The clinical and microbiological effects of non-surgical periodontal therapy in smokers and non-smokers


Abstract. 28 patients, 13 smokers and 15 non-smokers with untreated advanced periodontal disease, were subjected to a series of oral hygiene instructions and treated with non-surgical periodontal therapy. Baseline values regarding clinical data did not differ significantly between the groups. 6 months following therapy the full-mouth bleeding score among smokers was 36.5% as compared to 22.7% for non-smokers (p<0.05). Probing depth was reduced by 1.9 mm for smokers and by 2.5 mm for non-smokers. This difference was statistically significant (p<0.05). The level of P. gingivalis and P. intermedia/nigrescens was reduced in both groups as compared to baseline. A. actinomycetemcomitans, however demonstrated a slight increase in mean values at 6 months. This was especially notable for smokers in which A. actinomycetemcomitans were more difficult to eradicate. Conclusively, the microbiological response found in this study seems to be in conformity with the clinical response with little influence of the smoking habits.

During the past decade, a number of studies have focused on the association between smoking and periodontal disease. Smokers have been reported to exhibit a more severe periodontal disease as compared to non-smokers (Ismail et al. 1983, Bergström et al. 1991, Haber & Kent 1992, MacFarlane et al. 1992, Martinez-Canut et al. 1995), and cigarette smoking has been found to increase the risk of periodontitis (Grossi et al. 1994, 1995).

It has also been reported that healing following periodontal therapy is inferior among smokers as compared to non-smokers (Preber & Bergström 1985b, Preber & Bergström 1990, Ah et al. 1994, Preber et al. 1995, Grossi et al. 1996).

Conflicting results regarding the subgingival microflora in smokers and non-smokers have been reported. Preber et al. (1992) and Stoltenberg et al. (1993) were unable to detect any difference between smokers and non-smokers analysing the recovery of Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis and Prevotella intermedia. Zambon et al. (1996) demonstrated significantly higher proportions of A. actinomycetemcomitans, P. gingivalis and B. forsythus among current smokers.

The effect of non-surgical periodontal therapy on selective periodontal pathogens in smokers and non-smokers have been evaluated in a limited number of studies. Preber et al. (1995) were unable to detect differences between smokers and non-smokers one month following treatment, whereas Grossi et al. (1996) reported a less pronounced reduction of periodontal pathogens among smokers 3 months after treatment and significantly fewer smokers became negative for P. gingivalis and B. forsythus as a result of therapy.

As of now the information on a possible relationship between smoking and the subgingival microflora following treatment is limited and conflicting results have been reported. The aim of the present study was to compare the clinical and microbiological effects of scaling and rootplaning in sites with a probing pocket depth ≥6 mm, 6 months after treatment in smokers and non-smokers.

Material and Methods

28 medically non compromised patients (40-60 years of age) with untreated advanced periodontal disease were recruited from the waiting list at the Department of Periodontology, Public Dental Service in Kristianstad, Sweden. Patients with heart diseases, rheumatic fever, organ transplant, joint replacement, pregnancy and lactation, family history of diabetes, radiation therapy, immunosuppression or chemotherapy...
were not included in the study. The patients should demonstrate at least 3 sites with bleeding on probing (BOP) and a probing pocket depth of ≥6 mm at the initial examination. They should not have received any antibiotic therapy during the past 6 months. The patient group consisted of 13 smokers (≥15 cigarettes per day) and 15 non-smokers. The mean age was 43 years for smokers (range 29–72 years) and 45 years for non-smokers (range 26–73 years).

**Treatment**

Following patient and site selection patients eligible for the study were thoroughly informed, and those who volunteered for the study signed a consent form. The patients were subjected to a series of oral hygiene instructions. Subsequently, scaling and rootplanning were performed under local anaesthesia by a dental hygienist. The instrumentation was carried out until the operator felt that the root surfaces were adequately debrided. Three months after completion of the initial treatment the patients were recalled for reinforcement of the oral hygiene and supragingival rubbercup polishing of the teeth. At this visit no further subgingival debridement was performed. The results of therapy were evaluated after 6 months.

**Measurements**

Microbiological and clinical measurements were obtained at baseline and after 24 weeks. All recordings were made by the same examiner (SR) in the following sequence: (1) experimental site plaque index; (2) subgingival microbial sample; (3) experimental site probing depths and probing attachment level measurements; (4) experimental site BOP; (5) full mouth BOP; (6) full mouth plaque index.

**Local plaque index**

Supragingival plaque was registered as present or not present. A sterile curette was applied to the superficial surface at the gingival margin and moved coronally. If plaque was visible on the curette, the surface was scored as having plaque.

**Microbial samples and examination**

3 paperpoints were inserted into the periodontal pocket until definite resistance was met and left in place for 30 s. The samples were transferred by mail to the laboratory, and was processed on the following day. The vials containing the sample, transport medium and glass beads were thoroughly shaken in a mixer (Whirly-mixer, Fisons Scientific Apparatus Limited, Longbrough, Leicestershire, England) and diluted to 10⁻² and 10⁻⁴ in VMG I (Möller 1966). A volume of 0.1 ml from each dilution as well as from the undiluted sample was distributed on the surface of a TSBV agar plate (Trypticase-soy-agar with 75 mg/l of bacitracin and 5 mg/l of vancomycin (Slots 1982), and on a Brucella agar plate (BBL Microbiology Systems, Cockeysville, MD, USA) enriched with 5% defibrinated horse blood, 0.5% haemolyzed blood and 5 µg/l of menadione.

After 5 days of incubation of the TSBV agar plate in 10% CO₂, colony forming units with characteristics of *A. actinomycetemcomitans* were identified and enumerated. *A. actinomycetemcomitans* was defined as small adherent colonies showing a positive catalase reaction.

After 7–9 days of incubation in jars with 95% H₂ and 5% CO₂, the total viable count was determined from the Brucella agar plate. 1–5 colonies each of those with a characteristic appearance were subjected to Gram-staining procedures, lactose fermentation test, and to be suspended in a few drops of methanol to check for the ability to demonstrate red fluorescence in long-wave (360 nm) UV-light (Slots & Reynolds 1983). *Porphyromonas gingivalis* was identified as black or greenish-black colonies not demonstrating fluorescence, and *Prevotella intermedia/nigrrescens* as black colonies exhibiting dark red fluorescence and not fermenting lactose.

**Probing depths and probing attachment levels.** Measurements of probing pocket depths and probing attachment levels were made using an electronic pressure sensitive probe (Electronic Periodontal Probe model 200, Vine Valley Research, Middlex, NY, USA). A probe tip having 1 mm increments and 0.4 mm diameter was used. Each site was probed using a standard force of 0.50 N. Recordings were made to the nearest 0.5 mm. A vacuum adapted soft acrylic onlay was used to provide reference points for the probing attachment level measurements. Measurements from buccal and lingual sites were taken from the midpoint of these surfaces. For proximal areas the placement of the probe was guided by the interdental indentations of the thin onlay and the probe was directed toward the deepest point of the site.

**Bleeding on probing**

Bleeding upon probing was determined using the same standardised probe and probing force as described above and recorded for the entire dentition immediately following the experimental site probing measurements. Two types of bleeding scores were calculated: (i) full mouth bleeding score (%), based on the following 6 aspects of the teeth in

| Table 1. Clinical parameters at baseline and at 6 months in smokers and non-smokers (mean and SD) |
|-------------------------------------------------|-------------------------------|-------------------------------|---------------|-----------------|-----------------|
| Clinical parameter                              | Smokers (n=13)                | Non-smokers (n=15)            |               |                 |                 |
|                                                  | baseline                      | 6 months                      | baseline      | 6 months        | baseline        |
| full mouth plaque score (%)                      | 61.5±17.4                     | 22.8±11.6                    | 54.7±18.6     | 24.0±16.0       |                 |
| local site plaque index                         | 0.70±0.32                     | 0.28±0.25                    | 0.72±0.29     | 0.20±0.25       |                 |
| full mouth bleeding score (%)                    | 63.0±21.3                     | 36.5±19.9                    | 53.0±24.3     | 22.7±12.3       |                 |
| local site bleeding index                        | 0.97±0.06                     | 0.67±0.32                    | 0.98±0.06     | 0.70±0.25       |                 |
| pocket depth (mm)                               | 7.1±0.8                       | 5.2±0.9                      | 7.6±1.1       | 5.1±0.8         |                 |
| attachment level (mm)                           | 11.7±1.1                      | 10.7±1.2                     | 12.1±1.3      | 11.1±1.3        |                 |

*p<0.05.

| Table 2. Bleeding score (0, 1, 2, 3) at baseline, 6 months and difference between baseline and 6 months in a subsample of 16 individuals (7 smokers and 9 non-smokers) (mean and SD) |
|-------------------------------------------------|-------------------------------|-------------------------------|---------------|-----------------|-----------------|
| Smokers (n=7)                                   | Non-smokers (n=9)             | Difference Bl – 6 months      |               |                 |                 |
| baseline                                        | 6 months                      | baseline                      | 6 months      | smokers         | non-smokers     |
| 1.98±0.31                                       | 1.38±0.42                     | 2.43±0.43                     | 1.82±0.58     | −0.60±0.50      | −0.61±0.48      |

*p<0.05.
the mouth: mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual; (ii) experimental site bleeding score. Bleeding was recorded as present (1) or absent (0). A mean value for the experimental sites was calculated.

In a subsample of 16 individuals (7 smokers and 9 non-smokers), the degree of BOP was evaluated in 96 experimental sites before and after therapy. The bleeding was graded 1-3, grade 1 representing blood forming a light red dot at the gingival margin, grade 2 representing a line of blood at the gingival margin, and grade 3 being a drop of blood filling the entire approximal space.

Dental plaque
Plaque was disclosed using an erytosin dye (Rondell Red, LIC Dental, Upplands Väsby, Sweden). Areas adjacent to the gingival margin which exhibited a deep stain that could easily be removed with the side of the probe was scored as having plaque. Full mouth plaque scores based on 6 registrations per tooth were calculated in the same manner as for full mouth plaque scores.

**Statistical analysis**
The statistical significance of the difference between smokers and non-smokers was tested for each clinical and microbiological parameter using the Student t-test, analysis of covariance and by estimation of 95% confidence interval.

**Results**
Clinical data at baseline and at 6 months are presented in Table 1. Base-line values for the 2 groups did not differ significantly. Both groups had high plaque and bleeding scores. At baseline almost all experimental areas demonstrated BOP. Mean pocket depths were 7.6 mm for non-smokers and 7.1 mm for smokers. As a result of therapy plaque and full mouth bleeding index was reduced in both groups. At 6 months the difference in BOP between the groups was statistically significant (p<0.05). In the subsample based of 96 sites in 16 individuals, non-smokers demonstrated a more pronounced bleeding tendency as compared to smokers. This difference was statistically significant at baseline (p<0.05) (Table 2).

The probing pocket depth was reduced by 1.9 mm for smokers and by 2.5 mm by non-smokers. The reduction of probing pocket depth was statistically significant (p<0.05) comparing smokers and non-smokers (Table 3). The change of probing attachment levels amounted around 1 mm for both groups.

At baseline comparable amounts of the three selected subgingival micro-organisms were found among smokers and non-smokers (Table 4).

As a result of therapy the level of *P. gingivalis* and *P. intermedia/nigrescens* was reduced. However, *A. actinomycetemcomitans* demonstrated a slight increase in mean values at 6 months. This was especially notable for the smokers (Table 5). The numbers of positive sites before and after treatment are presented in Table 6. *P. intermedia/nigrescens* and *A. actinomycetemcomitans* were more often eradicated from sites in non-smokers.

### Discussion
The aim of this 6 months study was to evaluate, the clinical and microbiological effect of non-surgical periodontal therapy in smokers and non-smokers. At baseline, no significant differences in bleeding index was found between...
smokers and non-smokers. However, following therapy smokers demonstrated significantly more bleeding on probing (BOP). This is to some extent contradictory to other reports claiming that smokers do not exhibit gingivitis symptoms as clearly as non-smokers (Preber & Bergström 1985a, Bergström 1989, Danielsen et al. 1990, Preber et al. 1995). However, significantly more bleeding sites among smokers following therapy have previously been reported by Ah et al. (1994). Although significant differences were found in the study by Ah et al. (1994), the average difference between smokers and non-smokers was approximately 3%. Interestingly, in the subsample, in which the degree of bleeding was analysed, bleeding was more pronounced in non-smokers as compared to smokers. This is an indication of that BOP using 0.5 N pressure may be a blunt measurement to evaluate inflammatory changes in the gingiva. The concept of a graded bleeding score might be more accurate in order to distinguish the relative amounts of traumatic and inflammatory bleeding (Renvert et al. 1992). Although significant differences in bleeding tendency have been reported between smokers and non-smokers the clinical significance of this is still debatable.

Probing pocket depth reductions have been reported to be dependent on the initial probing pocket depth (Lindhe et al. 1982, Badersten et al. 1984). The significantly smaller probing pocket depth reductions obtained among smokers was not related to shallower initial probing pocket depth as compared to non-smokers. Inferior probing pocket depth reductions as a result of therapy among smokers have been reported by others (Preber & Bergström 1985b, Ah et al. 1994, Preber et al. 1995) and the results from this study support these findings. However, in spite of the greater probing pocket reductions obtained among non-smokers attachment level changes was somewhat more pronounced among smokers. One reason for the discrepancies between pocket reductions and attachment level changes may be the less pronounced morphological changes in the tissues among smokers. The difference in tissue response between smokers and non-smokers could to some degree be explained by the difference in gingival blood flow (Johnson et al. 1991) and number of blood vessels in the marginal gingiva (Bergström et al. 1988). In this study the amount of bleeding in the sub-sample was significantly lower at baseline among smokers as compared to non-smokers. An increase in gingival volume due to the increase in blood vessels and leakage of gingival fluid as a result of the bacterial load at the gingival margin seems to be less evident in smokers. This may explain the smaller probing pocket depth reductions as compared to non-smokers.

The assumption that differences in the subgingival microflora between smokers and non-smokers might exist was based on a report that smokers due to less bleeding would create a low oxygen level in the deep periodontal pockets (Kenny et al. 1975). It was not possible to detect P. gingivalis, P. intermedia/nigrescens or A. actinomycetemcomitans more frequently in test sites among smokers in the present study. This is in concordance with the reports by Preber et al. (1992) and Stoltenberg et al. (1993) but in disagreement with the report by Zambon et al. (1996). Zambon et al. (1996) however, used pooled samples from 1312 individuals which may account for the discrepancies in results.

In this study, the microbiological response to treatment, measured as the reduction of some selected bacterial markers 6 months after scaling and rootplaning, was found to be of the same magnitude in smokers and non-smokers. However, A. actinomycetemcomitans increased among the smokers as a percentage of the subgingival flora and was detected in the same number of sites 6 months after therapy. This finding is concordant with the results in the previously cited paper by Zambon et al. (1996), who reported current smokers to be 3.1 times more likely to be infected with A. actinomycetemcomitans. In our study we found A. actinomycetemcomitans to be more difficult to eradicate among smokers as compared to non-smokers although this difference was not statistically significant.

Conclusively, the microbiological response found in this study seems to be in conformity with the clinical response with little influence of the smoking habits.

Zusammenfassung
Die klinische und mikrobiologische Wirkung der nicht-chirurgischen Parodontaltherapie bei Rauchern und Nichtrauchern
Achtundzwanzig Patienten, 15 Raucher und 15 Nichtraucher, mit unbehandelter fortgeschrittener Parodontalerkrankung bekamen eine Reihe von Mundhygienemaßnahmen und wurden mittels nicht-chirurgischer Parodontaltherapie behandelt. Die klinischen Ausgangswerte unterschieden sich zwischen den Gruppen nicht signifikant. 6 Monate nach der Therapie lagen die Blutungswerte für das vollständige Gebiß bei 36.5% für die Raucher und 22.7% für die Nichtraucher (p<0.05). Die Sondierungstiefe war bei den Rauchern um 1.9 mm und bei den Nichtrauchern um 2.5 mm reduziert. Dieser Unterschied war statistisch signifikant (p<0.05). Im Vergleich zu den Ausgangswerten war der Anteil an P. gingivalis und P. intermedia/nigrescens in beiden Gruppen reduziert. Jedoch zeigte A. actinomycetemcomitans nach 6 Monaten einen leichten Anstieg der Mittelwerte. Dies wurde insbesondere bei Rauchern festgestellt, bei denen A. actinomycetemcomitans schwieriger zu entfernen war. Die Schlußfolgerung ist, daß die mikrobiologische Reaktion, die in dieser Studie gefunden wurde, konform mit der klinischen Entwicklung geht und nur gering von den Rauchgewohnheiten beeinflußt wird.

Résumé
Effets cliniques et microbiologiques du traitement parodontal non chirurgical chez les fumeurs et les non-fumeurs
28 patients atteints de maladie parodontale avancée, dont 13 fumeurs et 15 non-fumeurs, ont reçu une série d'instructions d'hygiène bucco-dentaire et un traitement parodontal non chirurgical. Les valeurs des données cliniques au début (baseline) ne diffèrent pas significativement d'un groupe à l'autre. 6 mois après le traitement, le score du saignement pour toute la bouche était de 36,5% chez les fumeurs, à comparer à 22,7% chez les non-fumeurs (p<0.05). La réduction de la profondeur de sondage était de 1,9 mm chez les fumeurs et de 2,5 mm chez les non-fumeurs. Cette différence était statistiquement significative (p<0.05). Le niveau de P. gingivalis et P. intermedia/nigrescens était réduit dans les deux groupes par rapport aux valeurs du début. Cependant, les valeurs moyennes de A. actinomycetemcomitans présentaient une légère augmentation à 6 mois. Ce fait était particulièrement visible chez les fumeurs, chez qui il était plus difficile d'obtenir l'éradication de A. actinomycetemcomitans. En conclusion, la réponse microbiologique mise en évidence dans cette étude semblait être conforme avec la réponse clinique, les habitudes concernant l'usage de tabac ayant peu d'influence à ce sujet.

References
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