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Impact of smoking on the clinical, microbiological and immunological parameters of adult patients with periodontitis

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Abstract

Objectives: The aim of the current study was to assess the impact of smoking on the clinical indices, the humoral immune response and the detection frequency of putative periodontal pathogens in patients with periodontitis cross-sectionally and following therapy.

Material and Methods: Clinical measurements, subgingival plaque samples, gingival crevicular fluid (GCF) and sera were collected from 40 untreated patients with moderate-to-advanced chronic periodontitis before and after treatment over a period of 6 months. The treatment consisted of the initial therapy of scaling and root planing. Smoking status was self-reported and was confirmed by cotinine enzyme inhibition assay (CEIA). Whole-mouth clinical measurements were recorded with a manual periodontal probe at baseline (BAS) and at 6 months (RAS). Selected-site analyses were performed on the deepest site in each quadrant before and after therapy and clinical indices were recorded with an electronic pressure-sensitive probe. GCF sample volume was quantified using the Periotron 6000. Polymerase chain reaction (PCR) was utilized to determine the presence of *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia*, *Treponema denticola* and *Tanerella forsythensis* in subgingival plaque. Enzyme-linked immunosorbent assay examined the systemic antibody titres to these bacteria, and thiocyanate disassociation determined the antibody avidity to these organisms.

Results: At baseline, smokers showed significantly less gingival inflammation and lower GCF volume compared with non-smokers. After treatment, a compromised clinical outcome was noted for smokers in terms of pocket depth reduction and gain in attachment levels. No significant differences in the detection of putative periodontal pathogens in subgingival plaque existed between smokers and non-smokers. A consistent trend was noted in that smokers had lower sera immunoglobulin G antibody titres to these organisms before and after treatment (statistically significant for *A. actinomycetemcomitans*). This pattern was less clear when antibody avidities were considered, revealing only small differences, if any, between the two groups of patients.

Conclusion: Current data indicate that smokers with periodontal disease have a suppressed inflammatory response, a significantly less favourable clinical outcome and seem to have an altered host antibody response to antigenic challenge than non-smokers. In contrast, the subgingival microflora of smokers appears similar to that of non-smokers.

Key words: antibody avidity; antibody titres; chronic periodontitis; clinical indices; periodontal pathogens; periodontal therapy; smoking

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Numerous studies have indicated that smoking is a significant risk factor for the development of periodontal disease (Kinane & Chestnutt 2000). Grossi et al. (1994, 1995) have shown that the severity of attachment loss highly correlated with the number of cigarettes smoked per day and the duration of smoking, and that the severity of bone loss was positively related to smoking experience.

Smoking was found to suppress overt clinical signs of gingival inflammation, as indicated by a reduced bleeding score in smokers (Preber & Bergström 1985). In addition, Darby et al. (2000) examined the periodontal status in chronic periodontitis and generalized aggressive periodontitis patients, and found that smokers in both disease groups exhibited significantly lower bleeding scores than non-smokers.

Significantly lower gingival crevicular fluid (GCF) volume was found for smokers than non-smokers, in periodontitis patients (Kinane & Radvar 1997).

Zambon et al. (1996) demonstrated by immunofluorescence that higher proportions of smokers harboured *Tanerella forsythensis*, *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* than did non-smokers and they were infected with higher mean levels of the majority of the test bacteria than were non-smokers. Controlling for the severity of periodontal disease, former and current smokers were 1.5 times more likely to be infected with *T. forsythensis* than non-smokers, and the greater risk seen in smokers for infection with this organism was dose-related. Similarly, Kamma et al. (1999) compared the microbial profiles of smokers and non-smokers in a group of patients with aggressive periodontitis using culture techniques. The analysis of the subgingival plaques revealed that a variety of suspected periodontal pathogens, including *T. forsythensis* and *P. gingivalis*, were found in significantly higher numbers and more frequently in smokers than non-smokers. In addition, another study demonstrated that former smokers exhibited a decreased risk of harbouring *A. actinomycetemcomitans* in saliva than non-smokers (odds ratio: 0.23), while current smokers displayed an increased risk of harbouring *Treponema denticola* in periodontal pockets than non-smokers (odds ratio: 4.61) (Umeda et al. 1998). These reports are in line with findings of other research groups, which showed that smoking affects the composition of the subgingival microbial flora (Eggert et al. 2001, van Winkelhoff et al. 2001). Conversely, Darby et al. (2000) failed to show any differences in the prevalence of five putative periodontal pathogens between smokers and non-smokers, either with chronic periodontitis or generalized aggressive periodontitis. Utilizing the checkerboard DNA–DNA hybridization technique, Boström et al. (2001) confirmed the previous data by showing that in chronic periodontitis patients smoking had little, if any, influence on the subgingival presence of several suspected periodontal pathogens. Despite the controversy in the literature over differences in the subgingival microflora between smokers and non-smokers, several studies have reported that plaque levels were similar between smokers and non-smokers (Kinane & Radvar 1997, Kamma et al. 1999, Darby et al. 2000, Haffajee & Socransky 2001a).

Smoking has been shown to have an adverse effect on fibroblast function (Raulin et al. 1988), chemotaxis and phagocytosis by neutrophils (Kenney et al. 1977, Kraal et al. 1977) and immunoglobulin (Ig) production (Holt 1987, Johnson et al. 1990). It has been shown that cigarette smoke results in reduced concentration of serum immunoglobulin G (IgG) antibodies (Andersen et al. 1982, Graswinckel et al. 2004). The deleterious effects of smoking and tobacco on the immune system are summarized in several reviews (Barbour et al. 1997, Kinane & Chestnutt 2000).

The effect of cigarette smoking on the outcome of scaling and root planing and adjunctive antimicrobial treatment has been evaluated (Kinane & Radvar 1997). Non-smokers showed greater pocket depth (PD) reduction and a trend for greater gain in attachment levels, indicating that a greater degree of recession occurred in smokers than non-smokers. Boström et al. (1998) showed a predominance of smokers among patients exhibiting loss of bone height after 5 years of maintenance. Haffajee et al. (1997) reported that pre-treatment *P. gingivalis*, *T. forsythensis* and *T. denticola* were equally prevalent among current and past smokers and subjects who had never smoked, and decreased significantly after periodontal therapy in past smokers and in subjects who had never smoked but increased in current smokers.

The aim of the current study was to assess the impact of smoking on the clinical indices, the humoral immune response and the subgingival microflora of patients before and after periodontal therapy.

Materials and Methods

Patient selection

Forty untreated chronic periodontitis patients, aged 31–70 years, were recruited from new referrals to Glasgow Dental Hospital and School and attended for the 6-month duration of the study. Each patient had at least two non-adjacent sites per quadrant with a PD of 5 mm or over and radiographic evidence of bone loss with no history of systemic disease or antibiotic therapy within the last 3 months or during the course of the study. Cigarette smoking status was self-reported by the patients at the screening visit and was confirmed by the cotinine enzyme inhibition assay (CEIA). Subjects were considered smokers if they had been smoking five or more cigarettes a day. All patients gave informed consent.

The demographic details of the patients recruited to the current study are shown in Table 1. We initially recruited 58 patients but 18 were excluded from the study for two reasons, namely failure to attend their appointment twice ($n = 10$) and intake of antibiotics during treatment ($n = 8$). One of the participants was prescribed antibiotics for a tooth abscess and the others for reasons not related to periodontal treatment.

Clinical interventions and experimental design

The clinical study design is described in our previously published report (Apatzidou & Kinane 2004a). In brief, after an initial screening visit for recruitment,

Table 1. Demographic details of smokers and non-smokers

	No. of subjects	Ethnic group	Age*	Females
Non-smokers	25	24 Caucasians, 1 Asian	45 (31–70)	11 (44%)
Smokers	15	14 Caucasians, 1 Asian	41 (32–67)	6 (40%)

Number (min–max) of cigarettes smoked per day: 15 (5–30) cigarettes/day.
*Median (min–max).

patients were randomly allocated into one of the two treatment groups based on a pre-determined randomization list made by computer and baseline measurements were recorded. Subsequently, same-day full-mouth scaling and root planing (FM-SRP) or quadrant scaling and root planing at two-weekly intervals (Q-SRP) were performed on each patient under local anaesthesia using an assortment of periodontal curettes (American Eagle, Gracey Access curettes, Missoula, MT, USA) and ultrasonic scalers (Cavitron, Dentsply, York, USA). During instrumentation, pockets were irrigated with saline and no use of disinfection i.e. antiseptics such as chlorhexidine was used during the active phase of treatment or post-operatively. During the 6 months of the study, oral hygiene instructions (OHIs) were re-inforced as needed. No teeth needed to be extracted during therapy.

Conventional full-mouth periodontal pocket charts were completed at baseline (BAS) and at the 6-month re-assessment (RAS). PD and clinical attachment levels (CALs) were determined at 6 sites/tooth to the nearest millimetre (mm) using a PCP 12 probe (Hu-Friedy Mfg Co, Chicago, IL, USA). Bleeding on probing (BOP) was also recorded dichotomously as present or absent after PD probing on each arch. The examiner had no access to previous recordings.

Furthermore, 1 site/quadrant with the deepest PD, and not less than 5 mm deep, and with no endodontic or furcation involvement was selected from each patient at BAS for selected-site clinical analysis. At each selected site, the modified gingival index (MGI) (Lobene et al. 1986), plaque index (PI) (Silness & Löe 1964), BOP, PD and relative attachment level (RAL) were recorded. Each tooth was air-dried, MGI was assessed and a periodontal probe was used to determine PI. PD and RAL were measured at each site using an electronic probe with a controlled force of $20 \times g$ (Florida probe; Gibbs et al. 1988) using the PD and disc probes, respectively. Each site was measured twice to assess the variability of the probing measurements. Clinical measurements were recorded from the computer screen by an assistant at BAS and at RAS. The operator was blinded to these recordings. BOP was recorded between PD measurements.

Furthermore, at these time points GCF samples were harvested and subgingival plaque samples were collected for the detection of five putative periodontal pathogens: *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythensis*, *Prevotella intermedia* and *T. denticola* by polymerase chain reaction (PCR). In addition, blood samples were collected from all participants to determine the serum antibody titres and avidity against the homologous organisms.

GCF sampling

GCF was sampled after PI and MGI were recorded, but prior to any other clinical measurements. GCF volumes were determined using Whatman grade 4 paper strips (2×13 mm) (Whatman Labsales Ltd. Maidstone, Kent, UK) and a measuring unit, Periotron 6000 (Harco, Winnipeg, MB, Canada), which was calibrated each time prior to GCF collection.

Plaque sampling and processing of plaque samples

Subgingival plaque samples were taken with a single vertical stroke, using a sterile hoe for each sample to prevent cross-contamination, as described previously (Apatzidou et al. 2004). Plaque samples were vortex mixed for 30 s and stored in a coded sterile tube containing 0.5 ml sterile MilliQ grade H_2O (Millipore UK Limited, Watford, UK) at -70°C until required. The PCR analysis was performed blindly.

Blood sampling and processing of sera samples

Twenty millilitres of venous blood were collected from the ante-cubital vein using the Vacutainer system (BD Vacutainer™, Plymouth, UK). Blood samples were allowed to clot overnight and serum was aliquoted and stored at -70°C for further analysis. The laboratory analysis was performed in a blind manner.

PCR

The PCR primers used in the current study are described in depth by Ashimoto et al. (1996) and Riggio et al. (1998). These species-specific primers targeted the 16S recombinant RNA (rRNA) of the bacteria. All primers were obtained from MWG-Biotech (Milton Keynes, UK).

PCR amplification reactions were carried out in a reaction mixture of 100 µl consisting of 10 µl sample lysate and 90 µl of reaction mixture containing $1 \times$ PCR buffer (10 mM Tris-HCl pH 9.0, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton® X-100), 2 U of Taq DNA polymerase (Promega, Southampton, UK), 0.2 mM deoxynucleoside triphosphates and 50 pmol of each primer. The primers were separated from the other components of the reaction mixture by a layer of wax (DyNAwax, Flowgen, Lichfield, UK). The wax layer prevented the PCR from starting until the wax had melted upon commencement of PCR cycling (''hot start'' PCR). PCR cycling was carried out in an OmniGene thermal cycler (Hybaid, Teddington, UK).

The cycling conditions for *P. intermedia* and *A. actinomycetemcomitans* comprised an initial denaturation step at 94°C for 5 min., 40 amplification cycles of denaturation at 94°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 *P. gingivalis*, *T. denticola* and *T. forsythensis* were as follows: initial denaturation at 94°C for 5 min., 35 amplification cycles of denaturation at 94°C for 1 min., annealing of primers at 60°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. as previously described by Ashimoto et al. (1996). The reaction products were either stored at -20°C or analysed immediately.

For the *T. denticola*, a synthetic positive control was constructed. A small fragment of the *T. denticola* 16S rRNA gene (79 bp) was amplified using the following nucleotide primers: 5′-TAA TAC CGA ATG TGC TCA TTT ACA TAA AGG TAA ATG AGG AAA GGA GCT-3′ (base position 193–244) and 5′-T CAA AGA AGC ATT CCC TCT TCT TCT TA-3′ (base position 508–482).

Analysis of PCR products

Ten microlitres of each reaction product were added to 1.5 µl of gel-loading dye (0.25% bromophenol blue, 50% glycerol, 100 mM ethylene diamine tetraacetic acid pH 8.0), electrophoresed on a 2% agarose gel containing ethidium bromide (0.5 µg/ml) and visualized and photographed using an ImageMaster video documentation system (Pharmacia Biotech, St. Albans, UK). A 100 bp DNA ladder (Pharmacia Biotech) was used as a molecular weight marker.

Enzyme-linked immunosorbent assay (ELISA)

Specific antibody titres were measured by ELISA as described previously (Ebersole et al. 1980), using formalized whole cells. *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. forsythensis* and *T. denticola* were prepared for coating the ELISA plates as described previously (Apatzidou & Kinane 2004b).

Optical densities were read using a Dynex Technologies MRX II plate reader (Dynex Technologies Ltd., West Sussex, UK) at 450 nm with a 630 nm reference wavelength. All sera samples collected from the same patient at different time points (BAS and RAS) were assayed in duplicate and on the same plate. Correction was made for non-specific binding and the averaged duplicate results were read from a reference line derived from serial dilutions of the reference positive control serum. Results were expressed as ELISA units (EU) (Gmür et al. 1986, Mooney et al. 1993).

Avidity analysis

The dissociation assay to determine antibody avidity was performed in a manner similar to the ELISA for the serum analysis described more fully in Apatzidou & Kinane (2004b).

Cotinine verification of smoking status

The assay was performed using the COZART serum cotinine assay kit (Abingdon, UK), following the manufacturer's instructions. Briefly, all reagents were warmed to room temperature. Ten microlitre dilutions of cotinine standards and serum (neat and 1/5 dilutions) were added to microplate wells in duplicate. Hundred microlitres of cotinine enzyme were then added to each well and the reaction was incubated for 30 min. at room temperature. The plate was washed four times with wash buffer and 100 µl of substrate reagent was added to each well, and the plate was incubated for 30 min. at room temperature. The reaction was terminated with 100 µl of stop solution and optical densities were read using a Dynex Technologies MRX II plate reader at 450 nm with a 630 nm reference wavelength. Sample readings were compared with a reference line derived from serial dilutions of the cotinine standards.

Statistical analysis of data

The clinical data were statistically analysed using Minitab statistical package (Minitab release 12, State College, PA, USA) and SPSS statistical software (SPSS 5, Chicago, IL, USA). The analyses were carried out using the patient as the experimental unit. Statistical significance was set at the 95% confidence level ($p < 0.05$) for hypothesis testing.

The average of PD, RAL, MGI, PI and GCF volume collected from the 4 selected sites per patient was used for the analysis. The two-sample *t*-test was used for the comparison of the whole mouth clinical indices and PD and RAL from the selected sites between smokers and non-smokers before and after treatment (BAS and RAS). Differences in patients and the frequency of sites (from 1 to 4) that were BOP positive or negative were compared between the groups using the χ^2 test, except when expected counts were less than five, where Fisher's exact test was used. The Mann–Whitney test was used to analyse MGI, PI and GCF volume differences between smokers and non-smokers and although non-parametric statistical analysis was performed, the mean values of these parameters are shown for illustration of effects. For the longitudinal analyses of the full-mouth and the site-specific clinical indices (PD and RAL) before and after treatment within each subgroup, the paired *t*-test was used. The Wilcoxon signed rank test was applied to assess changes in MGI, PI and GCF volume before and after treatment for each group.

The patient was scored positive for an organism if at least 1 out of the 4 sites harboured this organism. For each group, the McNemar test was used to compare the frequency of patients who harboured the specific organisms before and after treatment. Differences in patients and the frequency of sites (from 1 to 4) that were positive or negative for a species between smokers and non-smokers were analysed using the χ^2 test, except when expected counts were less than five, where Fisher's exact test was used.

The Mann–Whitney test was used for the comparison of serum IgG titres and IgG avidity between smokers and non-smokers at baseline and after treatment. Within each subgroup, the Wilcoxon signed rank test was used to assess changes before and after therapy. Similarly, the Mann–Whitney test was used for the comparison of serum cotinine levels between the two groups of patients.

Results

Tables 2 and 3 demonstrate clinical data collected from the whole mouth and from the selected sites, respectively, in 15 smokers and 25 non-smokers. Statistically significant improvements in all clinical indices were found post-operatively in each group ($p < 0.001$). Table 2 shows that, at BAS, the whole-mouth clinical parameters were similar for smokers and non-smokers. Similarly, no significant differences between the two groups were found at RAS. Table 3

Table 2. Whole-mouth clinical indices ($N_{\text{n-sm}} = 25$, $N_{\text{sm}} = 15$)

	BAS	RAS	Change (BAS − RAS)
PD (mm)			
N-sm	4.4 ± 0.6	2.5 ± 0.3	1.9 ± 0.6*
Sm	4.4 ± 0.7	2.7 ± 0.3	1.7 ± 0.5*
CAL (mm)			
N-sm	4.9 ± 0.8	3.7 ± 0.8	1.2 ± 0.5*
Sm	5.3 ± 1.1	4.2 ± 1.1	1.1 ± 0.5*
BOP (%)			
N-sm	70.6 ± 18.7	11.6 ± 5.8	59.0 ± 19.8*
Sm	67.3 ± 16.6	12.1 ± 7.9	55.2 ± 16.7*
No. of sites >5 mm			
N-sm	68.1 ± 21.3	8.4 ± 7.1	59.7 ± 18.8*
Sm	69.3 ± 26.8	8.7 ± 7.5	60.6 ± 24.1*

Mean ± SD.

Number (min–max) of teeth: 27 (18–32) for n-sm and 27 (20–30) for sm.

No statistically significant differences were noted between n-sm and sm ($p > 0.05$).

n-sm, non-smokers; sm, smokers; BAS, baseline; RAS, re-assessment at 6 months; PD, pocket depth; CAL, clinical attachment level; BOP, bleeding on probing.

*$p < 0.001$; *p*-values represent longitudinal changes from baseline within each group.

demonstrates that at the selected sites a compromised treatment outcome was seen for smokers, in terms of PD reduction ($p = 0.0005$) and gain in RAL ($p = 0.0009$). At RAS, smokers presented with higher PD ($p = 0.0015$) and attachment loss ($p = 0.0350$) than did non-smokers (Table 3).

Lower MGI ($p = 0.030$), but similar PI, was found for smokers at BAS (Fig. 1). At this time point, although BOP was less severe in smokers than non-smokers (fewer smokers presented with 4 bleeding sites), this failed to reach statistical significance ($p = 0.070$) (Fig. 2). Figure 1 compares the GCF volume (μl/30 s) between smokers and non-smokers and shows that at BAS, GCF volume was significantly lower for smokers ($p = 0.017$). After treatment, no statistically significant differences were found between the two groups (Figs 1 and 2), while the longitudinal analysis of these clinical parameters within each group showed significant improvements in all of them ($p < 0.010$).

The PCR analysis revealed that despite the differences between smokers and non-smokers in the percentages of patients and sites positive for the five putative periodontal pathogens, none of these differences reached statistical significance (Figs 3–7). Post-therapy, a marked reduction in the detection frequency of all of the test organisms was seen, which reached statistical significance for the majority of them ($p < 0.05$).

Although serum IgG titres (EU) to all putative pathogens were lower for smokers at BAS, this observation failed to reach statistical significance (Table 4). At RAS, smokers retained lower antibody levels to all bacteria tested compared with non-smokers, and this was statistically significant for *A. actinomycetemcomitans* ($p = 0.04$). Both groups demonstrated a trend towards reduced IgG antibody titres at RAS from BAS, reaching statistical significance for only some of the species (Table 4). No significant differences in IgG avidity (M at ID_{50}) were seen between non-smokers and smokers pre- and post-therapy (Table 4). The longitudinal analysis of the data revealed a significant increase in IgG antibody avidity to *A. actinomycetemcomitans* at RAS from BAS in both groups ($p < 0.05$) (Table 4).

Cigarette smoking status was self-reported by the patients and was confirmed by the CEIA. Subjects were considered to be smokers if they smoked five or more cigarettes per day. The range of cotinine levels was 3.2–28.6 ng/ml in non-smokers and 43.0–3267.0 ng/ml in smokers, with a median of 11.2 ng/ml and 1848.0 ng/ml, respectively.

Table 3. Selected-site clinical indices ($N_{n\text{-}sm} = 25$, $N_{sm} = 15$)

	BAS	RAS	Change (BAS – RAS)
PD (mm)			
N-sm	6.2 ± 0.8	3.1 ± 0.4**	3.1 ± 0.9***†
Sm	5.9 ± 0.6	3.6 ± 0.5**	2.3 ± 0.6***†
RAL (mm)			
N-sm	13.8 ± 1.6	12.4 ± 1.4*	1.4 ± 0.5***†
Sm	14.2 ± 1.4	13.5 ± 1.6*	0.7 ± 0.5***†

Mean ± SD.

n-sm, non-smokers; sm, smokers; BAS, baseline; RAS, re-assessment at 6 months; PD, pocket depth; RAL, relative attachment level.

*$p < 0.050$;

**$p < 0.010$;

***$p < 0.001$; *p*-values represent differences between non-smokers and smokers;

†$p < 0.001$; *p*-values represent longitudinal changes from baseline within each group.

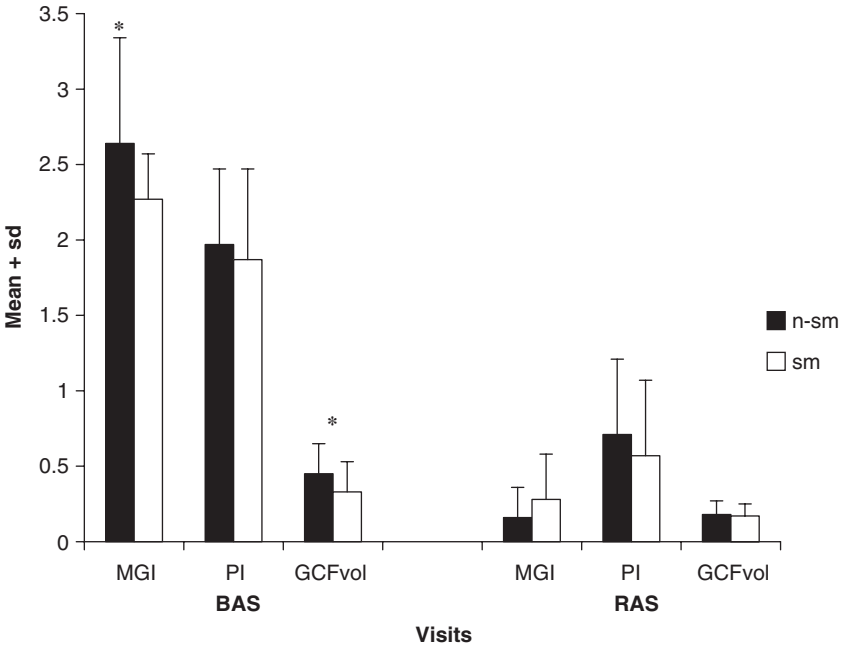

Fig. 1. MGI, PI and GCF volume (μl/30 s) for non-smokers (25) and smokers (15). MGI, Modified Gingival Index; PI, Plaque Index; GCF_{vol}, gingival crevicular fluid volume. *$p < 0.05$; *p*-values represent differences between non-smokers and smokers; n-sm, non-smokers; sm, smokers; BAS, baseline; RAS, re-assessment at 6 months.

Discussion

Comparison of baseline parameters between non-smokers and smokers

In a previous study, we have shown that both treatment modalities, same-day FM-SRP and Q-SRP at two-weekly intervals, were equally efficacious with no significant differences in clinical indices between the two treatments at 6 months (Apatzidou & Kinane 2004a). However, the General Linear Model analysis demonstrated a significant three-way interaction among the following fixed factors: treatment modality, smoking and visit effect on PD and RAL of the selected sites ($p < 0.001$). It was difficult to interpret the effect of each factor on the selected-site clinical indices, but it appeared that smoking was the predominant factor in this three-way interaction. Based on these findings, we analysed the pre- and post-

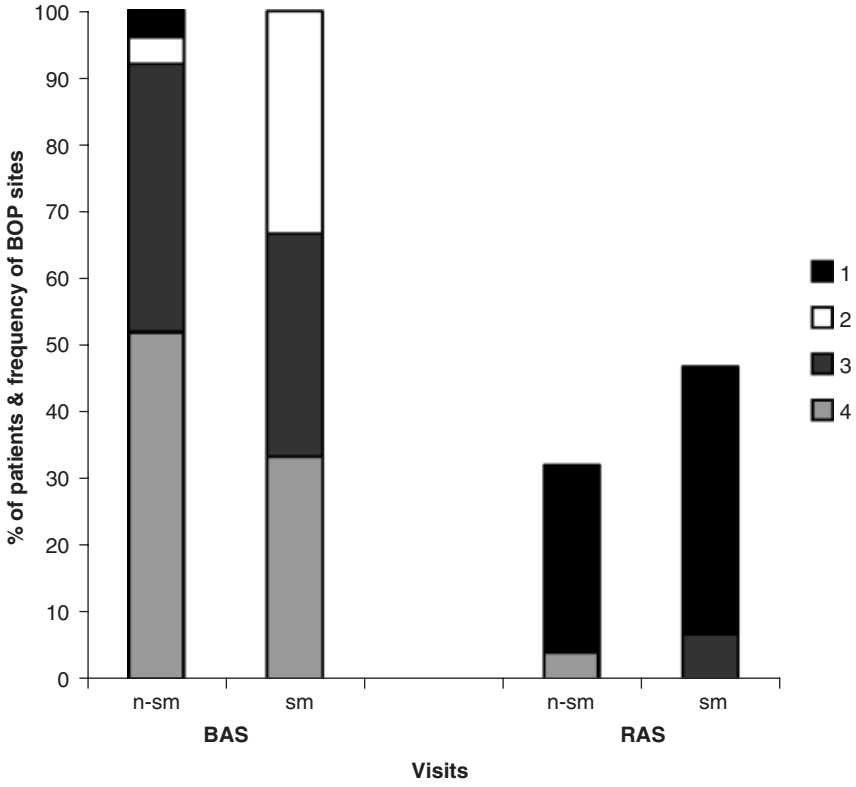

Fig. 2. Percentage of patients and the frequency of sites (from 1 to 4) that are BOP positive in non-smokers (25) and smokers (15). No statistically significant differences were noted between non-smokers and smokers ($p = 0.07$ at BAS); n-sm, non-smokers; sm, smokers; BAS, baseline; RAS, re-assessment at 6 months; BOP, bleeding on probing.

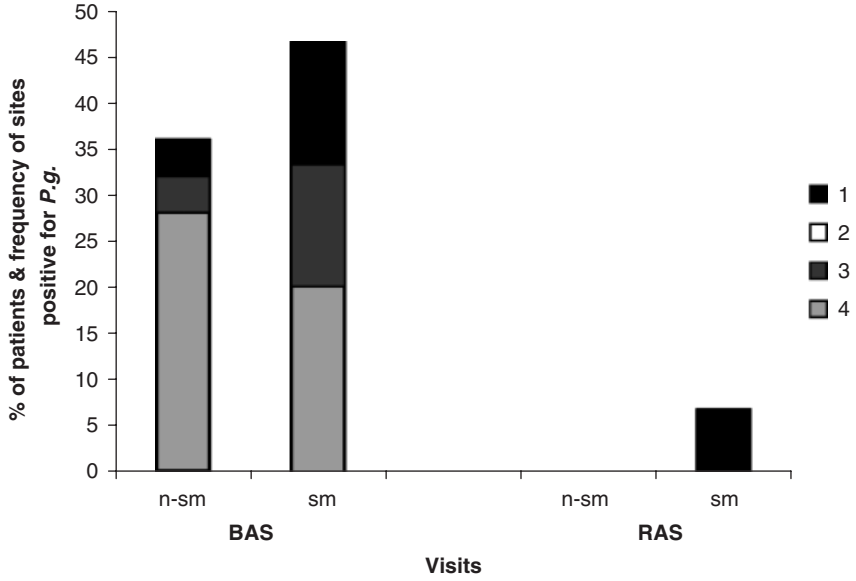

Fig. 3. Percentage of patients and the frequency of sites (from 1 to 4) that harbour *Porphyromonas gingivalis* in non-smokers (25) and smokers (15). No statistically significant differences were noted between non-smokers and smokers ($p > 0.05$); both smokers and non-smokers who were positive for this species decreased significantly after treatment ($p < 0.05$); n-sm, non-smokers; sm, smokers; BAS, baseline; RAS, re-assessment at 6 months.

treatment data to assess the impact of smoking on the treatment outcome.

At a site-specific level, smokers showed significantly lower MGI and GCF volume with a trend towards lower BOP and PD, but greater RAL at BAS compared with non-smokers. The finding that smoking suppresses the inflammatory response to plaque challenge, and therefore masks clinical signs of gingival inflammation, confirms previous observations in periodontitis patients (Preber & Bergström 1985, Darby et al. 2000, Bergström & Boström 2001) and in experimental gingivitis-induced patients (Danielsen et al. 1990, Lie et al. 1998). The lower GCF volume found in smokers is in accord with previous findings from our laboratory (Kinane & Radvar 1997). Similarly, other investigations showed that in periodontally healthy subjects, GCF volume was lower among smokers than non-smokers (Holmes 1990, Persson et al. 1999).

No differences in plaque levels were found between smokers and non-smokers, which is consistent with other reports (Kinane & Radvar 1997, Kamma et al. 1999, Darby et al. 2000, Haffajee & Socransky 2001a), implying that the harmful effects of smoking on periodontal health may not be associated with plaque accumulation and poor oral hygiene (Bergström & Eliasson 1987a).

The present study demonstrated that despite the differences in the prevalence of microorganisms between the two groups of patients, these were not found to be statistically significant, which is in agreement with other reports that failed to show an altered subgingival microbiota in smokers (Preber et al. 1992, Stoltenberg et al. 1993, Darby et al. 2000, Boström et al. 2001). Lie et al. (1998) did not attribute the lower bleeding scores seen in smokers after the induction of experimental gingivitis to microbiological differences between smokers and non-smokers. Nevertheless, there are conflicting reports that smokers harbour specific pathogens at higher levels and frequencies than non-smokers (Zambon et al. 1996, Kamma et al. 1999), and that they are at increased risk for bacterial infection (Zambon et al. 1996, Umeda et al. 1998, van Winkelhoff et al. 2001). Disagreement in the subgingival microbial composition between smokers and non-smokers among various studies could be explained by differences in patient populations, microbial sampling techni-

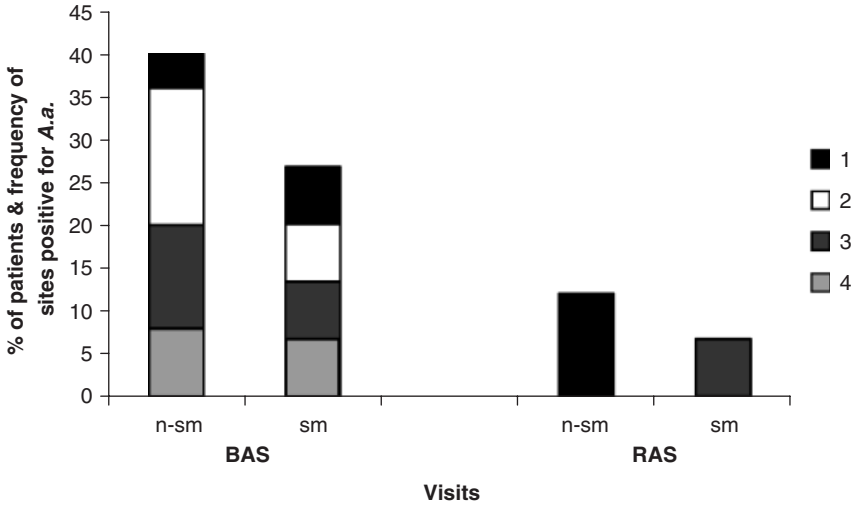

Fig. 4. Percentage of patients and the frequency of sites (from 1 to 4) that harbour *Actinobacillus actinomycetemcomitans* in non-smokers (25) and smokers (15). No statistically significant differences were noted between non-smokers and smokers ($p > 0.05$); only non-smokers who were positive for this species decreased significantly after treatment ($p < 0.05$); n-sm, non-smokers; sm, smokers; BAS, baseline; RAS, re-assessment at 6 months.

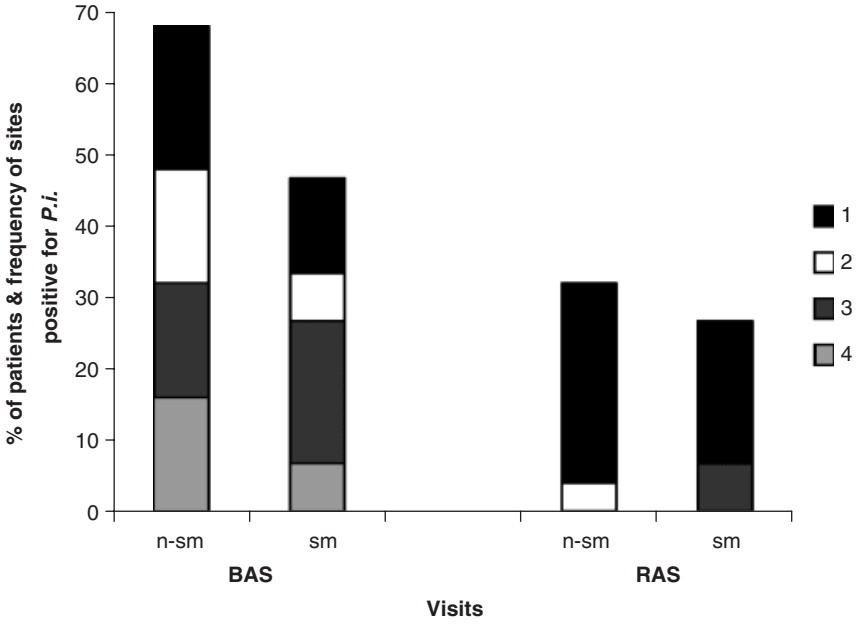

Fig. 5. Percentage of patients and the frequency of sites (from 1 to 4) that harbour *Prevotella intermedia* in non-smokers (25) and smokers (15). No statistically significant differences were noted between non-smokers and smokers ($p > 0.05$); only non-smokers who were positive for this species decreased significantly after treatment ($p < 0.05$); n-sm, non-smokers; sm, smokers; BAS, baseline; RAS, re-assessment at 6 months.

ques, number of samples studied, detection methods of putative pathogens, species examined and also differences in evaluation and expression of data (counts *versus* proportions *versus* site or subject prevalence) (Haffajee & Socransky 2001b).

There is evidence that smoking has a systemic effect on the immune response (Kenney et al. 1977, Andersen et al. 1982, Holt 1987, Graswinckel et al. 2004). Present data showed a consistent trend for lower serum antibody titres against all microorganisms in smokers than non-smokers pre- and post-therapy, although this observation did not reach statistical significance for any of the bacteria tested prior to therapy. The lower sera antibody levels imply that smoking has the potential to modify the host antibody response and this agrees with previous observations (Haber 1994). This pattern of decreased levels of antibodies was less clear when antibody avidities were considered, showing small differences, if any, between the two groups of patients. It has been shown in other studies that smoking has an impact on serum Ig levels, but this effect seems to be both race and serum IgG subclass specific (Quinn et al. 1996, 1998, Gunsolley et al. 1997, Tangada et al. 1997). It is possible that B-cells are functionally compromised by having reduced proliferative responses to oral pathogens, and thus resulting in reduced production of serum Ig's (Mooney et al. 2001).

It was interesting to note that the majority of the subjects who dropped out of the study were smokers (60%) and this agrees with previous findings (Boström et al. 1998, Jansson & Hagström 2002), indicating lack of compliance in smokers.

Comparison of longitudinal parameters between non-smokers and smokers

Periodontal therapy undoubtedly improves clinical parameters, decreases the antigenic load and consequently the antibody levels to specific organisms. The current study showed a significant reduction in the median antibody titres to some of the test organisms and a concomitant decrease in the mean clinical parameters and the detection of the homologous organisms at 6 months. These results agree with data reported by other investigators (Tolo et al. 1982, Naito et al. 1985, Mouton et al. 1987, Aukhil et al. 1988, Murray et al. 1989, Horibe et al. 1995). However, the wide variation in the subjects' antibody response to treatment made the interpretation of results difficult.

Successful treatment results in the elimination of the aetiological agents and maturation of the immune system to produce antibodies of high avidity (Chen et al. 1991). Data reported here show that treatment resulted in lower levels of antibodies to some of the bacteria tested, but avidity remained the same for the majority of the organ-

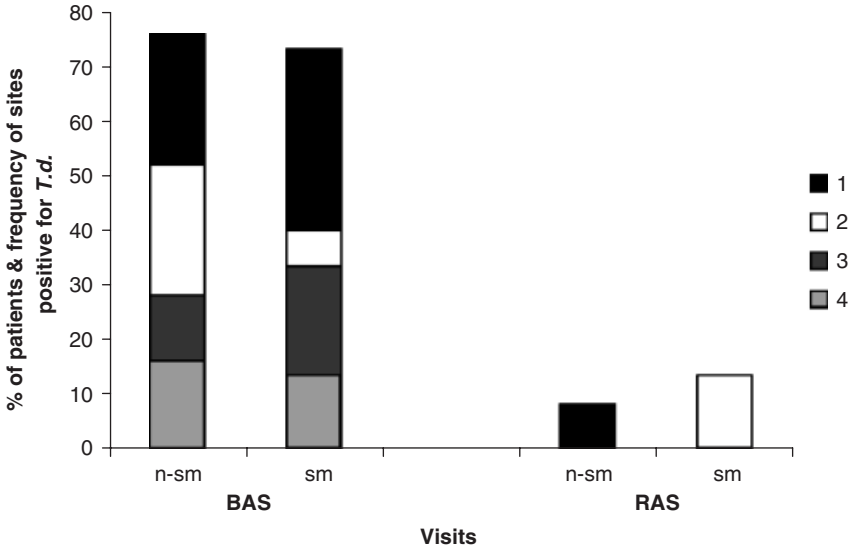

Fig. 6. Percentage of patients and the frequency of sites (from 1 to 4) that harbour *Treponema denticola* in non-smokers (25) and smokers (15). No statistically significant differences were noted between non-smokers and smokers ($p > 0.05$); both smokers and non-smokers who were positive for this species decreased significantly after treatment ($p < 0.05$); n-sm, non-smokers; sm, smokers; BAS, baseline; RAS, re-assessment at 6 months.

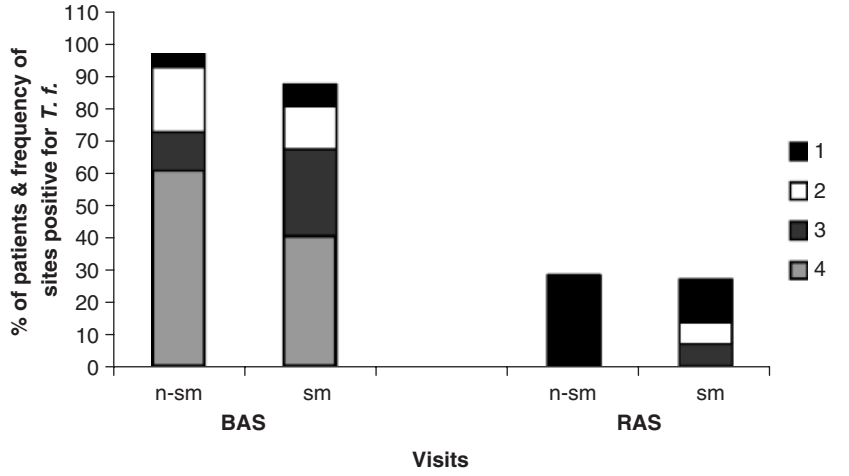

Fig. 7. Percentage of patients and the frequency of sites (from 1 to 4) that harbour *Tanerella forsythensis* in non-smokers (25) and smokers (15). No statistically significant differences were noted between non-smokers and smokers ($p > 0.05$); both smokers and non-smokers who were positive for this species decreased significantly after treatment ($p < 0.05$); n-sm, non-smokers; sm, smokers; BAS, baseline; RAS, re-assessment at 6 months.

isms. It was of interest to note that antibody avidity increased significantly to a major putative periodontal pathogen, *A. actinomycetemcomitans*, in both groups at 6 months, indicating a beneficial effect of periodontal therapy on the host antibody response. Another study from our laboratory showed that despite the clinical improvement seen post-therapy, there were no significant post-treatment effects on the humoral immune response other than a reduction in the avidity of antibodies to *P. gingivalis* and *P. intermedia* (Darby et al. 2001). This finding may indicate a failure of the host response to produce adequate levels of biologically functional antibodies after treatment and it is possible that this poor host response makes patients susceptible to future disease progression. The present findings imply that antibody avidity may be a very dynamic process, with fluctuations that are difficult to detect with conventional laboratory techniques, or that a period of time longer than the monitoring period of this study (6 months) is required to document the maturation of the immune system.

Significant reductions in the percentage of patients positive for most of the bacteria were seen after therapy. However, the majority of the organisms were still detected post-scaling but in significantly lower frequencies than baseline. This is in agreement with other reports that showed that scaling and root planing lowers the numbers of selected periodontal pathogens, but is unlikely to eliminate these species from any subject (Haffajee et al. 1997, Cugini et al. 2000). The results of the present study agree with those by Doungudomdacha et al. (2001), who showed a significant decrease but not eradication of the test organisms post-treatment. The presence of organisms at sites that were considered clinically healthy implied that the mere presence of a putative pathogen is not indicative of the presence or recurrence of disease. However, these pathogenic mechanisms still need clarification and more evidence is required to explain these concepts.

The current report demonstrated that, following therapy, selected sites of smokers exhibited less clinical improvement, in terms of PD and RAL reductions than did those of non-smokers. Marked reductions in the detection frequency of putative periodontal pathogens were seen at 6 months with no significant differences between smokers and non-smokers. After treatment, smokers maintained lower serum antibody titres compared with non-smokers, and this was significant for *A. actinomycetemcomitans*.

These results re-inforce the concept that smoking results in a compromised clinical outcome following treatment. (Preber et al. 1995, Kaldahl et al. 1996, Haffajee et al. 1997, Kinane & Radvar 1997, Renvert et al. 1998) and seems to have an effect on the host antibody response to suspected periodontal pathogens. These findings may be detrimental in the long-term prognosis of smokers with periodontal disease. The present data agree with other reports, which found no differences in the presence of pathogens in supra- or subgingival plaque between smokers and non-smokers after treatment (Preber et al. 1995, Boström et al. 1998, Renvert et al. 1998).

Table 4. Comparison of IgG antibody titres and IgG antibody avidities between n-sm (25) and sm (15)

	P. gingivalis	*A. actinomycetemcomitans*	*P. intermedia*	*T. denticola*	*T. forsythensis*
Serum IgG titres					
BAS					
N-sm	109 (54, 716)	73 (20, 1536)	277 (128, 663)	15 (8, 77)	34 (15, 975)
Sm	70 (21, 516)	55 (9, 118)	94 (26, 497)	12 (3, 38)	28 (14, 135)
RAS					
N-sm	85 (29, 531)	58 (25, 691)*	321 (85, 475)	22 (6, 34)	36 (15, 498)
Sm	46 (19, 549)	22 (9, 159)*	129 (42, 392)	13 (1, 20)	31 (13, 94)
BAS-RAS					
N-sm	58 (− 27, 376)†	15 (− 10, 242)	26 (− 44, 257)	4 (0, 21)†	2 (− 5, 90)
Sm	18 (− 5, 391)	2 (− 5, 46)	33 (− 22, 116)	3 (0, 27)†	8 (− 1, 63)†
IgG avidities					
BAS					
N-sm	0.50 (0.32, 0.68)	0.44 (0.37, 0.66)	0.83 (0.62, 1.00)	0.31 (0.26 0.43)	0.43 (0.30, 0.66)
Sm	0.46 (0.28, 0.58)	0.37 (0.33, 0.50)	0.85 (0.65, 1.00)	0.32 (0.23, 0.58)	0.41 (0.29, 0.58)
RAS					
N-sm	0.58 (0.30, 0.80)	0.43 (0.36, 0.83)	0.85 (0.67, 0.98)	0.35 (0.30 0.44)	0.48 (0.31, 0.68)
Sm	0.49 (0.32, 0.67)	0.55 (0.32, 0.68)	0.76 (0.70, 1.18)	0.39 (0.24, 0.48)	0.38 (0.27, 1.10)
BAS-RAS					
N-sm	0.01 (− 0.24, 0.06)	− 0.04 (− 0.22, 0.04)†	0.01 (− 0.18, 0.08)	− 0.03 (− 0.11, 0.04)	0.00 (− 0.11, 0.06)
Sm	− 0.05 (− 0.11, 0.03)	− 0.09 (− 0.28, 0.02)†	0.06 (− 0.19, 0.20)	0.00 (− 0.07, 0.08)	− 0.04 (− 0.52, 0.03)

Serum IgG titres are expressed as ELISA units (EU); IgG avidity is expressed as molarity (M) of ammonium thiocyanate at ID_{50}.
Median (interquartile range).
n-sm, non-smokers; sm, smokers; BAS, baseline; RAS, re-assessment at 6 months; IgG, immunoglobulin G.
Bacteria tested: *Porphyromonas gingivalis*; *Actinobacillus actinomycetemcomitans*; *Prevotella intermedia*; *Treponema denticola*; *Tanerella forsythensis*.
*$p = 0.04$; p-values represent differences between non-smokers and smokers.
†$p < 0.05$; p-values represent longitudinal changes from baseline within each group.

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