Journal of Clinical Periodontology
Official Journal of The European Federation of Periodontology
Founded by the British, Dutch, French, German, Scandinavian, and Swiss Societies of Periodontology

J Clin Periodontol 2009; 36: 229–239 doi: 10.1111/j.1600-051X.2008.01371.x

Journal of Clinical Periodontology

Effects of smoking on healing response to non-surgical periodontal therapy: a multilevel modelling analysis

Chi Pui Wan, W. Keung Leung, May C. M. Wong, Ruby M. S. Wong, Peng Wan, Edward C. M. Lo and Esmonde F. Corbet

Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR, China

Wan CP, Leung WK, Wong MCM, Wong RMS, Wan P, Lo ECM, Corbet EF. Effects of smoking on healing response to non-surgical periodontal therapy: a multilevel modelling analysis. J Clin Periodontol 2009; 36: 229–239. doi: 10.1111/j.1600-051X.2008.01371.x.

Abstract

Aim: To investigate the factors predicting non-surgical periodontal treatment responses using multilevel multiple regression.

Material and Methods: Forty men (mean 45.6 years) were recruited; 20 were smokers. A 12-month reduction in probing pocket depth (PPD) and gain in probing attachment level (PAL) of 5814 sites were analysed, with 594 being initially diseased sites (initial PPD $\geqslant$ 5 mm).

Results: Variance Component models showed that site-level variations contributed about 70–90% of the total variance. About a 10% reduction of the total variations of PPD reduction in initially diseased sites was achieved with the inclusion of the 10 predictors in the multilevel multiple regression. Multilevel multiple regression showed that three predictors, subject level: non-smokers; tooth-level: anterior teeth; and site level: sites without plaque at baseline, were significantly associated with a greater reduction in PPD in initially diseased sites over the 12-month study period ($p < 0.05$). No consistent predictor was found for PAL gain.

Conclusion: Multilevel analysis was applied on periodontal treatment response data. Smokers showed less favourable PPD reduction at deep sites after non-surgical periodontal therapy.

Key words: models; periodontitis; smoking; statistical

Accepted for publication 21 November 2008

Smoking is considered as a well-established risk factor for periodontal diseases, a chronic infectious disorder caused by bacterial plaque characterized by destruction of tooth-supporting tissue (Page & Kornman 1997). Smokers have increased risks of experiencing periodontal attachment loss (Grossi et al. 1994, Haffajee & Socransky 2001a, Susin et al. 2004, Ng & Leung 2006), radiographic bone loss (Grossi et al. 1995, Bergström 2004, Baljoon et al. 2005) and tooth loss post-treatment (Leung et al. 2006, Matuliene et al. 2008). Smokers are found to harbour a higher number of periodontal pathogens (Haffajee & Socransky 2001b, van Winkelhoff et al. 2001).

Besides alterations of the periodontal microflora, smoking has been shown to adversely affect the host immune response in various respects, including impaired neutrophil function (Mariggio et al. 2001, Güntsch et al. 2006), lowered immunoglobulin production (Mooney et al. 2001, Apatzidou et al. 2005), reduced fibroblast function (Raulin et al. 1988), altered inflammatory mediator production (Boström et al. 1998, 1999, Giannopoulou et al., 2003) and vasoconstrictive effects of tissue exposed to cigarette smoke (Mirbod et al. 2001).

Non-surgical mechanical periodontal therapy, including oral hygiene instruction, scaling and root planing, is an effective treatment modality for periodontal disease (Van der Weijden & Timmerman 2002, Sanz & Teughels

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests. The work described in this paper was partially supported by grants from the Research Grants Council of the Hong Kong Special Administrative Region, China (HKU 7331/00M) and Merck Sharp & Dohme (Asia) Limited (26004077).

2008); however, numerous studies have indicated that smokers generally show less favourable improvements in response to non-surgical therapy (Preber & Bergström 1986, Preber et al. 1995, Renvert et al. 1998, Jin et al. 2000). A systematic review evaluating the effect of smoking on non-surgical periodontal therapy (Labriola et al. 2005) found that the mean difference in probing pocket depth (PPD) reduction with an initial probing depth of 5 mm or more would be 0.433 mm, favouring non-smokers. On the other hand, the same meta-analysis showed that there was no evidence of a difference in clinical attachment level gain between smokers and non-smokers after non-surgical periodontal therapy, although a review of clinical evidence (Heasman et al. 2006) suggests that the majority of studies do show that smokers gain less clinical attachment gain in response to periodontal therapy. It is known that achieving optimal treatment responses to non-surgical periodontal therapy in smokers is a challenging task and that the treatment outcome of the therapy may vary from patient to patient and may also vary among different teeth and tooth sites. It would be beneficial to understand factors at the patient, tooth and site level that may affect these variations in treatment response in both smokers and non-smokers.

Since the early 1990s, researchers have questioned the utility of a single-level statistical analysis of site- or tooth-level data in periodontal clinical trials because the correlation among sites and/or teeth within subjects invalidate these methods. In applying single-level statistical analysis to periodontal data, many earlier publications chose to present average sites' measurements generated on a subject level. However, such an approach may not explicitly reflect the site-specific nature of periodontal disease (Albandar & Goldstein 1992, Gilthorpe et al. 2000a, b, 2001). Application of multilevel modelling analysis, which takes the clustering effect of periodontal research data into consideration, may provide a more accurate explanation of the natural hierarchical structure of the clinical findings of periodontitis and the healing responses after periodontal therapy. Recently, two reports adopted such an approach in their periodontal trial data analysis (Tomasi et al. 2007, Matuliene et al. 2008).

In the present prospective study, the clinical healing responses of two groups of male Chinese subjects: smokers or non-smokers – matched according to age, pre-operative oral hygiene levels and periodontal disease severity – were recorded after non-surgical periodontal therapy. The aim of this study was to compare the 12-month healing response of male Chinese smokers and non-smokers with chronic periodontitis after non-surgical mechanical periodontal therapy using multilevel modelling analysis. The clinical data would be analysed at the site level. The null hypothesis of this clinical trial is that there is no difference in the healing responses after non-surgical mechanical periodontal therapy of periodontitis-affected male Chinese smokers and non-smokers.

Materials and Methods

Sample size determination

This clinical study targeted subjects with chronic periodontitis who were otherwise systemically healthy. The sample size for the study was computed as follows: in a study among the same local population, patients with chronic periodontitis showed 4.6 mm of PPD reduction at 12 months after non-surgical therapy, with a standard deviation (SD) of 1.6 mm (Tong et al. 2003). Assuming that the SD would be the same for smokers and an expected difference of PPD reduction at the initially diseased sites between smokers and non-smokers of 2 mm, 20 subjects in each group were required to allow such a difference to be detected.

Patient selection and screening

New male patients attending the Reception Clinic of the Prince Philip Dental Hospital, Faculty of Dentistry, The University of Hong Kong, and fulfilling the inclusion criteria were recruited to participate in the study. The target sample size was at least 22 subjects for each group, to allow for retention of 20 subjects in each group at 12 months. For inclusion, patients had to be free of systemic disease, not undergoing orthodontic treatment and displaying the following features:

1. Thirty-five to 64-year-old ethnic Chinese with untreated chronic periodontitis.
2. Smokers with a smoking habit of $\geqslant 10$ cigarettes per day for at least 10 years and expressing no interest in quitting smoking in the coming 12 months.
3. Non-smokers with a smoking history of never having smoked.
4. At least 16 standing teeth, with at least one tooth having PPD $\geqslant 5$ mm in each quadrant, excluding the third molars.

Subjects were excluded if the patient interview revealed:

1. known systemic diseases,
2. a history of taking systemic antibiotics in the preceding 30 days and
3. history of dental treatment, other than oral hygiene instructions, in the preceding 30 days.

The target sample size for each group was achieved 6 months after the commencement of recruitment.

Patient management and non-surgical mechanical periodontal treatment

The clinical study was carried out in the Periodontology Clinic, Prince Philip Dental Hospital, Faculty of Dentistry, The University of Hong Kong. Emergency treatment such as extraction, caries stabilization and initial endodontic therapy, if necessary, was completed before the non-surgical periodontal treatment. Six tooth sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual) of each standing tooth were included in this study. One member of the research team (W. K. L.) verified the eligibility of all subjects and ensured that all necessary pre-treatment preparations had been carried out. Receptionists of the Periodontology Clinic were then instructed to arrange the non-surgical periodontal treatment appointments (four to six visits) under local anaesthesia for all subjects to be delivered by a group of six experienced dental hygienists within an 8-week period. Both smokers and non-smokers received the same non-surgical periodontal treatment, namely oral hygiene instruction regarding brushing and inter-dental cleaning, followed by quadrant-wise debridement under local anaesthesia. Two research group members (P. W. and R. M. S. W.) at the end of the last dental hygienist treatment appointment independently assessed the quality of

the hygienists' care clinically to ensure the completeness of the non-surgical periodontal therapy.

Any residual periodontal problems at conclusion of the study at 12 months, namely any sites with residual PPD $\geqslant$ 5 mm, were followed up and appropriate periodontal treatment, e.g. re-root planing or surgical treatment was arranged and carried out without delay. Smoking subjects were again reminded of the deleterious effects resulting from their continued smoking.

Clinical examination

This was a 12-month prospective clinical study. Clinical parameters were obtained from the patients at baseline and at 3, 6 and 12 months after completion of non-surgical therapy. All clinical examinations were performed by one examiner (C. P. W.).

PPD and probing attachment level (PAL) were measured and recorded for six sites of each tooth, excluding third molars. Custom-made polyethylene occlusal stents were made for each patient as reference guides for reproducibility of probing sites and for measurement of PAL throughout the study. Except for initial baseline PAL data, which were collected using a manual periodontal probe (PCP-UNC 15, Hu-Friedy probe®, Chicago, IL, USA) (Cheng et al. 2008), each site was probed with an automated controlled-force periodontal probe, Florida Probe® (Florida Probe Corporation, Gainesville, FL, USA). Probe tips were 0.45 mm in diameter and manufactured from implant-grade titanium. The resolution of 0.2 mm could be detected with a controlled force of 15 *g*. The presence of plaque was recorded dichotomously as the presence or absence of plaque according to detection of plaque deposits determined by running the tip of a periodontal probe along the tooth surface at the gingival margin of each site. Bleeding on probing (BOP) was designated as positive if bleeding occurred within 10 s after periodontal probing using the electronic probe.

Ethics

The research protocol was approved by the Ethics Committee, Faculty of Dentistry, The University of Hong Kong. Written informed consent was obtained from all participants before the commencement of the study.

Data analysis

Routine statistical analysis

The data collected were entered into a computer and analysed using the statistical software package (SPSS). For comparing the difference in the healing response between smokers and non-smokers at the subject level, the primary efficacy measure was change in PPD and change in PAL and the secondary efficacy measures included PI%, BOP% and percentage of sites $\geqslant$5.0 mm. The significance level was set at $p < 0.0017$ for multiple comparisons at the 3-, 6- and 12-month recalls within groups or between groups. Differences between groups and between different time-points within groups were tested by the Mann–Whitney *U*-test and the Wilcoxon signed rank test, respectively.

Multilevel analysis

In order to account for the hierarchical structure of periodontal disease measurements, site measurements clustered around individual teeth and then teeth clustered within subjects, analysis using a multilevel approach was adopted in this study (Gilthorpe et al. 2000b). PPD reductions at the site level at 3, 6 and 12 months (compared with baseline PPD) were analysed using multilevel multiple regressions. A three-level random intercept regression model was constructed: site at level 1, tooth at level 2 and subject at level 3. Variance Components models (with no independent variables included) were obtained initially to investigate the variance of the PPD reductions across all the three levels. At different levels the random effects were assumed to be uncorrelated and followed normal distributions. Subsequently, 10 independent variables, with five on the subject level, two on the tooth level and three on the site level, were included in the multilevel multiple regression model. The five subject-level variables were smoking (non-smoker *versus* smoker), age (in years), number of missing teeth at baseline, percentage sites with plaque at baseline and percentage sites with BOP at baseline. The two tooth-level variables considered in the regression model were the tooth position (posterior [premolars and molars] *versus* anterior [incisors and canines]) and arch (lower *versus* upper). The three site-level variables were presence or absence of plaque at baseline, presence or absence of BOP at baseline and surface (lingual *versus* buccal). All the continuous variables were centred (subtracted from the mean) before the analysis. The analyses of the gain in PAL at 3, 6 and 12 months were performed in a similar manner: three-level regression models were considered with 10 independent variables. All the analyses were performed using the software MLwiN 2.1 (Rasbash et al. 2000). The level of significance was set at 0.05.

In order to focus on the factors affecting the change of PPD and PAL of initially diseased sites (sites with PPD $\geqslant$ 5.0 mm at baseline), the above-mentioned multilevel multiple regressions were repeated for initially diseased sites only. Again, the level of significance was set to 0.05.

Results

Routine statistical analysis

Change of PPD and PAL at all sites

In the present study, 23 non-smokers and 23 smokers were recruited. Forty of the enrolled subjects completed the study, three subjects being lost to follow-up in both the smoker and the non-smoker groups. One smoker and three non-smokers could not attend the scheduled recalls due to a contemporaneous conflict with their job time tables. Two smokers quit smoking, one for personal reasons and the other having been diagnosed to be suffering from hypertension, was successfully counselled to quit smoking by his physician.

The mean age of the smokers and non-smokers who completed the study was 46.2 ± 6.8 and 45.0 ± 5.9 years, respectively. Regarding the tobacco consumption of smokers, six were light smokers while the remaining 14 were moderate smokers (Grossi et al. 1994). Their smoking-pack-years were 20.8 ± 8.7, ranging from 10 to 30. The mean number of missing teeth (excluding third molars) was 3.9 ± 2.9 teeth for smokers and 3.7 ± 2.8 teeth for non-smokers ($p > 0.05$). Other clinical data are shown in Table 1. There was no difference between non-smokers and smokers in the percentage of plaque, mean full-mouth PPD, mean full-mouth PAL and percentage of sites with PPD $\geqslant$ 5 mm at baseline. Both groups showed poor oral hygiene and a high percentage of sites with BOP at baseline, while smokers exhibited signifi-

Table 1. Subject-level clinical parameters over study period

	Non-smokers ($n = 20$)				Smokers ($n = 20$)			
	baseline	months post-treatment			baseline	months post-treatment		
		3	6	12		3	6	12
Full-mouth plaque%	75.45 ± 14.95	40.70 ± 17.21	32.81 ± 17.21	26.55 ± 14.19	77.36 ± 10.96	35.21 ± 23.50	26.36 ± 13.61	33.79 ± 15.07
Full-mouth BOP%	73.45 ± 21.02	42.01 ± 15.53	37.95 ± 15.40	24.92 ± 10.44	**54.32 ± 13.68**	32.04 ± 11.73	***23.97 ± 9.65***	26.91 ± 10.85
Full-mouth mean PPD (mm)	2.82 ± 0.73	1.95 ± 0.42	1.82 ± 0.31	1.71 ± 0.28	2.89 ± 0.52	2.06 ± 0.37	1.99 ± 0.34	2.01 ± 0.38
Full-mouth mean PAL (mm)*	3.69 ± 0.97	–	–	–	3.71 ± 0.68	–	–	–
PPD reduction (mm)	–	0.88 ± 0.57	1.00 ± 0.55	1.11 ± 0.69	–	0.83 ± 0.28	0.91 ± 0.28	0.89 ± 0.32
PAL gain (mm)	–	0.18 ± 0.48	0.33 ± 0.54	0.50 ± 0.52	–	0.28 ± 0.18	0.26 ± 0.21	0.31 ± 0.42
% of pocket ⩾5.0 mm	11.43 ± 12.14	1.98 ± 2.13	1.15 ± 1.52	0.80 ± 0.94	9.98 ± 9.69	2.76 ± 3.01	2.52 ± 2.68	***3.37 ± 3.24***
Diseased site mean PPD (mm)	5.94 ± 0.47	3.29 ± 0.57	2.89 ± 0.39	2.49 ± 0.50	5.85 ± 0.48	3.51 ± 0.71	***3.46 ± 0.54***	3.00 ± 0.80
Diseased site mean PAL (mm)*	6.86 ± 0.88	–	–	–	6.61 ± 0.64	–	–	–
Diseased site PPD reduction (mm)	–	2.65 ± 0.66	3.05 ± 0.61	3.45 ± 0.62	–	2.33 ± 0.50	***2.38 ± 0.57***	2.84 ± 0.75
Diseased site plaque%	86.48 ± 14.67	65.24 ± 26.59	50.22 ± 26.83	42.45 ± 25.57	92.53 ± 10.24	53.23 ± 29.31	45.70 ± 23.07	58.27 ± 21.86
Diseased site BOP%	90.25 ± 15.86	65.81 ± 21.94	55.74 ± 19.73	35.86 ± 22.49	**71.89 ± 19.54**	44.92 ± 22.47	36.68 ± 20.88	42.42 ± 21.54

*Measured manually by PCP-UNC 15, Hu-Friedy probe (Cheng et al. 2008); all other measurements of PPD and PAL used Florida Probe®.
Bold Fonts: statistically significance between groups regarding data at baseline ($p < 0.05$).
Bold and italic fonts: statistically significance between groups after adjustment for multiple comparison ($p < 0.0017$).
BOP, bleeding on probing; PAL, probing attachment level; PPD, probing pocket depth.

cantly less bleeding compared with non-smokers ($p = 0.003$).

Table 1 shows the change of subject-level clinical parameters over the study period. Throughout the course of the study, both non-smokers and smokers achieved favourable improvements in their plaque control. This was demonstrated by significant reductions of Pl% at 3, 6 and 12 months compared with the baseline in both groups. By 12 months, the mean Pl% was reduced to $<34\%$.

In addition, in response to non-surgical mechanical periodontal therapy, both groups showed significant reductions in the mean full-mouth BOP% compared with the baseline. By 12 months, the mean BOP% was reduced to $<27\%$.

During the 12-month study period, the full-mouth mean PPD in both groups was found to be significantly reduced when compared with the baseline. Moreover, both groups showed PAL gains compared with the baseline. However, there was no significant difference in the mean full-mouth PPD reduction and the mean full-mouth PAL gain between non-smokers and smokers. Also, the proportion of sites with PPD $\geqslant$ 5.0 mm was significantly reduced after the non-surgical periodontal therapy in both smokers and non-smokers. However, at 12 months, smokers showed less favourable results in terms of significantly higher percentage residual pockets (PPD $\geqslant$ 5.0 mm) than non-smokers (Table 1).

Change of PPD and PAL at initially diseased sites

For the 594 sites with initial PPD $\geqslant$ 5.0 mm, the mean PPD at these initially diseased sites was 5.85 ± 0.48 mm in smokers and was 5.94 ± 0.47 mm in non-smokers. Both smokers and non-smokers showed significant reductions of PPD at 3, 6 and 12 months when compared with the baseline ($p < 0.001$) (Table 1). In smokers, the PPD at initially diseased sites reduced from 5.85 ± 0.48 mm at the baseline to 3.00 ± 0.80 mm at 12 months. In non-smokers, the corresponding PPD change was from 5.94 ± 0.47 mm at the baseline to 2.49 ± 0.50 mm at 12 months (Table 1). On comparing the two groups, non-smokers showed significantly greater PPD reduction at 6 months ($p < 0.0017$) (Fig. 1).

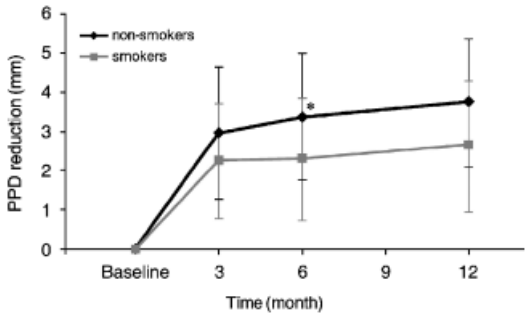

Fig. 1. Change in probing pocket depth (PPD; ± SD) of sites with PPD ⩾ 5.0 mm at baseline. *Statistically significant differences between groups after adjustment for multiple comparisons ($p < 0.001$).

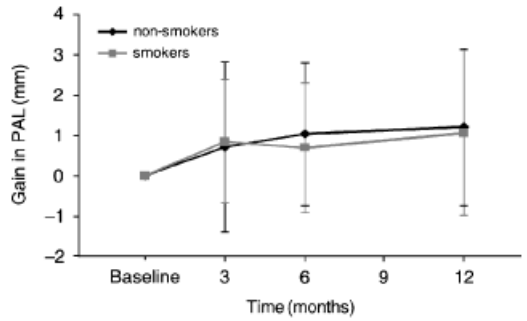

Fig. 2. Change in PAL (± SD) of sites with PPD ⩾ 5.0 mm at baseline. PAL, probing attachment level; PPD, probing pocket depth; SD, standard deviation.

The change in PAL at initially diseased sites of the two groups is shown in Fig. 2. No significant difference between smokers and non-smokers was detected at any time point.

Multilevel statistical analysis

Change of PPD at all sites

Altogether, 5814 sites distributed on 969 teeth in these 40 subjects were included for the analysis of reduction in PPD at 3, 6 and 12 months.

The overall mean reductions in PPD at 3, 6 and 12 months were 0.85, 0.95 and 1.00 mm, respectively (Table 2). The Variance Component models showed that significant variations existed at all three levels of the multilevel structure (all 95% confidence intervals did not cover the value of 0). Site-level variation contributed about 80% of the total variation in reduction in PPD at 3, 6 and 12 months.

Ten independent variables were included in the multilevel multiple regression and the random intercept models with significant variables only are shown in Table 3. The intercept in the model for the reduction in 3-month PPD was 0.62 mm. This indicates that the mean reduction in PPD at 3 months was 0.62 mm for buccal sites from lower anterior teeth with absence of plaque and BOP at baseline in smokers with a mean age of 45.58 years, with a mean 3.78 missing teeth and a mean 63.89% sites with BOP and 77.11% with plaque at baseline.

From the random intercept models for all sites, there was no statistically significant difference in PPD reduction between non-smokers and smokers throughout the study period ($p < 0.05$).

Consistently, sites on incisors and canines, on lingual aspects, sites with presence of plaque and BOP at baseline, as well as sites from subjects with higher percentages of sites with BOP showed significantly greater reduction in PPD at 3, 6 and 12 months.

The variances at each level were reduced by the inclusion of the ten variables. The total variances of the models were reduced by 7%, 8% and 9%, respectively, for reduction in PPD at 3, 6 and 12 months when compared with the corresponding Variance Components models.

Change of PAL at all sites

Again, 5814 sites distributed on 969 teeth in all the 40 subjects were included for the analyses of gain in PAL at 3, 6 and 12 months.

The overall mean gains in PAL at 3, 6 and 12 months were 0.24, 0.30 and 0.37 mm, respectively (Table 2). The Variance Component models showed that significant variations existed at all three levels of the multilevel structure (all 95% confidence intervals did not cover the value of 0), except for the tooth level at 12 months. Site-level variation contributed from 80% to 90% of the total variation in gain in PAL at 3, 6 and 12 months.

From the regression models (Table 3), it was found that there was no significant difference in the gain in PAL at 3, 6 and 12 months between smokers and non-smokers. Consistently, sites on lingual surfaces showed significantly greater gains in PAL at 3, 6 and 12 months ($p < 0.001$). Moreover, sites on anterior teeth showed a slightly greater PAL gain at 6 and 12 months ($p < 0.001$).

The variations at the three levels were reduced by 0–30% with the inclusion of the 10 variables. The total variances of the models were reduced only by 2–4% for the gain in PAL at 3, 6 and 12 months when compared with the corresponding Variance Components models.

Change in PPD at initially diseased sites

Altogether, 594 sites with initial PPD ⩾ 5 mm, distributed on 324 teeth in these 40 subjects, were included for the analyses of reduction in PPD of initially diseased sites at 3, 6 and 12 months.

The overall mean reductions in PPD of initially diseased sites at 3, 6 and 12 months were 2.55, 2.77 and 3.16 mm, respectively (Table 4). The Variance Component models showed that significant variations existed at all three levels of the multilevel structure (all 95% confidence intervals did not cover the value of 0), except for subject level at 3 months. Site-level variation contributed about 70–80% of the total variation in reduction in PPD at 3, 6 and 12 months.

Similar to the analysis for all sites, 10 independent variables were included in the multilevel multiple regressions, and the results of random intercept models are shown in Table 5. From the regression models, initially diseased sites of non-smokers consistently showed greater PPD reduction at 3, 6 and 12 months (0.41, 0.79 and 0.68 mm. respectively, $p < 0.05$).

Table 2. Variance Components models for reduction in PPD and gain in PAL for all sites

	Reduction in PPD			Gain in PAL		
	3-month	6-month	12-month	3-month	6-month	12-month
Mean (intercept)	0.85 (0.72, 0.99)	0.95 (0.82, 1.08)	1.00 (0.83, 0.16)	0.24 (0.13, 0.34)	0.30 (0.17, 0.42)	0.37 (0.23, 0.51)
Variance						
Subject (level-3)	0.18 (0.09, 0.26)	0.17 (0.09, 0.25)	0.27 (0.14, 0.39)	0.11 (0.06, 0.17)	0.14 (0.07, 0.21)	0.18 (0.10, 0.27)
Tooth (level-2)	0.15 (0.12, 0.18)	0.14 (0.10, 0.17)	0.15 (0.11, 0.19)	0.11 (0.08, 0.14)	0.13 (0.10, 016)	0.03 (0.00, 0.07)
Site (level-1)	1.15 (1.10, 1.20)	1.21 (1.16, 1.26)	1.53 (1.47, 1.59)	1.24 (1.19, 1.29)	1.22 (1.17, 1.27)	1.89 (1.82, 1.97)
Total variance	1.48	1.51	1.95	1.46	1.50	2.11
% total variance						
Subject (level-3)	12	11	14	8	10	9
Tooth (level-2)	10	9	8	7	9	1
Site (level-1)	78	80	78	85	81	90

95% confidence intervals in parentheses.
PAL, probing attachment level; PPD, probing pocket depth.

Table 3. Random intercept models for reduction in PPD and gain in PAL for all sites

Variables	Reduction in PPD			Gain in PAL		
	3-month estimate (SE)	6-month estimate (SE)	12-month estimate (SE)	3-month estimate (SE)	6-month estimate (SE)	12-month estimate (SE)
Intercept	***0.62 ± 0.10***	***0.66 ± 0.09***	***0.60 ± 0.11***	0.13 ± 0.09	0.14 ± 0.10	**0.22 ± 0.10**
Subject-level						
Smoking (non-smoker *versus* smoker)	− 0.11 ± 0.13	− 0.10 ± 0.12	− 0.01 ± 0.15	− 0.15 ± 0.12	− 0.04 ± 0.14	0.06 ± 0.14
Age at baseline	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	< 0.01 ± 0.01
Number of missing teeth	0.02 ± 0.02	0.03 ± 0.02	**0.06 ± 0.03**	0.01 ± 0.02	− 0.01 ± 0.02	< 0.01 ± 0.02
% of sites with plaque at baseline	< 0.01 ± < 0.01	< 0.01 ± < 0.01	− 0.003 ± < 0.01	< 0.01 ± < 0.01	− 0.01 ± < 0.01	− 0.01 ± 0.01
% of sites with BOP at baseline	***< 0.01 ± < 0.01***	***< 0.01 ± < 0.01***	***0.01 ± < 0.01***	< 0.01 ± < 0.01	0.01 ± < 0.01	**0.01 ± < 0.01**
Tooth-level						
Tooth position (post. *versus* ant.)	***− 0.10 ± 0.04***	***− 0.11 ± 0.04***	***− 0.14 ± 0.04***	< 0.01 ± 0.04	***− 0.13 ± 0.04***	***− 0.15 ± 0.04***
Arch (lower *versus* upper)	− 0.06 ± 0.04	− 0.04 ± 0.04	0.01 ± 0.04	− 0.04 ± 0.04	0.02 ± 0.04	0.06 ± 0.04
Site-level						
Presence of plaque at baseline	***0.21 ± 0.04***	***0.25 ± 0.04***	***0.30 ± 0.04***	0.08 ± 0.04	**0.08 ± 0.04**	0.06 ± 0.05
Presence of BOP at baseline	***0.26 ± 0.04***	***0.29 ± 0.04***	***0.32 ± 0.04***	**0.07 ± 0.04**	***0.10 ± 0.04***	0.02 ± 0.04
Surface (lingual *versus* buccal)	***0.10 ± 0.03***	***0.10 ± 0.03***	***0.09 ± 0.03***	***0.19 ± 0.03***	***0.23 ± 0.03***	***0.23 ± 0.04***
Variance						
Subject	0.11	0.10	0.14	0.10	0.13	0.13
Tooth	0.14	0.12	0.14	0.11	0.13	0.03
Site	1.12	1.17	1.49	1.22	1.20	1.87
Total variance	1.38	1.40	1.77	1.43	1.36	2.03
% reduction in variance (compared with Variance Component models in Table 2)						
Subject	34	41	46	11	9	30
Tooth	6	9	9	0	0	0
Site	2	3	3	1	2	1
Total variance	7	8	9	2	2	4

Bold fonts: $p < 0.05$; Bold and italic fonts: $p < 0.001$.
BOP, bleeding on probing; PAL, probing attachment level; PPD, probing pocket depth.

In accordance with analysis of all sites, initially diseased sites from anterior teeth were found to have undergone a significantly greater reduction in PPD at 3, 6 and 12 months ($p < 0.05$). In contrast to the results of the analysis of all sites, initially diseased sites on lingual aspects with the presence of plaque at baseline showed less PPD reduction at 3, 6 and 12 months ($p < 0.05$).

In the analysis of the initially diseased sites, the total variances of the models were reduced by only 9–13%, respectively, for reduction in PPD at 3, 6 and 12 months when compared with

Table 4. Variance Components models for reduction in PPD and gain in PAL for initially diseased sites*

	Reduction in PPD			Gain in PAL		
	3-month	6-month	12-month	3-month	6-month	12-month
Mean (intercept)	2.55 (2.35, 2.74)	2.77 (2.55, 3.00)	3.16 (2.91, 3,42)	0.80 (0.63, 0.97)	0.83 (0.62, 1.03)	1.21 (0.98, 1.44)
Variance						
Subject (level-3)	0.15 (− 0.01, 0.32)	0.24 (0.03, 0.45)	0.40 (0.11, 0.68)	0.00 (− 0.10, 0.11)	0.17 (− 0.01, 0.36)	0.16 (− 0.06, 0.37)
Tooth (level-2)	0.35 (0.07, 0.63)	0.38 (0.10, 0.67)	0.43 (0.14, 0.71)	0.83 (0.41, 1.25)	0.39 (0.07, 0.70)	0.73 (0.28, 1.18)
Site (level-1)	2.07 (1.74, 2.39)	2.03 (1.71, 2.34)	1.90 (1.60, 2.20)	2.55 (2.15, 2.96)	2.32 (1.96, 2.68)	3.03 (2.55, 3.50)
Total variance	2.57	2.65	2.73	3.38	2.88	3.91
% total variance						
Subject (level-3)	6	9	14	0	6	4
Tooth (level-2)	14	15	16	25	14	19
Site (level-1)	80	76	70	75	80	77

95% confidence intervals in parentheses.
*Baseline PPD $\geqslant$ 5.0 mm.
PAL, probing attachment level; PPD, probing pocket depth.

Table 5. Final multilevel multiple regression random intercept models for reduction in PPD and gain in PAL for initially diseased sites*

Variables	Reduction in PPD			Gain in PAL		
	3-month estimate (SE)	6-month estimate (SE)	12-month estimate (SE)	3-month estimate (SE)	6-month estimate (SE)	12-month estimate (SE)
Intercept	***3.45 ± 0.27***	***3.26 ± 0.27***	***3.65 ± 0.28***	***1.71 ± 0.31***	***1.49 ± 0.29***	***2.12 ± 0.34***
Subject-level						
Smoking (non-smoker *versus* smoker)	**0.41 ± 0.20**	***0.79 ± 0.20***	***0.68 ± 0.24***	− 0.19 ± 0.22	− 0.09 ± 0.22	− 0.13 ± 0.25
Age at baseline	0.01 ± 0.02	0.02 ± 0.02	< 0.01 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	< 0.01 ± 0.02
Number of missing teeth	< 0.01 ± 0.03	< 0.01 ± 0.03	0.06 ± 0.04	− 0.02 ± 0.03	− 0.04 ± 0.04	< 0.01 ± 0.04
% of sites with plaque at baseline	< 0.01 ± < 0.01	< 0.01 ± < 0.01	**− 0.02 ± < 0.01**	− 0.002 ± < 0.01	− 0.01 ± < 0.01	**− 0.02 ± < 0.01**
% of sites with BOP at baseline	< 0.01 ± < 0.01	< 0.01 ± < 0.01	< 0.01 ± < 0.01	< 0.01 ± < 0.01	< 0.01 ± < 0.01	< 0.01 ± < 0.01
Tooth-level						
Tooth position (post. *versus* ant.)	**− 0.35 ± 0.15**	***− 0.48 ± 0.14***	**− 0.35 ± 0.15**	− 0.23 ± 0.18	**− 0.31 ± 0.16**	− 0.36 ± 0.19
Arch (lower *versus* upper)	− 0.02 ± 0.14	− 0.02 ± 0.14	0.06 ± 0.14	− 0.25 ± 0.17	− 0.06 ± 0.15	− 0.12 ± 0.18
Site-level						
Presence of plaque at baseline	***− 0.55 ± 0.19***	**− 0.44 ± 0.19**	***− 0.45 ± 0.19***	**− 0.48 ± 0.22**	**− 0.45 ± 0.20**	− 0.25 ± 0.24
Presence of BOP at baseline	− 0.21 ± 0.20	0.10 ± 0.19	− 0.16 ± 0.20	− 0.14 ± 0.23	− 0.03 ± 0.21	− 0.41 ± 0.25
Surface (lingual *versus* buccal)	***− 0.39 ± 0.13***	***− 0.42 ± 0.13***	− 0.20 ± 0.13	− 0.07 ± 0.15	< 0.01 ± 0.14	− 0.09 ± 0.16
Variance						
Subject	0.11	0.05	0.16	0.00	0.07	0.06
Tooth	0.14	0.32	0.41	0.81	0.38	0.76
Site	1.12	1.94	1.85	2.50	2.30	2.97
Total variance	1.38	2.30	2.42	3.31	2.75	3.78
% reduction in variance (compared with Variance Component models in Table 4)						
Subject	79	80	60	100	57	64
Tooth	− 8	17	4	3	1	− 3
Site	6	4	3	2	1	2
Total variance	9	13	11	2	5	3

Bold fonts: $p < 0.05$; Bold and italic fonts: $p < 0.001$.
*Baseline PPD $\geqslant$ 5.0 mm.

the corresponding Variance Component models.

Change in PAL at initially diseased sites

The 594 initially diseased sites on 324 teeth in the 40 patients were included for the analyses of gain in PAL at 3, 6 and 12 months.

From the Variance Component models, the overall mean gains in PAL at 3, 6 and 12 months were 0.80, 0.83 and 1.21 mm, respectively (Table 4). Significant variations existed at tooth and site levels but not at the subject level of the multilevel structure (all 95% confidence intervals did not cover the value of 0) at 3, 6 and 12 months. Site-level variation contributed most of the variation in gain in PAL at 3, 6 and 12 months, ranging from 75% to 80%.

After the inclusion of the 10 variables, the total variances of the models were reduced by 2–5% for the gain in PAL at 3, 6 and 12 months when

compared with the corresponding Variance Component models.

From the regression models (Table 5), it was found that there was no significant difference between smokers and non-smokers in the gain in PAL at 3, 6 and 12 months for initially diseased sites ($p > 0.05$). Only subjects with higher percentage of sites with plaque at baseline showed slightly less PAL gain at 12 months ($p < 0.05$).

For tooth-level variables, only tooth position showed a significant effect on gain in PAL of initially diseased sites at 6 months. Sites from anterior teeth had significantly greater gain in PAL than sites on posterior teeth at 6 months ($p < 0.05$).

For the site level, it was found that only sites with absence of plaque at baseline showed greater PAL gain at 3 and 6 months ($p < 0.05$), while the effects of other variables were insignificant.

Discussion

Previous studies have generally demonstrated that smokers have an increased risk of periodontal destruction and less favourable healing in response to non-surgical periodontal therapy (Preber & Bergström 1986, Preber et al. 1995, Renvert et al. 1998, Jin et al. 2000). However, the factors affecting the variability of treatment outcomes among different smoking patients and at different sites within individual smokers are still not fully understood.

In much of periodontal research, statistical methods have been applied that generally ignore the fact that many observations are correlated, by combining all site observations into a mean value. Site-level observations are not truly independent (Hujoel et al. 1990). Sites are clustered around a tooth and teeth are clustered in individuals. It is therefore inappropriate to analyse the site- or subject-level observations using single-level, univariate statistical methods because the correlation among sites and/or teeth within an individual invalidates these statistical methods. Consequently, statistical analysis with the assumption that the site observations are independent would generate potentially misleading results (Tu et al. 2004).

Consequently, statistical analysis undertaken on the assumption that site observations are independent could generate potentially misleading interpretations of results (Tu et al. 2004).

A recent study used a multilevel approach to investigate the factors affecting the probability of ''pocket closure'' for diseased sites 3 months after two separate regimes of non-surgical periodontal therapy (Tomasi et al. 2007). However, ''pocket closure'' is not the only healing response to non-surgical therapy. Therefore, the present study aimed, using multilevel modelling analysis, to investigate the possible factors affecting the response of non-surgical periodontal therapy in male Chinese smokers and non-smokers in terms of both PPD reduction and PAL gain.

In the present study, the results generated from traditional, routine statistical analysis are also presented. It was found that smokers showed less favourable responses after non-surgical therapy. At 12 months, smokers presented with a significantly higher percentage of residual pockets (Table 1). Additionally, smokers showed less PPD reduction in sites with initial PPD $\geqslant$ 5 mm (Fig. 1). However, there was no statistically significant difference in the gain in PAL in initially diseased sites between smokers and non-smokers (Fig. 2). This is in agreement with a recent systematic review on the effect of smoking on non-surgical therapy (Labriola et al. 2005), although a review of clinical evidence suggests that the majority of studies do show that clinical attachment gain in response to periodontal therapy is impaired in smokers (Heasman et al. 2006).

In order to account for the natural hierarchical structure of periodontal disease measurements, the present study adopted multilevel multiple regressions to analyse reductions in PPD and gains in PAL compared with the baseline at 3, 6 and 12 months following non-surgical periodontal therapy. The Variance Component models of our study clearly showed that a significant variation existed at most of the levels in the hierarchical structure at all time points (Tables 2 and 4). This indicates that subject-, tooth- and site-level factors are all responsible for the outcome variations of PPD reduction and change in PAL in response to non-surgical periodontal therapy. In addition, this once more demonstrated that analysis that ignores the natural hierarchical structure of periodontal data might provide some inaccurate results. However, this is still a common data management approach in contemporary periodontal research.

The advantage of a multilevel approach can be identified in the difference between routine subject-level analysis, shown in Table 1, and the multilevel regression result, shown in Table 5. Routine univariate statistical analysis showed the difference of PPD reduction in initially diseased sites smokers and non-smokers to be significant only at 6 months ($p < 0.0017$) and marginally insignificant at 12 months ($p = 0.008$). On the other hand, the multilevel regression for initially diseased sites (Table 5) showed that sites from non-smokers achieved a significantly greater PPD reduction throughout the study period.

Tables 2 and 4 demonstrate that the site-level factors contributed around 70–80% of the total variance in healing outcomes, whereas tooth and subject levels only contributed the remaining 20–30%. This implies that most of the variations in outcomes to non-surgical periodontal therapy level result from factors acting at the site level. This is in agreement with a recent study also assessing the relative contribution of multilevel variation for the outcome of subgingival debridement (D'Aiuto et al. 2005) and with a report on both non-surgical and surgical therapy in single-rooted teeth (Kim et al. 2007), both of which found that site-level factors had a much greater impact than subject-level factors. Indeed, if tooth loss or tooth retention is the true outcome measure of significance after periodontal therapy, it is worth noting that tooth-level factors have been shown to be more important than subject-level factors in an analysis that factored in tooth- and patient-level features (Muzzi et al. 2006).

In the multilevel multiple regression models (Tables 3 and 5), 10 independent variables were included. The percentage reduction in variance compared with Variance Component models indicates the amount of variation that could be explained by the 10 independent variables introduced. For PPD reduction, the independent variables used in the present study achieved about a 10% reduction in variance at the 3-, 6- and 12-month re-examinations. Some variables such as the presence of BOP at baseline and the mean percentage of sites with BOP at the baseline only seem to influence the variance for PPD reduction in general for all sites but do not influence the PPD reduction of initially diseased sites, which mostly exhibited BOP at baseline.

Only 2–5% of variance reductions were obtained for gain in PAL in all sites and in initially diseased sites using the same 10 independent variables (Tables 3 and 5). It is rational to presume that factors affecting PPD reduction in response to non-surgical periodontal therapy are different from those influencing PAL gain. Further study involving further independent variables is warranted for investigating the factors affecting gain in PAL after non-surgical periodontal therapy.

By means of multilevel modelling analysis, besides analysing which variables significantly affect the results of non-surgical periodontal therapy, an understanding of the effects of these individual factors can be gained. In the regression model, utilizing data from 5814 sites of 969 teeth from 40 subjects for all sites (Table 3), sites on anterior teeth, sites with presence of plaque and BOP at baseline, sites on lingual aspects and sites from subjects with higher full-mouth mean BOP% consistently showed greater PPD reduction.

From Table 3, it appears that the effect of percentage of sites with BOP at baseline on PPD reduction is clinically insignificant (0.01 mm). However, if a subject's baseline BOP% were to be increased by 1%, the PPD reduction of sites in that subject would have been 0.01 mm greater. Hence, if a subject presents with 50% higher BOP% at the baseline, the PPD reduction of sites in that subject would all be 0.5 mm greater. Hence, greater reductions in PPD can be expected in those presenting with poorer plaque control, and this may be of clinical importance.

It is generally believed that deeper initial pockets show more PPD reduction. However, researchers have questioned whether the correlation of PPD reduction and baseline PPD measurement is only due to ''mathematical coupling'' (Tu et al. 2002, 2005). Because the objective of the present study was not to test the relationship between change and the initial value of PPD and PAL, but to focus on the effect of smoking on response after non-surgical periodontal therapy in terms of PPD reduction and PAL gain, independent variables such as initial PPD and PAL at baseline and full-mouth, mean PPD and PAL at baseline were not included in the analysis (Tu et al. 2004). Other multilevel analysis, strategies for investigating the relationship between change and initial values, are available to address this issue (Blance et al. 2005, Tu et al. 2005, Tu & Gilthorpe 2007).

In treating patients with chronic periodontitis, it may be important to focus attention on the response of diseased sites with periodontal pockets rather than gingivitis sites or healthy sites with no increase in PPD. In the present study, a separate set of multilevel multiple regressions was performed to investigate the effects of variables on PPD reduction and PAL gain in sites with baseline PPD $\geqslant$ 5 mm. Non-smokers showed consistently greater PPD reduction at initially diseased sites throughout the study (Table 5). The differences were 0.41, 0.79 and 0.68 mm at the 3-, 6- and 12-month recalls, respectively. These results are in agreement with a previous study demonstrating that smokers from the same population generally have less favourable PPD reduction post-treatment (Jin et al. 2000) and implies that the effect of smoking is to reduce the PPD reduction in sites with baseline PPD $\geqslant$ 5 mm by 0.41, 0.79 and 0.68 mm at 3, 6 and 12 months post-therapy, respectively. However, it is important to note that smoking status as a subject-level variable was considered in a dichotomous fashion, i.e. if the patient is a current smoker or a never smoker. Future studies could include a quantitative measurement such as pack-years and also include former smokers in investigating any dose related or residual effect of cigarette smoking on periodontal healing.

In addition, initially diseased sites from anterior teeth and diseased sites with absence of plaque at baseline were found to undergo greater PPD reduction throughout the course of the study in response to non-surgical periodontal therapy.

In the present study, we have applied the multilevel statistical analysis of the periodontal data derived from investigating treatment responses after non-surgical therapy in smokers and non-smokers. This approach has yielded new insights into and a better understanding of the result of non-surgical periodontal treatment, and has allowed a comparison of the treatment responses in Chinese male smokers and non-smokers.

Conclusion

The present study adds to the evidence that smokers generally show less favourable responses after non-surgical mechanical periodontal therapy in terms of pocket depth reduction. Use of multilevel modelling allowed determination of the impact of tooth position and site-level factors on healing responses to non-surgical periodontal therapy in both smokers and non-smokers. Most of the variations were found to be associated with site-level variables. On the basis of this study, future studies with larger sample sizes and focusing on different site-level variables are warranted.

Acknowledgements

We thank Alan Wong for assistance in data processing.

References

Albandar, J. M. & Goldstein, H. (1992) Multilevel statistical models in studies of periodontal diseases. *Journal of Periodontology* **63**, 690–695.

Apatzidou, D. A., Riggio, M. P. & Kinane, D. F. (2005) Impact of smoking on the clinical, microbiological and immunological parameters of adult patients with periodontitis. *Journal of Clinical Periodontology* **32**, 973–983.

Baljoon, M., Natto, S. & Bergström, J. (2005) Long-term effect of smoking on vertical periodontal bone loss. *Journal of Clinical Periodontology* **32**, 789–797.

Bergström, J. (2004) Influence of tobacco smoking on periodontal bone height. Long-term observations and a hypothesis. *Journal of Clinical Periodontology* **31**, 260–266.

Blance, A., Tu, Y. K. & Gilthorpe, M. S. (2005) A multilevel modelling solution to mathematical coupling. *Statistical Methods in Medical Research* **14**, 553–65.

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

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

Cheng, R. H., Leung, W. K. & Corbet, E. F. (2008) Non-surgical periodontal therapy with adjunctive chlorhexidine use in adults with down syndrome: a prospective case series. *Journal of Periodontology* **79**, 379–385.

D'Aiuto, F., Ready, D., Parkar, M. & Tonetti, M. S. (2005) Relative contribution of patient-, tooth-, and site-associated variability on the clinical outcomes of subgingival debridement. I. Probing depths. *Journal of Periodontology* **76**, 398–405.

Giannopoulou, C., Kamma, J. J. & Mombelli, A. (2003) Effect of inflammation, smoking and stress on gingival crevicular fluid

cytokine level. *Journal of Clinical Periodontology* **30**, 145–153.

Gilthorpe, M. S., Griffiths, G. S., Maddick, I. H. & Zamzuri, A. T. (2000a) The application of multilevel modelling to periodontal research data. *Community Dental Health* **17**, 227–235.

Gilthorpe, M. S., Griffiths, G. S., Maddick, I. H. & Zamzuri, A. T. (2001) An application of multilevel modelling to longitudinal periodontal research data. *Community Dental Health* **18**, 79–86.

Gilthorpe, M. S., Maddick, I. H. & Petrie, A. (2000b) Introduction to multilevel modelling in dental research. *Community Dental Health* **17**, 222–226.

Grossi, S. G., Genco, R. J., Machtei, E. E., Ho, A. W., Koch, G., Dunford, R., Zambon, J. J. & Hausmann, E. (1995) Assessment of risk for periodontal disease. II. Risk indicators for alveolar bone loss. *Journal of Periodontology* **66**, 23–29.

Grossi, S. G., Zambon, J. J., Ho, A. W., Koch, G., Dunford, R. G., Machtei, E. E., Norderyd, O. M. & Genco, R. J. (1994) Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. *Journal of Periodontology* **65**, 260–267.

Güntsch, A., Erler, M., Preshaw, P. M., Sigusch, B. W., Klinger, G. & Glockmann, E. (2006) Effect of smoking on crevicular polymorphonuclear neutrophil function in periodontally healthy subjects. *Journal of Periodontal Research* **41**, 184–188.

Haffajee, A. D. & Socransky, S. S. (2001a) Relationship of cigarette smoking to attachment level profiles. *Journal of Clinical Periodontology* **28**, 283–295.

Haffajee, A. D. & Socransky, S. S. (2001b) Relationship of cigarette smoking to the subgingival microbiota. *Journal of Clinical Periodontology* **28**, 377–388.

Heasman, L., Stacey, F., Preshaw, P. M., McCracken, G. I., Hepburn, S. & Heasman, P. A. (2006) The effect of smoking on periodontal treatment response: a review of clinical evidence. *Journal of Clinical Periodontology* **33**, 241–253.

Hujoel, P. P., Loesche, W. J. & DeRouen, T. A. (1990) Assessment of relationships between site-specific variables. *Journal of Periodontology* **61**, 368–372.

Jin, L., Wong, K. Y., Leung, W. K. & Corbet, E. F. (2000) Comparison of treatment response patterns following scaling and root planing in smokers and non-smokers with untreated adult periodontitis. *Journal of Clinical Dentistry* **11**, 35–41.

Kim, T.-S., Schenk, A., Lungeanu, D., Reitmeir, P. & Eickholz, P. (2007) Nonsurgical and surgical periodontal therapy in single-rooted teeth. *Clinical Oral Investigations* **11**, 391–399.

Labriola, A., Needleman, I. & Moles, D. R. (2005) Systematic review of the effect of smoking on non-surgical periodontal therapy. *Periodontology 2000* **37**, 124–137.

Leung, W. K., Ng, D. K. C., Jin, L. & Corbet, E. F. (2006) Tooth loss in treated periodontitis patients responsible for their supportive care arrangements. *Journal of Clinical Periodontology* **33**, 265–275.

Mariggio, M. A., Guida, L., Laforgia, A., Santacroce, R., Curci, E., Montemurro, P. & Fumarulo, R. (2001) Nicotine effects on polymorphonuclear cell apoptosis and lipopolysaccharide-induced monocyte functions. A possible role in periodontal disease? *Journal of Periodontal Research* **36**, 32–39.

Matuliene, G., Pjetursson, B. E., Salvi, G. E., Schmidlin, K., Brägger, U., Zwahlen, M. & Lang, N. P. (2008) Influence of residual pockets on progression of periodontitis and tooth loss: results after 11 years of maintenance. *Journal of Clinical Periodontology* **35**, 685–695.

Mirbod, S. M., Ahing, S. I. & Pruthi, V. K. (2001) Immunohistochemical study of vestibular gingival blood vessel density and internal circumference in smokers and non-smokers. *Journal of Periodontology* **72**, 1318–1323.

Mooney, J., Hodge, P. J. & Kinane, D. F. (2001) Humoral immune response in early-onset periodontitis: influence of smoking. *Journal of Periodontal Research* **36**, 227–232.

Muzzi, L., Nieri, M., Cattabriga, M., Rotundo, R., Cairo, F. & Pini-Prato, G. P. (2006) The potential prognostic value of some periodontal factors for tooth loss: a retrospective multilevel analysis on periodontal patients treated and maintained over 10 years. *Journal of Periodontology* **77**, 2084–2089.

Ng, S. K. & Leung, W. K. (2006) A community study on the relationship between stress, coping, affective dispositions and periodontal attachment loss. *Community Dentistry and Oral Epidemiology* **34**, 252–266.

Page, R. C. & Kornman, K. S. (1997) The pathogenesis of human periodontitis: an introduction. *Periodontology 2000* **14**, 9–11.

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

Preber, H., Linder, L. & Bergström, J. (1995) Periodontal healing and periopathogenic microflora in smokers and non-smokers. *Journal of Clinical Periodontology* **22**, 946–952.

Rasbash, J., Browne, W., Goldstein, H., Yang, M., Plewis, I., Healy, M., Woodhouse, G., Draper, D., Langford, I. & Lewis, T. (2000) *A User's Guide to MLwiN. Version 2.1. Multilevel Models Project*. London: Institute of Education, University of London.

Raulin, L. A., McPherson, J. C. III, McQuade, M. J. & Hanson, B. S. (1988) The effect of nicotine on the attachment of human fibroblasts to glass and human root surfaces in vitro. *Journal of Periodontology* **59**, 318–325.

Renvert, S., Dahlén, G. & Wikström, M. (1998) The clinical and microbiological effects of non-surgical periodontal therapy in smokers and non-smokers. *Journal of Clinical Periodontology* **25**, 153–157.

Sanz, M. & Teughels, W. (2008) Innovations in non-surgical periodontal therapy: Consensus Report of the Sixth European Workshop on Periodontology. *Journal of Clinical Periodontology* **35**, 3–7.

Susin, C., Oppermann, R. V., Haugejorden, O. & Albandar, J. M. (2004) Periodontal attachment loss attributable to cigarette smoking in an urban Brazilian population. *Journal of Clinical Periodontology* **31**, 951–958.

Tomasi, C., Leyland, A. H. & Wennström, J. L. (2007) Factors influencing the outcome of non-surgical periodontal treatment: a multilevel approach. *Journal of Clinical Periodontology* **34**, 682–690.

Tong, K. S., Zee, K.-Y., Lee, D. H. & Corbet, E. F. (2003) Clinical responses to mechanical periodontal treatment in Chinese chronic periodontitis patients with and without *Actinobacillus actinomycetemcomitans*. *Journal of Periodontology* **74**, 1582–1588.

Tu, Y.K, Baelum, V. & Gilthorpe, M. S. (2005) The problem of analysing the relationship between change and initial value in oral health research. *European Journal of Oral Sciences* **113**, 271–278.

Tu, Y. K. & Gilthorpe, M. S. (2007) Revisiting the relation between change and initial value: a review and evaluation. *Statistics in Medicine* **26**, 443–457.

Tu, Y. K., Gilthorpe, M. S. & Griffiths, G. S. (2002) Is reduction of pocket probing depth correlated with the baseline value or is it ''mathematical coupling''? *Journal of Dental Research* **81**, 722–726.

Tu, Y.K, Gilthorpe, M. S., Griffiths, G. S., Maddick, I. H., Eaton, K. A. & Johnson, N. W. (2004) The application of multilevel modeling in the analysis of longitudinal periodontal data–part II: changes in disease levels over time. *Journal of Periodontology* **75**, 137–145.

Van der Weijden, G. A. & Timmerman, M. F. (2002) A systematic review on the clinical efficacy of subgingival debridement in the treatment of chronic periodontitis. *Journal of Clinical Periodontology* **29** (Suppl 3), 55–71.

van Winkelhoff, A. J., Bosch-Tijhof, C. J., Winkel, E. G. & van der Reijden, W. A. (2001) Smoking affects the subgingival microflora in periodontitis. *Journal of Periodontology* **72**, 666–671.

Address:
W. K. Leung
Room 3B39
34 Hospital Road
Prince Philip Dental Hospital
Faculty of Dentistry
The University of Hong Kong
Hong Kong SAR
China
E-mail: ewkleung@hkucc.hku.hk

Clinical Relevance

Scientific rationale for the study: It would be useful for clinicians to be able to predict the outcomes of non-surgical mechanical periodontal therapy based on clinical data. The hierarchical structure of periodontal disease measurements, sites' measurements clustered around teeth and then teeth clustered within individuals applies to periodontal disease clinical findings and to outcomes of periodontal therapy; hence, a multilevel analysis approach is adopted in this study.

Principal Findings: About 70–90% of the total variance was found to be contributed by site-level variation. From the multilevel regression models of initially diseased site, anterior teeth without plaque at baseline in non-smokers were significantly associated with greater PPD reduction throughout the 12-month study period. Non-smokers showed 0.68 ± 0.24 mm more PPD reduction in initially diseased site at 12 months.

Practical implications: Multilevel analysis revealed that diseased sites without plaque at the baseline, from anterior teeth, in non-smokers were found to respond favourably throughout the 12 months post-treatment. Such an analysis strategy could be applied to evaluate outcomes of other periodontal treatment modalities.