetraacetic acid, 10% Triton X-100) and boiled for 5 min. Ten microlitres of this lysate was used in each PCR reaction.

The primers used for the various PCR analyses are shown in Table 2 with the size of the amplification product. The primers for *A. actinomycetemcomitans* targeted the leucotoxin gene as previously described (Goncharoff et al. 1993), and the primers for *Porphyromonas gingivalis* targeted the fimbrillin gene, as described by Watanabe & Frommel (1996). The primers for *Prevotella intermedia* were previously described by Riggio et al. (1998), targeting the 16sRNA nucleotide region. The *Tannerella forsythensis* and *Treponema denticola* primers were as previously described by Slots et al. (1995), and targeted the 16sRNA nucleotide region.

PCR amplification was carried out in a reaction volume of 100 µl consisting of 10 µl sample lysate and 90 µl of reaction mixture containing 1 × PCR buffer (10 mM Tris-HCl pH 8.8, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1%Triton X-100), 2 U of Dynazyme DNA polymerase (Flowgen, Sittingbourne, UK), 0.2 mM dNTPs (dATP, dCTP, dGTP, dTTP) and 50 pmol of each primer. The primers were separated from the other components of the reaction mixture by a layer of wax (''hot start'' PCR) preventing the reaction from starting until the wax had melted upon commencement of PCR cycling. PCR cycling was carried out in an OmniGene thermal cycler (Hybaid, Teddington, UK). The cycling conditions for *A. actinomycetemcomitans* and *Prevotella intermedia* were initial denaturation for 5 min. at 95°C, 35 amplification cycles of denaturation at 95°C for 1 min., annealing of primers at 55°C for 1 min. and primer extension at 72°C for 1.5 min., followed by a final extension step at 72°C for 10 min. The cycling conditions for the other organisms were the same except that in the amplification cycles the primer extension step lasted for 1 min. The reaction products were either stored at -20°C or analysed immediately. Negative and positive controls were included in each batch of samples being analysed by PCR. The negative control was a 90 µl reaction mixture with the sample replaced by 10 µl of sterile water. The positive control contained 100 ng of genomic DNA from the relevant organism in 90 µl of reaction mixture, with sterile water added to make up the volume to 100 µl.

Analysis of PCR products

Twenty microlitres of each reaction product was fractionated on a 2% agarose gel containing ethidium bromide (0.5 µg/ml), using a 100 bp DNA ladder (Life Technologies, Paisley, UK) as a size marker, and visualized and photographed using an Imagemaster video documentation system (Pharmacia Biotech, St Albans, Herts, UK).

Statistical analysis of data

The clinical and microbiological data for each site and patient were statistically analysed using Minitab statistical package (Minitab, release 12, Minitab Inc., State College, PA, USA) and SPSS statistical software (SPSS, version 5, SPSS Inc., Chicago, IL, USA). The duplicate recordings of PD and attachment level measurements were averaged for each site. BOP, Supp and prevalence of the microorganisms are presented as percentages, but the raw data and not the percentages were used in the analysis. All patient/site data were used in the analyses. No patients were excluded from the analyses on the grounds of poor response to therapy.

The data were analysed for differences between smoker and non-smoker CP and GAgP groups before and after treatment. The Mann–Whitney test was used to analyse MGI and PLI scores, and two sample *t*-tests for PD, and RAL. The differences between BOP, Supp and all microorganisms were assessed using the χ^2 test, except when expected counts were less than five where the Fisher's exact test was used. The Bonferroni correction was used to confirm any significant values arising from multiple comparisons. The clinical parameters from the site and the average for the patient gave the same result, so the patient data has been quoted.

Table 2. Sequences, expected product size, target and references for PCR primers

Primer pairs (5′–3′)	Amplicon length (bp)	Target	Reference
Porphyromonas gingivalis			
ATAATGGAGAACAGCAGGGAA		Fimbrillin	Watanabe
TCTTGCCAACCAGTTCCATTGC	131	gene	& Frommel (1996)
Prevotella intermedia			
CCTAATACCCGATGTTGTCCACA		16S RNA	Riggio et al. (1996)
AAGGAGTCAACATCTCTGTATCC	855		
Actinobacillus actinomycetemcomitans			
GGAATTCCTAGGTATTGCGAAACAAT		Leukotoxin	Goncharoff
GGAATTCCTGAAATTAAGCTGG	262	gene	et al. (1993)
Tannerella forsythensis			
GCGTATGTAACCTGCCCGCA		16S RNA	Slots et al. (1995)
TGCTTCAGTGTCAGTTATACCT	641		
Treponema denticola			
TAATACCGAATGTGCTCATTTACAT		16S RNA	Slots et al. (1995)
TCAAAGAAGCATTCCCTCTTCTTCTTA	316		

PCR, polymerase chain reaction; bp, base pairs.

Results

CP subjects

Change in response to SRP

CP non-smokers had significantly greater reductions in BOP (49% compared with 12.5%, p 0.02) and PD (1.7 (± 1.4) mm compared with 1.0 (± 1.3) mm, $p = 0.007$) (Table 3). Non-smokers also had greater improvements in MGI, PLI, Supp and RAL. These differences were not significant. However, the microbial analysis shows similar changes in *Porphyromonas gingivalis* percentages for both groups and a significantly greater reduction in non-smokers for *Prevotella intermedia* (23.8% compared with

Table 3. Comparison of CP smoker and non-smoker patients showing pre-treatment, post-SRP and change in clinical parameters (reduction is a positive value and an increase is a negative value)

Clinical parameters	Pre-SRP		*p*-value	Post-SRP		*p*-value	Smokers	Change non-smokers	*p*-value
	smoker	non-smoker		smoker	non-smoker				
MGI	2.3 (± 0.7)	2.4 (± 07)	0.56	1.3 (± 0.8)	1.2 (± 0.9)	0.35	0.97 (± 1.1)	1.2 (± 1.0)	0.37
PLI	1.5 (± 1.1)	1.5 (± 0.9)	0.96	0.8 (± 0.8)	1.1 (± 0.9)	0.1	0.3 (± 1.0)	0.4 (± 1.1)	0.17
BOP (%)	69	92.5	0.001	56	44	0.23	12.5	49	0.02
Supp (%)	28	29	0.95	12.5	6	0.27	15.6	22.5	0.86
PD (mm)	5.9 (± 1.5)	5.9 (± 1.2)	0.79	4.9 (± 1.4)	4.2 (± 1.6)	0.028	1.0 (± 1.3)	1.7 (± 1.4)	0.007
RAL (mm)	14.1 (± 2.2)	13.3 (± 2.2)	0.12	13.8 (± 12.1)	12.7 (± 2.3)	0.017	0.3 (± 1.0)	0.7 (± 1.3)	0.09

CP, chronic periodontitis; SRP, scaling and root planing; MGI, modified gingival index; PLI, plaque index; BOP, bleeding on probing; Supp, suppuration; PD, probing depth; RAL, relative attachment level.

Table 4. Comparison of CP smoker and non-smoker patients showing pre-treatment, post-SRP and change in percentage of positive sites (reduction is a positive value and an increase is a negative value)

Microbial parameters (%)	Pre-SRP		*p*-value	Post-SRP		*p*-value	Smokers	Change non-smokers	*p*-value
	smoker	non-smoker		smoker	non-smoker				
Porphyromonas gingivalis	50	41.3	0.4	40.6	31.3	0.34	9.4	10	0.7
Prevotella intermedia	62.5	52.5	0.34	43.8	28.8	0.13	18.8	23.8	0.015
Tannerella forsythensis	40.6	61.3	0.05	65.6	25	<0.001	− 25	36.3	<0.001
Actinobacillus actinomycetemcomitans	0	2.5	0.63	0	0	1	0	2.5	0.63
Treponema denticola	56.3	35	0.04	25	16.3	0.28	31.3	18.8	0.22

CP, chronic periodontitis; SRP, scaling and root planing.

18.8%, $p = 0.015$) (Table 4). There was an increase in *Tannerella forsythensis* in smokers and a decrease in non-smokers, which was significant ($p < 0.001$). There was no change in *A. actinomycetemcomitans* for smokers and only a slight change for non-smokers.

Post-SRP

Post-treatment CP smoking and non-smoking clinical and microbial parameters are shown in Table 3. Smokers had significantly deeper pockets (4.9 (± 1.4) mm compared with 4.2 (± 1.6) mm, $p = 0.03$) and greater relative attachment loss (13.8 (± 2.1) mm compared with 12.7 (± 2.3) mm, $p = 0.02$). This group also had slightly more gingival inflammation, BOP and Supp, but less plaque than non-smokers. However, these differences were not significant.

The microbial analysis showed a significantly increased prevalence of *Tannerella forsythensis* in smokers (65.6% compared with 25%, $p < 0.0001$) (Table 4). *Porphyromonas gingivalis*, *Prevotella intermedia* and *Treponema denticola* were also more frequently detected but not significantly. *A. actinomycetemcomitans* was not detected.

GAgP subjects

Change in response to SRP

The change in pocket depth was the only statistically significantly different clinical parameter between GAgP smokers and non-smokers (1.3 (± 1.0) and 2.4 (± 1.2) mm, $p < 0.001$) (Table 5). Non-smokers had greater reductions in MGI, BOP, Supp and RAL.

There were significant differences when the changes in the test organisms were analysed on a site basis, with significantly greater reduction for *Prevotella intermedia* in non-smokers and a significant decreases in *Porphyromonas gingivalis* and *Tannerella forsythensis* in smokers (Table 6).

Post-SRP

GAgP smokers demonstrated significantly higher pocket depths after treatment (5.4 (± 1.1) and 4.3 (± 1.1) mm, $p < 0.01$) (Table 5). This group also showed greater RAL plus lower MGI and PLI scores compared with non-smokers, which was only significantly different for MGI ($p = 0.01$).

The microbiological analysis also showed lower *Porphyromonas gingivalis* and higher *Prevotella intermedia*, which were significant in smokers (Table 7). *Porphyromonas gingivalis* was found in 11.1% of smokers and 37.5% of non-smokers, $p = 0.011$. *Prevotella intermedia* was found at 30.6% of smoker sites but absent in non-smoker sites and the *p*-value was 0.0006. *Prevotella intermedia* and *A. actinomycetemcomitans* were eliminated in non-smokers. The difference between *A. actinomycetemcomitans* was just above significance, $p = 0.052$. *Tannerella forsythensis* was detected more frequently in non-smokers and *Treponema denticola* in smokers, but not significantly.

Discussion

The analyses compared smokers with non-smokers in the total patient population and for both disease groups separately. We made the decision to include former smokers in the non-smoking group as all former smokers had given up at least 2 years previously, and there is evidence that given sufficient time responses to periodontal therapy in former smokers becomes similar to never smokers (Grossi et al. 1997).

Clinical parameters

For both disease groups these non-smokers had a significantly greater reduction

Table 5. Comparison of GAgP smoker and non-smoker patients showing pre-treatment, post-SRP and change in clinical parameters (reduction is a positive value and an increase is a negative value)

Clinical parameters (%)	Pre-SRP		*p*-value	Post-SRP		*p*-value	Smokers	Change non-smokers	*p*-value
	smoker	non-smoker		smoker	non-smoker				
MGI	1.7 (± 0.9)	2.2 (± 0.9)	0.01	0.5 (± 0.7)	1.0 (± 0.9)	0.01	1.2 (± 0.9)	1.3 (± 1.0)	0.7
PLI	1.1 (± 1.1)	1.3 (± 1.1)	0.26	0.5 (± 0.8)	0.8 (± 0.9)	0.087	0.6 (± 1.2)	0.5 (± 1.2)	0.86
BOP (%)	58	84.4	0.019	31	34.4	0.74	28	50	0.14
Supp (%)	33	37.5	0.72	2.8	3.1	0.93	31	34.4	0.74
PD (mm)	6.7 (± 1.2)	6.7 (± 0.9)	0.92	5.4 (± 1.1)	4.3 (± 1.1)	<0.001	1.3 (± 1.0)	2.4 (± 1.2)	<0.001
RAL (mm)	14.2 (± 1.3)	13.9 (± 1.5)	0.45	13.0 (± 1.5)	12.4 (± 2.0)	0.24	1.2 (± 1.0)	1.4 (± 1.2)	0.38

GAgP, generalized aggressive periodontitis; SRP, scaling and root planing; MGI, modified gingival index; PLI, plaque index; BOP, bleeding on probing; Supp, suppuration; PD, probing depth; RAL, relative attachment level.

Table 6. Comparison of GAgP smoker and non-smoker patients showing pre-treatment, post-SRP and change in percentage of positive sites (reduction is a positive value and an increase is a negative value)

Microbial parameters (%)	Pre-SRP		*p*-value	Post-SRP		*p*-value	Smokers	Change non-smokers	*p*-value
	smoker	non-smoker		smoker	non-smoker				
Porphyromonas gingivalis	52.8	56.3	0.77	11.1	37.5	0.011	41.7	18.8	0.02
Prevotella intermedia	55.6	46.7	0.47	30.6	0	0.0006	25	46.9	0.028
Tannerella forsythensis	91.7	65.6	0.098	27.8	34.3	0.56	63.9	31.3	0.015
Actinobacillus actinomycetemcomitans	16.7	12.5	0.63	11.1	0	0.052	5.6	12.5	0.18
Treponema denticola	36.1	56.3	0.096	5.6	3.1	0.63	30.6	53.1	0.068

GAgP, generalized aggressive periodontitis; SRP, scaling and root planing.

in pocket depth following therapy. Previous studies have reported a poorer response in smokers to SRP and the current report is in agreement (Preber & Bergstrom, 1986b, Ah et al. 1994, Preber et al. 1995, Kaldahl et al. 1996b, Kinane & Radvar, 1997, Grossi et al. 1997, Machtei et al. 1998). After treatment CP patients who smoked had significantly greater relative attachment loss. Kaldahl et al. (1996b) and Ah et al. (1994) reported similar findings in their patient population. Oral hygiene (as measured by PLI) was slightly better in smokers after treatment and so the poorer response to therapy may not be due to oral hygiene levels (Preber & Bergstrom 1986b). Non-smokers also showed a markedly greater reduction in BOP. These are consistent with a reduced response to therapy in smoker subjects.

The poorer response of smokers to therapy is probably the result of the general effect of smoking on the host, which compromises the response to periodontal treatment. The mechanisms of healing among smokers may be impaired, especially if fibroblast and osteoblast functions are suppressed, which may result in poorer tissue repair (Barbour et al. 1997).

In addition, the greater reduction in pocket depth in non-smokers but similar attachment loss changes are perhaps because of the greater reduction in gingival swelling of non-smokers. Non-smokers exhibit more signs of gingival inflammation (than smokers) and when this resolves it may lead to some recession, which favours a greater decrease in pocket depth over smokers (Kinane & Chestnutt 2000). The less favourable healing response in smokers was not related to any difference in the plaque control level. This is in agreement with studies by Ismail et al. (1983) and Tonetti et al. (1995).

Microbiological parameters

Following therapy at smoker sites in CP subjects, the change in percentage of positive sites for *Tannerella forsythensis* was significantly lower and there were a significantly higher percentage of positive sites for *Tannerella forsythensis*, even after correction for multiple comparisons. Whereas GAgP smoker sites showed a significantly higher prevalence of *Prevotella intermedia* post-therapy compared with non-smoker sites and non-smokers a significantly higher prevalence of *Porphyromonas gingivalis*.

The reduction in *Tannerella forsythensis* in CP subjects is similar to that found by Grossi et al. (1997) They reported a lower reduction in *Tannerella forsythensis* levels in smokers, but also for *Porphyromonas gingivalis*. However the finding in this study of greater *Porphyromonas gingivalis* reduction is supported by Haffajee et al. (1997), who found similar results comparing smoker and non-smoker groups. Preber et al. (1995) reported almost total eradication of *A. actinomycetemcomitans* but similar levels of reduction for smokers and non-smokers for *A. actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia* in response to a favourable clinical outcome after therapy. Renvert et al. (1998) reported slight decreases in the levels of *Porphyromonas gingivalis* and *Prevotella intermedia/P. nigrescens* after SRP. They suggested that the microbiological response found seemed to be in conformity with the clinical response with little influence of the smoking habits. However this study used culture to examine the flora, which may account for the differences. Interestingly CP smokers showed an increase in *Tannerella forsythensis* prevalence whereas GAgP smokers had a significantly much greater reduction in the same organism. This may suggest less thorough debridement of CP sites although a good clinical response and reduction in bacterial prevalences overall were produced. It also suggests that there may

be biological differences in the responses of smoker CP and GAgP subjects to periodontal therapy. Exactly what these would be requires further investigation and may reflect underlying differences between the aetiology and pathogenesis of the two groups.

The differences reported in the pocket depths between CP and GAgP smokers and non-smokers may account for some of the differences in the microflora after treatment. The deeper pocket depths in smokers, which are more anaerobic than non-smokers (Loesche et al. 1983), may favour an anaerobic flora whereas the shallower less anaerobic pockets after treatment in non-smokers may not be a suitable environment for the persistence of an anaerobic flora. It is possible that the differences could be because of the differing plaque control levels between smokers and non-smokers, but given that plaque control was poorer in the study in non-smokers, who had the better improvement in the microflora, this is unlikely.

The poorer host response against infectious agents in smokers may also account for the findings. The host immune response is adversely affected in smokers (Seymour 1991; Lamster 1992). In smokers there are significantly increased numbers of peripheral blood leucocytes especially neutrophils (Barbour et al. 1997). However, smoking has been shown to affect neutrophil chemotaxis, adherence, phagocytosis and function (Eichel & Shahrik 1969, Noble & Penny 1975, Kenney et al. 1977, Kraal et al. 1977, Kraal and Kenney 1979, MacFarlane et al. 1992), both systemically and locally in the periodontium. Low levels of nicotine are chemotactic (Totti et al. 1994) whereas high levels inhibit phagocytosis (Ryder 1994). There is an enhanced oxygen metabolism and dysfunctional regulation of extracellular proteases (Barbour et al. 1997). A detrimental effect on the respiratory burst has also been reported (Codd et al. 1987, Kalra et al. 1991). The proportion of circulating natural killer cells is decreased in smokers (Tollerud et al. 1991) and the cytotoxic activity is dose-dependently reduced (Hersey et al. 1983, Ginns et al. 1982, Hughes et al. 1985, Phillips et al. 1985). Systemically there appears to be an increase in macrophage numbers but a decrease in function (Hoogsteden et al. 1991). There is inhibition of phagocytosis (Ortega et al. 1994) and the oxidative burst (Skold et al. 1993) from these cells; however, *in vitro* exposure to nicotine seems to suppress the ability of macrophages to kill oral pathogens (Pabst et al. 1995). Plasma vitamin C levels are reduced in smokers, possibly reflecting the ability of phagocytes to control oral pathogens (Tribble et al. 1993, van Antwerpen et al. 1993). In addition there is impaired production of IgA, IgG and IgM. These could all be potentially important factors in protecting the host tissues from re-infection during the healing process. The combination of these may result in inadequate clearance of oral pathogens, and so account for the increased prevalences of the test bacteria in smokers compared with non-smokers after SRP.

In conclusion, the reduced improvement in PD following therapy in smokers compared with non-smokers may reflect the systemic effects of smoking on both the host response and the healing process. The inferior reduction in the microflora and greater post-therapy prevalence of periodontal pathogens in smokers may reflect a poorer clearance of these organisms which may be because of a complex interplay of smoking on microbes and local and systemic host response and healing processes. These detrimental consequences for smokers appear consistent in both aggressive and CP.

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