Progression and Treatment of Chronic Adult Periodontitis

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Background: The periodontal status of 41 medically healthy adults with untreated chronic periodontitis was monitored before and after scaling and root planing (SRP).

Methods: During a 6-month pretreatment phase, clinical measurements, digital subtraction radiography (DSR) analysis of alveolar bone, and measurement of gingival crevicular fluid (GCF) prostaglandin E_2 (PGE_2) levels were undertaken. SRP was provided during a 1-month treatment phase. Clinical, radiographic, and biochemical analyses were repeated in a 6-month post-treatment healing period.

Results: Pretreatment: no clinically significant changes in mean plaque indices (PI), probing depths (PD), bleeding on probing (BOP), or relative clinical attachment levels (CAL) were detected ($P > 0.05$). DSR revealed small but statistically significant bone height (0.04 mm) and mass (0.97 mg) loss ($P < 0.001$). GCF PGE_2 levels gradually increased from 38.8 ng/ml at month 1 to 79.4 ng/ml at month 6. Post-treatment: statistically and clinically significant reductions were observed in mean PI, BOP, and PD ($P < 0.05$). A statistically significant reduction in CAL was noted ($P < 0.05$). The trend towards progressive bone loss was halted and reversed, and a statistically significant decrease in GCF PGE_2 concentrations was detected ($P < 0.001$). Smokers, non-smokers, and ex-smokers did not differ significantly in PI, BOP, CAL, radiographic, or biochemical parameters at any time. Mean PD was significantly greater in current smokers than in non- and ex-smokers ($P < 0.005$). PD reduced comparably in all 3 smoking subgroups following treatment ($P < 0.01$).

Conclusions: Conventional clinical measurements failed to identify disease progression over a 6-month period. Significant improvements were observed in clinical parameters after SRP, and a trend towards progressive bone loss was halted and reversed. Regular and frequent maintenance visits are important following treatment to maintain improvements in clinical parameters. Smokers had deeper probing depths than non- and ex-smokers, but pockets were reduced significantly and comparably in all 3 smoking subgroups following efficacious treatment. *J Periodontol 1999;70:1209-1220.*

KEY WORDS

Disease progression; follow-up studies; periodontal diseases/therapy; planing; scaling; wound healing.

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Periodontitis is one of the most common chronic diseases of adults. In the United Kingdom, 69% of dentate adults have early signs of the disease in at least one site, and only 5% are completely free of any of the clinical signs of chronic adult periodontal disease (CAPD).[1] In the United States, over 90% of dentate individuals aged 13 years or older have experienced some clinical loss of attachment (LOA), although only 15% demonstrate more severe attachment loss (≥5 mm).[2]

Clinical measurements and radiographs are used to determine both disease status and the response of the tissues to treatment. Routine clinical techniques are relatively insensitive and non-specific in determining disease progression over short periods of time. The precise pattern of periodontal disease progression, therefore, remains elusive. Early studies demonstrated a continuous, linear progression,[3,4] although this was supplanted in the mid-1980s by the random burst model.[5] More recently, the use of increasingly sensitive and accurate periodontal probes has shown that the prevalence of disease activity is dependent on the threshold used for identifying whether LOA has occurred.[6]

Conventional radiographs are also relatively insensitive for the determination of alveolar bone changes over short periods of time. CAPD progresses slowly, and bone destruction over months rather than years is

unlikely to be detected using routine radiographic techniques. Digital subtraction radiography (DSR) offers definite advantages over conventional radiography. It can be used to assess sensitively very small bone changes, both qualitatively and quantitatively. The superiority of DSR over conventional radiography in the detection of small osseous changes has been shown in vitro,[7-11] in animal experiments,[12] and in human clinical trials.[13,14] For example, in a study designed to compare the ability of examiners to detect bone lesions when using either conventional radiography or DSR, lesions 0.49 mm deep could be identified using DSR with near perfect accuracy, whereas a similar degree of accuracy was not obtained with conventional techniques until lesions were approximately 3 times as deep.[8]

Connective tissue LOA arises from a combination of direct injury sustained from bacterial products and activation of local immune-inflammatory mechanisms that lead to the release of inflammatory mediators. These biochemical mediators, including products of the cyclooxygenase pathway of arachidonic acid metabolism, appear to be of fundamental importance in determining disease progression. For example, prostaglandin E_2 (PGE_2) has been implicated as a key inflammatory mediator in periodontal disease[15-21] and causes decreased collagen synthesis by fibroblasts[22] and stimulates osteoclastic bone resorption.[23] Gingival crevicular fluid (GCF) PGE_2 levels have been shown to correlate with the clinical expression and rate of progression of periodontitis,[18,24] and GCF sampling provides a non-invasive and sensitive means of monitoring soft tissue levels of PGE_2 at specific periodontally involved sites.

The response of the periodontal tissues to non-surgical periodontal treatment has been documented in many clinical studies.[25-27] Clinically, effective treatment results in resolution of inflammation, reduction of probing depths, and gain of clinical attachment. The precise nature of changes in GCF PGE_2 levels and radiographic bone status occurring within a short period following treatment is less clearly documented, however.

The aim of this 13-month, prospective, longitudinal study was to monitor the short-term progression of untreated CAPD and the response of the periodontal tissues to non-surgical treatment (scaling and root planing, SRP). Clinical measurements, DSR analysis of alveolar bone status, and monitoring of GCF PGE_2 levels were undertaken to investigate disease progression and tissue response.

MATERIALS AND METHODS

This was a 13-month longitudinal study for which ethical approval was obtained from the Joint Ethics Committee of the University of Newcastle upon Tyne and the Newcastle and North Tyneside Health Authorities. The study was conducted under the European Community guidelines of good clinical practice (GCP),[28] and written informed consent was obtained from all subjects prior to participation.

Study Cohort

Male and female patients, aged 30 years or older, in good general health, with untreated, moderately advanced CAPD were recruited. Subjects were designated as either current smokers, non-smokers (never smoked) or ex-smokers (stopped smoking at least 2 years prior to study commencement). Each patient had a minimum of 16 natural teeth, with 8 or more periodontally involved clinical sites (test sites) in the posterior dentition but not on teeth serving as abutments to fixed or removable dental prostheses. All test sites exhibited bleeding on probing (BOP), radiographic alveolar bone loss, clinical LOA, and 5 to 8 mm probing depths (PD). Subjects were excluded if there was evidence of: 1) pregnancy; 2) exposure to topically or systemically administered non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics, or steroids within 30 days prior to screening, or any other drugs that could affect study completion or safety; 3) presence of any systemic illness or condition which could influence the response of the periodontal tissues to the accumulation of dental plaque (for example, insulin-dependent diabetes mellitus); 4) presence of any serious or debilitating oral conditions (for example, extensive, non-restored caries, oral candidiasis, chronic auto-immune disorders); 5) orthodontic therapy within the previous year; 6) previous periodontal surgery; 7) subgingival periodontal instrumentation within the previous year; 8) supragingival scaling or prophylaxis in the last month; or 9) any condition requiring the use of prophylactic antibiotics prior to invasive dental treatment.

Study Design

The study comprised pretreatment monitoring (6 months), treatment (1 month), and post-treatment evaluation (6 months). Eight test sites were designated per subject. All test sites were interproximal sites in the posterior dentition, not including pockets around third molars. Sites at the distal aspect of second molars were eligible only if there was an adjacent fully erupted third molar. The clinical parameters, measured at months 0 (baseline), 3, 6, 10, and 13, were plaque indices (PI), PD, BOP, and relative clinical attachment levels (CAL). GCF samples for PGE_2 analysis were taken at monthly intervals throughout the study and were always collected prior to periodontal probing. Standardized vertical bite-wing radiographs were exposed at months 0, 3, 6, and 13. Radiographs were also exposed randomly at one of the visits during the post-treatment period (between

months 7 and 12, inclusive). During the interval between months 6 and 7 (the treatment period), all participants received an intensive course of non-surgical therapy (SRP). Treatment was completed over a maximum of 4 visits within this period, each appointment lasting approximately 60 minutes. Oral hygiene instruction was prescribed according to individual needs, and SRP was undertaken using a combination of hand and ultrasonic instruments for approximately 6 minutes per tooth. At completion of the study (month 13), a full-mouth prophylaxis was provided and further interventional therapy was provided where indicated.

Clinical and Laboratory Methods

The presence of plaque was recorded at 6 sites per tooth using the Silness and Löe plaque index.[29] A constant-force periodontal probe‖ was used to measure probing depths with a 20g probing force.[30] Immediately following probing, each site was observed for approximately 10 seconds to determine whether bleeding occurred. An automated probe¶ was used to monitor changes in clinical attachment levels at test sites, which were measured from a fixed reference point (the occlusal surfaces of the teeth) to the probe tip, assumed to be at the base of the pocket. The probe was set at 20g probing force with a resolution of 0.1 mm. A first pass of all sites was performed, then a second reading obtained from each site (double pass technique). The median of these 2 scores was the recorded measurement. If these varied by more than 1.0 mm, a third reading was taken[31] and the median of the 3 scores was used for analysis.[32]

Radiographic Examination

Standardized vertical bite-wing radiographs were exposed at test sites using a cephalostat.#[7] A double-packed, ekta speed film** in a vertical bite-wing holder†† was placed in the mouth. An aluminum reference wedge held the film in place and also served as a bite block. Films were exposed for 1 second at 15 mA and 90 kVp, then mounted, labeled and analyzed by digital subtraction analysis.[33] Changes in bone height and bone mass relative to baseline were recorded. The threshold for detection of bone height change at any one site was 0.08 mm.[34]

PGE_2 Analysis

At test sites, GCF was sampled with filter strips[18] and quantified.[35‡‡] Filter papers were then wrapped in sterile aluminum foil and placed into labeled cryovials, which were immersed in liquid nitrogen prior to storage in a –70°C freezer. A commercially available competitive enzyme immunoassay (EIA) kit§§ was used to determine GCF PGE_2 levels. Immediately prior to assay, the stored filter papers were removed from the freezer, unwrapped, and each paper was placed into an individual, uniquely labeled 0.5 ml microcentrifuge tube. Assay buffer (containing aprotinin) was pipetted onto each filter paper strip. The tubes were allowed to stand at room temperature for 30 minutes, with vortexing every 5 minutes to facilitate extraction of the sample from the paper. Aliquots of the extracted sample were then used in the assay, which was performed immediately after extraction. Quantification of PGE_2 was achieved by comparison with a standard curve generated from known amounts of PGE_2 which had gone through the assay procedure. All assay runs included extraction efficiency and quality control standards. Mouth median GCF PGE_2 levels were calculated as the summary statistic for the individual patient.

Study Safety

The health of the oral soft tissues was assessed at each appointment, and the periodontal status of both test and non-test sites was evaluated on a regular basis. In the event that either relative CAL, PD, or alveolar bone loss (as determined by sequential radiographs) increased by more than 2.0 mm from baseline, the affected site was exited from the study and treated immediately.

Each subject had up to 4 vertical bite-wing radiographs exposed at each radiographic examination. Subjects had 5 radiographic examinations in total (months 0, 3, 6, random month 7 through 12, and 13). The majority of subjects required only 2 radiographs to be exposed at each examination, and all radiographs were taken using ekta-speed film and a collimated beam. Copies of radiographs were sent to the subjects' general dental practitioners to prevent unnecessary duplication of radiographic exposures.

Statistical Analyses

The subject was the unit of statistical analysis unless otherwise specified. The full-mouth assessments were designed to provide an indication of the general level of inflammation and show the impact of therapy. The full-mouth variables were based on measurements taken from each evaluative site in the subject's mouth. The test site variables were based on measurements taken from each evaluative test site. Summary statistics were calculated, and pairwise analyses (one sample paired *t* tests) were conducted to identify statistically significant differences between data recorded at successive time points. Analysis of variance (ANOVA) was used to compare data from the smoking subgroups across indi-

‖ True Pressure Sensitive probe, Ivoclar-Vivadent Ltd., Meridian South, Leicester, UK.
¶ Florida disk probe, Florida Probe Corporation, Gainesville, FL.
# Gendex GX-Ceph, Gendex Corp., Milwaukee, WI.
** Kodak Ektaspeed, Eastman Kodak Co., Rochester, NY.
†† Rinn vertical bite-wing holder, Rinn Corp., Elgin, IL.
‡‡ Periotron 6000, ProFlow Inc., Amityville, NY.
§§ Assay Designs Correlate EIA, Assay Designs Inc., Ann Arbor, MI.

vidual time points, and, when appropriate (P <0.05), independent samples t tests were used to identify statistically significant differences between these subgroups.

RESULTS

Forty-one patients with untreated CAPD completed baseline (27 female, 14 male). One participant was Asian and all others were Caucasian. The mean age at baseline was 43 years (range 30 to 64 years). Six subjects withdrew from the study subsequent to completing baseline: 1 subject moved; 2 were unable to attend appointments; and 3 were lost to follow up. Data collected from these subjects up to the date of withdrawal were considered valid and were used in statistical analyses. Of the 41 participants, 12 were non-smokers, 14 were ex-smokers, and 15 were current smokers (smoking an average of 16 cigarettes per day, range 3 to 40 cigarettes per day). In view of the demographics of non-, ex-, and current smokers, a retrospective decision was made to additionally analyze data from the smoking subgroups separately, although this was not part of the original study protocol or design. Smoking status was not a consideration during subject enrollment.

Plaque Score

Mean plaque scores during the pretreatment phase did not differ significantly (month 0 = 1.27, month 3 = 1.32, month 6 = 1.28; P >0.05) (Fig. 1). Mean plaque scores during the post-treatment phase were lower than those in the pretreatment phase (month 7 = 0.56, month 10 = 0.57, month 13 = 0.50). Mean PI at months 7, 10, and 13 were all statistically significantly lower than at month 6: between months 7 and 6, Δ = –0.71, 95% CI = –0.83, –0.59; P = 0.0001; between months 10 and 6, Δ = –0.72, 95% CI = –0.84, –0.60; P = 0.0001; and between months 13 and 6, Δ = –0.76, 95% CI = –0.90, –0.62; P = 0.0001.

Probing Depth

During the pretreatment phase, the full-mouth mean PD at month 6 (3.96 mm) was lower than that at month 0 (4.07 mm), although this difference was not statistically significant (Δ = –0.08 mm, 95% CI = –0.17 mm, 0.02 mm; P = 0.127) (Fig. 2). A highly significant reduction in mean full-mouth PD was observed from month 6 to month 13 (3.24 mm) (Δ = –0.68 mm, 95% CI = –0.79 mm, –0.57 mm, P = 0.0001).

The mean test site PD measurements at months 0, 3, and 6 were 5.73 mm, 5.65 mm, and 5.51 mm, respectively (Fig. 3). The difference between months 3 and 0 was not statistically significant (Δ = –0.08 mm, 95% CI = –0.22 mm, 0.06 mm; P = 0.266), whereas the difference between months 6 and 0 was statistically significant (Δ = –0.20 mm, 95% CI = –0.39 mm, –0.01 mm; P = 0.037). The means at months 10 and 13 were 4.33 mm and 4.17 mm, respectively. The difference between months 10 and 6 was statistically significant (Δ = –1.12 mm, 95% CI = –1.33 mm, –0.92 mm;

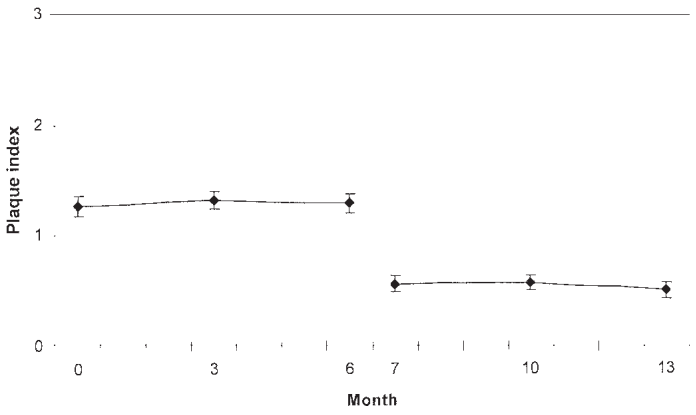

Figure 1.
Full-mouth mean plaque indices (± SEM).

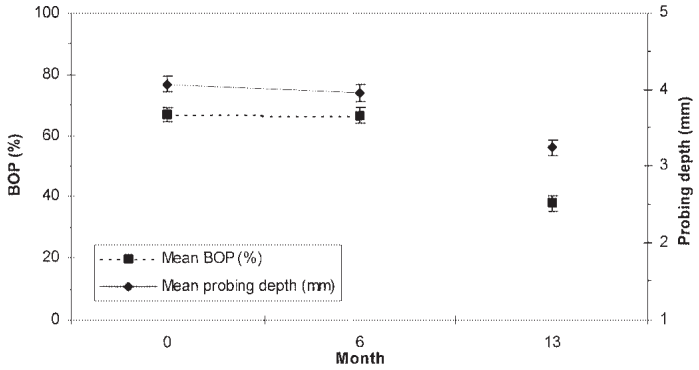

Figure 2.
Full-mouth mean bleeding on probing (BOP) scores and probing depths (± SEM).

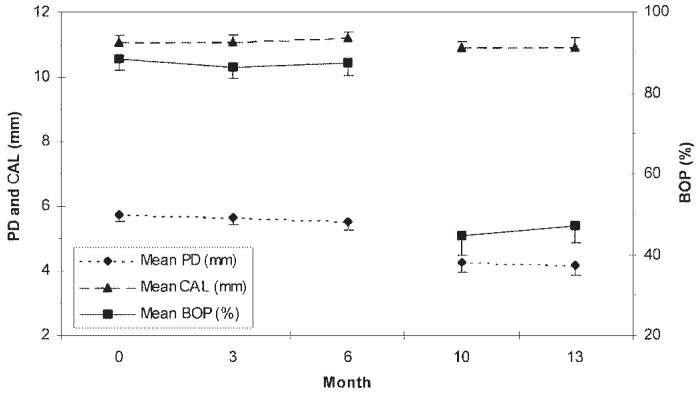

Figure 3.
Test site mean probing depths (PD), bleeding on probing (BOP), and relative clinical attachment levels (CAL) (± SEM).

$P = 0.0001$), as was the difference between months 13 and 6 ($\Delta = -1.30$ mm, 95% CI = –1.55 mm, –1.05 mm; $P = 0.0001$).

Bleeding on Probing

No statistically significant difference in full-mouth mean BOP scores was detected between months 0 (67%) and 6 (67%) ($\Delta = 0\%$, 95% CI = –3%, 3%; $P = 0.966$) (Fig. 2). The mean was lower at month 13 (38%) compared to month 6, and this difference was statistically significant ($\Delta = -27\%$, 95% CI = –32%, –22%; $P = 0.0001$).

Mean test site BOP scores at months 0, 3, and 6 were 88%, 87%, and 88%, respectively (Fig. 3). No statistically significant differences in test site BOP were observed in the pretreatment phase ($P = 0.762$). During the post-treatment period, a highly significant reduction in mean test site BOP was observed ($P < 0.001$). The means at months 10 and 13 were 45% and 47%, respectively. The difference between months 10 and 6 was statistically significant ($\Delta = -42\%$, 95% CI = –54%, –30%; $P = 0.0001$), as was the difference between months 13 and 6 ($\Delta = -39\%$, 95% CI = –51%, –27%; $P = 0.0001$).

Relative Clinical Attachment Level

The mean test site CAL measurements at months 0, 3, and 6 were 11.09, 11.09, and 11.17 mm, respectively (Fig. 3). No statistically significant changes in CAL were observed in the pretreatment phase ($P = 0.116$). The means at months 10 and 13 were 10.90 mm and 10.92 mm, respectively. The difference between months 10 and 6 was statistically significant ($\Delta = -0.23$ mm, 95% CI = –0.38 mm, –0.08 mm; $P = 0.004$), as was the difference between months 13 and 6 ($\Delta = -0.19$ mm, 95% CI = –0.37 mm, –0.02 mm; $P = 0.033$). Thus, there was a small, but statistically significant reduction in CAL as a result of treatment. Site-specific analysis of CAL changes occurring from month 0 to 13 revealed that they appeared to follow a normal distribution. Approximately 21% of sites remained unchanged, 37% demonstrated evidence of attachment loss, and 42% showed attachment gain.

Radiographic Analysis

Statistically significant changes in mean test site alveolar bone height and mass relative to baseline were detected. Mean reductions in bone height from baseline occurred during the pretreatment phase. The difference between months 3 and 0 was statistically significant ($\Delta = -0.02$ mm, 95% CI = –0.03 mm, –0.01 mm; $P = 0.0001$), as was the difference between months 6 and 0 ($\Delta = -0.04$ mm, 95% CI = –0.05 mm, –0.03 mm; $P = 0.0001$). Bone mass loss was also detected at months 3 and 6 relative to month 0. The difference between months 3 and 0 was statistically significant ($\Delta = -0.51$ mg, 95% CI = –0.73 mg, –0.30 mg; $P = 0.0001$), as was the difference between months 6 and 0 ($\Delta = -0.97$ mg, 95% CI = –1.27 mg, –0.67 mg; $P = 0.0001$).

Following treatment, statistically significant bone height and mass gain occurred when alveolar bone status at month 13 was compared with month 6. From month 6 to month 13, significant bone height gain of 0.03 mm (95% CI = 0.00 mm, 0.06 mm; $P = 0.028$) and bone mass gain of 0.89 mg (95% CI = 0.44 mg, 1.52 mg; $P = 0.001$) were detected. Thus, at month 13 there was a mean reduction in bone height compared to baseline of 0.01 mm, and a mean reduction in bone mass of 0.08 mg.

With regards to the random radiographic examination undertaken at one of the post-treatment appointments (months 7 through 12, inclusive), the month 8 radiographic examination was chosen retrospectively for further analysis as this visit occurred 1 month following completion of treatment. It was considered desirable to obtain information relating to any bone changes occurring immediately after SRP. Seven study subjects were randomly selected to generate the subgroup who participated in the month 8 examination. In the 1 month interval following completion of SRP, significant bone height gain of 0.032 mm ($P = 0.015$) and bone mass gain of 0.96 mg ($P = 0.007$) were observed relative to month 6.

Site-specific analyses revealed that of all radiographic sites studied, only 29.9% showed evidence of bone change in the pretreatment period between months 0 and 6. At one site in one patient, a bone gain was measured (+0.08 mm, +2.36 mg). All other sites with bone changes from baseline demonstrated a loss (height loss range: –0.08 to –0.41 mm, mass loss range: –1.01 to –9.63 mg). Overall, the mean (±SD) bone changes at sites which lost bone were –0.137 mm (±0.065 mm) and –3.202 mg (±2.107 mg) over the pretreatment 6 months. Retrospective additional analyses showed that over the entire duration of the study, 30.9% of sites showed evidence of bone change at month 13 relative to baseline. The pattern of bone change over the whole study appeared to follow a normal distribution, with approximately equal numbers of sites gaining or losing bone.

To investigate the relationship between attachment levels and alveolar bone status, site-specific bone height and mass changes and relative CAL changes occurring in the pretreatment period were calculated (data not shown). There was no correlation between the parameters, and this was confirmed when the product moment correlation coefficient was applied to the data (bone height change versus CAL change, $r = 0.03$, $P = 0.586$; bone mass change versus CAL change, $r = -0.01$, $P = 0.889$). Some sites demonstrated a gain in relative CAL and a simultaneous loss of bone height/mass. The majority of sites, which

showed no change in bone height/mass, exhibited either gains or losses in relative CAL. However, bone height and mass changes occurring during the pretreatment period were well correlated, (r = 0.94, P<0.001).

A similar analysis was undertaken of site-specific bone height and mass changes and relative CAL changes occurring over the entire 13-month study period (data not shown). Again, there were no correlations between CAL changes and bone changes (bone height change versus CAL change, r = 0.06, P = 0.332; bone mass change versus CAL change, r = 0.10, P = 0.116). Bone height change and bone mass change occurring over the entire study correlated well, however (r = 0.93, P<0.001).

Biochemical Observations

Due to a technical failure, some of the samples from month 0 were irretrievably lost, and therefore, biochemical data from month 0 were not included in the analysis. In the pretreatment phase, a gradual increase in GCF PGE_2 concentrations was observed from month 1 (38.8 ng/ml) to month 6 (79.4 ng/ml) (P = 0.0001) (Table 1). Resultant pairwise comparisons suggested that at the 0.05 significance level, PGE_2 concentrations at months 1, 2, and 3 were significantly lower than those at months 4, 5, and 6. The highest GCF PGE_2 concentration was recorded at month 6, immediately prior to treatment. A statistically significant decrease in PGE_2 levels was detected from month 6 to 7 (46.5 ng/ml) (Δ = –32.9 ng/ml, 95% CI = –50.81 ng/ml, –15.05 ng/ml; P = 0.0009). GCF PGE_2 levels recorded throughout the remainder of the post-treatment phase (months 8 through 13) were all significantly lower than those recorded at month 6 (P<0.03).

The Effect of Smoking

No statistically significant differences in full-mouth mean PI or BOP were noted between non-, ex-, and current smokers at any time point in the study (ANOVA, P>0.289) (Fig. 4). However, statistically significant differences were observed in mean full-mouth PD between the smoking subgroups at months 0, 6, and 13 (ANOVA, P<0.001) (Fig. 4). At month 0, mean PD in current smokers (4.55 mm) was significantly deeper than those in both non- (3.72 mm) and ex-smokers (3.85 mm) (P <0.005). No significant differences were observed at any time between mean PD in non- and ex-smokers (P >0.263). All smoking subgroups demonstrated significant reductions in probing depths from month 6 to 13: non-smokers (Δ = 0.55 mm, 95% CI = 0.34 mm, 0.75 mm; P = 0.0001); ex-smokers (Δ = 0.67 mm, 95% CI = 0.48 mm, 0.87 mm; P = 0.0001); current smokers (Δ = 0.78 mm, 95% CI = 0.58 mm, 0.99 mm; P = 0.0001).

Table 1.

Means of Mouth Median Crevicular Fluid PGE_2 Concentrations

Month	GCF [PGE_2] (ng/ml) Mean ± SEM (range)
1	38.81 ± 3.55 (15.10-75.75)
2	35.29 ± 4.23 (8.35-85.95)
3	42.20 ± 3.59 (8.05-101.25)
4	63.70 ± 7.51 (11.45-199.50)
5	63.12 ± 9.94 (6.50-301.40)
6	79.36 ± 6.54 (15.95-181.00)
7	46.54 ± 4.99 (17.30-126.70)
8	43.26 ± 3.91 (12.05-132.90)
9	65.32 ± 3.77 (28.70-134.20)
10	52.25 ± 3.08 (9.30-94.45)
11	45.50 ± 3.17 (20.25-93.60)
12	44.73 ± 2.82 (17.90-85.65)
13	44.94 ± 2.86 (18.65-82.15)

No statistically significant differences in mean test site PD were noted between non-, ex-, and current smokers at months 0 and 3 (ANOVA, P >0.481) (means at month 0: non-smokers = 5.69 mm; ex-smokers = 5.72 mm; current smokers = 5.77 mm). However, at months 6, 10, and 13, mean test site probing depths in smokers were significantly deeper than those in ex-smokers (P <0.030) (Fig. 5). At month 6, the mean PD in current smokers (5.92 mm) was also significantly deeper than that in non-smokers (5.30 mm) and also ex-smokers (5.30 mm) (P = 0.048). No statistically significant differences between current and non-smokers were observed at months 10 or 13 (P >0.065). No significant differences were observed at any time point between mean test site PD in non- and ex-smokers (P >0.225). All smoking subgroups demonstrated statistically and clinically significant reductions in test site PD from month 6 to 13: non-smokers (Δ = 1.10 mm, 95% CI = 0.45 mm, 1.76 mm; P = 0.004); ex-smokers (Δ = 1.50 mm, 95% CI = 1.07 mm, 1.94 mm; P = 0.0001); and current smokers (Δ = 1.27 mm, 95% CI = 0.89 mm, 1.65 mm; P = 0.0001). Mean test site PD at month 13 was 4.14 mm in non-smokers, 3.68 mm in ex-smokers, and 4.64 mm in current smokers.

No significant differences in mean test site BOP scores were recorded at any time point in the smoking subgroups (ANOVA, P >0.094). Similarly, at test

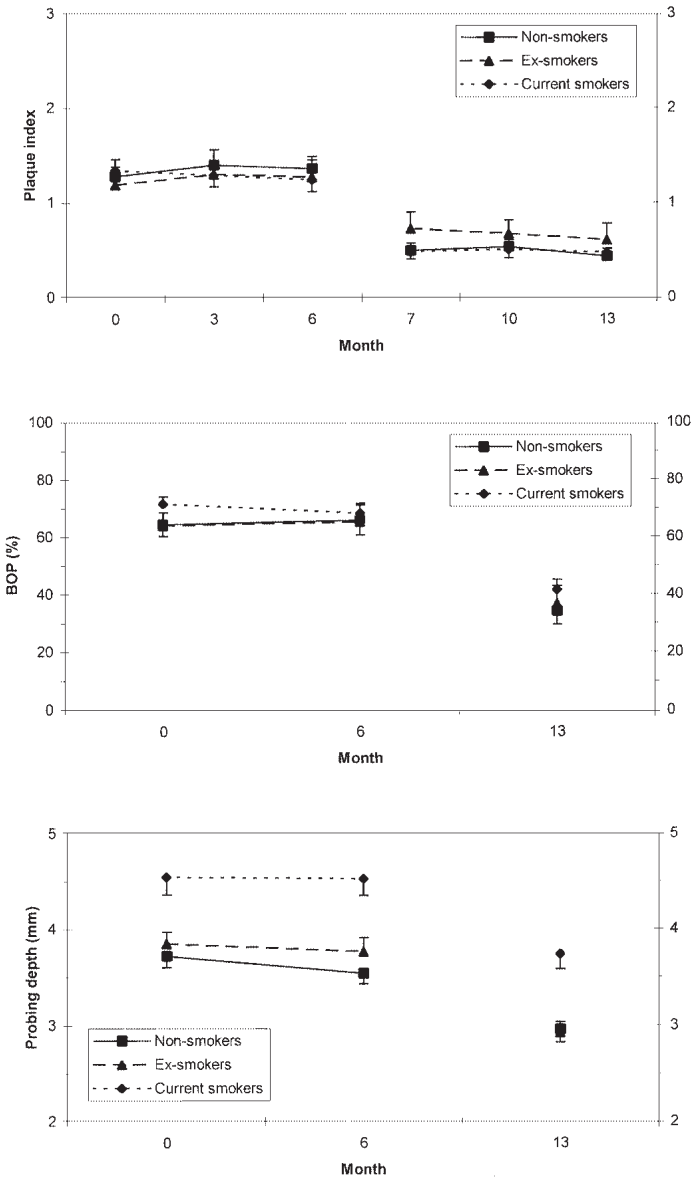

Figure 4.
Full-mouth mean plaque indices, bleeding on probing (BOP), and probing depths (± SEM) according to smoking status.

sites, there were no significant differences between mean relative CAL in non-, ex-, and current smokers (ANOVA, $P > 0.221$). Analysis of alveolar bone height and mass changes from baseline revealed that the smoking subgroups did not differ significantly from each other (ANOVA, $P > 0.185$), and furthermore, there were no significant differences between means of mouth median GCF PGE_2 concentrations recorded at any time point in the 3 smoking subgroups (ANOVA, $P > 0.05$).

DISCUSSION

The aim of this longitudinal study was to gain additional information regarding changes in clinical variables, radiographic alveolar bone status, and GCF PGE_2 levels in

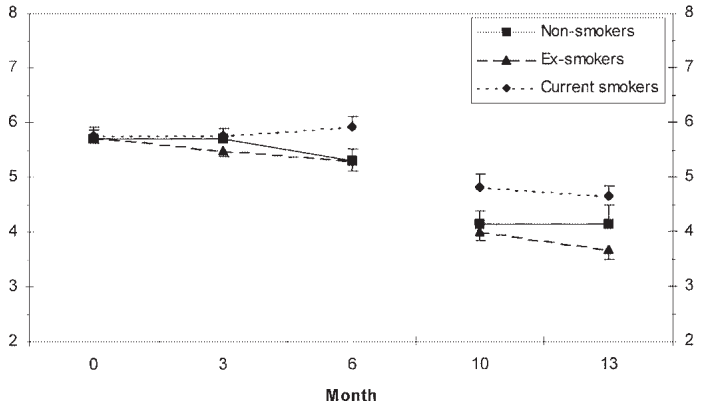

Figure 5.
Test site mean probing depths (± SEM): the effect of smoking.

patients with untreated CAPD prior to and following non-surgical treatment. The withdrawal rate of 15% was considered acceptable, with 35 of the 41 patients who completed baseline reaching month 13.

In the pretreatment phase, no significant changes were observed in whole-mouth PI, PD, or BOP, an expected finding, as no periodontal therapy was provided during this time. Similarly, at test sites, there were no significant changes in mean BOP or CAL. A statistically significant reduction in mean test site PD was observed from month 0 to 6 ($P = 0.037$), although this change was very small (0.20 mm) and not considered clinically significant. Thus, in the pretreatment period, there was no clear evidence of improvement in clinical status that might have been associated simply with participation in a clinical trial.[36] However, statistically and clinically significant changes in all clinical parameters (with the exception of CAL, which achieved statistical significance only) were detected following treatment. Mean plaque scores were significantly lower after treatment than before treatment. The improved standard of oral hygiene in the post-treatment period is attributable to the high frequency (monthly) of recall and maintenance appointments in the latter 6 months of the study. This finding lends support to the concept that regular, frequent, and high-quality supportive periodontal care is essential to promote healing and to minimize recolonization of the periodontal tissues by plaque bacteria during the post-treatment period.[37] Further evidence for resolution of inflammation following treatment is provided by the decreases in mean whole-mouth and test site BOP scores.

Mean full-mouth PD decreased significantly from 3.96 mm at month 6 to 3.24 mm at month 13 ($P = 0.0001$). Over the same time frame, test site PD was reduced significantly from 5.51 mm at month 6 to 4.17 mm at month 13 ($P = 0.0001$). These findings are comparable to those previously reported in similar

studies investigating the response of the periodontal tissues to non-surgical treatment.[25,26] In our study, there was a clinically significant reduction in PD but not in relative CAL as a result of treatment, thus confirming that gingival shrinkage is the most important factor in probing depth reduction after non-surgical therapy.[27,38] When considering individual sites, there was evidence of change in relative CAL. In the pretreatment period, the mean site-specific CAL change from month 0 to 6 was –0.11 mm (95% CI = –0.03, 0.24 mm, $P = 0.116$). That is, an overall loss of attachment of approximately 0.1 mm (equating to 0.2 mm per year) occurred in the pretreatment phase.

Small, but statistically significant, loss of mean alveolar bone height and mass was detected in the pretreatment period as determined by sensitive DSR. The observed bone height loss of 0.04 mm per 6 months is equivalent to 0.08 mm per year. Following treatment, small but statistically significant bone height and mass gain occurred as early as month 8 and this bone gain was maintained until month 13. Thus, progressive bone destruction detected in the pretreatment phase was halted and reversed by non-surgical treatment. The dynamic processes of bone metabolism may favor osteoblastic bone deposition within a very short period following treatment. Caution must be employed when interpreting these data, however, as only a small subset of the trial population participated in the month 8 radiographic examination. Furthermore, while achieving statistical significance, these changes cannot be considered to be clinically significant in individual patients over this short time. A clinically (as opposed to statistically) significant change can be defined as one which can easily be identified in an individual patient by a dentist using routine chairside diagnostic techniques.[39]

The mean bone height change in the pretreatment period (equivalent to 0.08 mm per year) is less than that reported in previous studies of untreated periodontitis patients, which has ranged from 0.16 mm per year[40] to 0.63 mm in 6 months.[41] These contrasting rates of bone loss are probably due to the different study populations and, for example, differing ethnic backgrounds, periodontal microflora, smoking status, and systemic disease. Such potential variables make direct comparisons between clinical studies difficult, and it is thus essential that the trial population is precisely defined in longitudinal studies of disease progression. Additional site-specific analyses of alveolar bone changes in the pretreatment phase revealed that 30% of sites showed evidence of bone destruction, with approximately two-thirds of sites remaining unchanged. This lends support to both the random burst model of periodontal disease progression[5] and to data which confirm that bone loss, as well as attachment loss, proceeds in an episodic manner.[42]

Alveolar bone loss has been previously advocated as a gold standard for identifying periodontal disease progression, together with clinical LOA.[43] Clearly, the magnitude of mean bone change in this study (0.04 mm bone height loss in 6 months) was very small, and if clinical CAL changes do reflect underlying bone changes, then a change of this size would be well below the threshold of detection of even the most sensitive clinical probe. The lack of correlation between CAL changes and bone height changes is likely due to the different sensitivities and specificities of the two measuring systems. DSR is very sensitive and accurate in quantifying small bone changes over time.[44] The automated probe system used in this study is less sensitive and is subject to manual errors which may lead to erroneous assessment of true attachment changes irrespective of the double pass technique. A poor correlation between CAL and radiographic bone changes has also been reported in a study of 79 patients with periodontitis.[45] These authors suggested that changes in clinical attachment levels and radiographic bone levels progress somewhat independently, particularly in the short term. It is therefore essential that both variables are measured in longitudinal clinical studies and are not interpreted independently. In another study of untreated periodontitis patients, however, radiographic bone changes and CAL changes were shown to be closely correlated.[43] This study used DSR to measure bone changes and an automated periodontal probe with the capability to detect the CEJ and a reported error in measuring attachment changes of 0.4 mm.[46] Again, comparisons between studies in which different probes were used should only be made with extreme caution.

GCF PGE_2 concentrations exhibited wide variations throughout the study. In the pretreatment phase, a gradual increase in GCF PGE_2 levels was observed to month 6, at which point PGE_2 levels peaked. This was followed by a statistically significant reduction in PGE_2 levels post-treatment at month 7. In the post-treatment period, GCF PGE_2 levels were stable and were maintained in the region of 40 to 50 ng/ml (with the exception of month 9 when levels reached 65.3 ng/ml). The gradual increase in GCF PGE_2 levels in the pretreatment period is difficult to explain, although an increase in GCF PGE_2 levels in untreated populations has been reported elsewhere. In a randomized, placebo-controlled investigation of the efficacy of topical ketorolac and systemic flurbiprofen on the inhibition of alveolar bone loss in humans, subjects who received placebo demonstrated an increase in GCF PGE_2 concentrations from approximately 25 ng/ml at baseline to 49 ng/ml after 6 months.[41] A similar finding was reported in a study of naturally occurring periodontitis in beagles.[47] In animals

receiving placebo, GCF PGE_2 levels increased from 315.8 ng/ml at baseline to 1663.1 ng/ml at month 6.

Elevated GCF PGE_2 levels have previously been proposed as a predictive risk factor for periodontal disease activity.[20] Patients with a pooled GCF PGE_2 level greater than 66.2 ng/ml were found to be 47 times more likely to have LOA than patients with GCF PGE_2 levels lower than 66.2 ng/ml.[20] The concept of using a particular GCF PGE_2 concentration as a threshold for placing patients into high- or low-risk categories is appealing. In our study, the month 1 mean (±SEM) GCF PGE_2 concentration at those sites which exhibited no bone change during the 6 months pretreatment (44.29 ± 2.37 ng/ml) did not differ significantly from that observed at sites at which subsequent bone loss did occur (53.36 ± 7.21 ng/ml) (independent samples *t* test, $P = 0.135$). In our population, therefore, GCF PGE_2 concentrations could not be used confidently as a predictive risk indicator for future alveolar bone destruction, although the number of sites which lost bone was relatively small.

Cigarette smoking is a potent risk factor for many systemic diseases, and a number of recent studies have demonstrated clearly that smoking is a risk factor for periodontitis.[40,48,49] While the pathogenesis of periodontitis in smokers is incompletely understood, epidemiological data suggest that the effect of smoking on the periodontal tissues is direct[50,51] and not simply related to increased plaque levels. In the present study, there were no significant differences between current, ex-, and non-smokers with regard to mean plaque scores. Whole-mouth and test site BOP also did not differ significantly between the smoking subgroups. Previous studies have documented reduced gingival bleeding in smokers,[52] a finding usually attributed to the local vasoconstrictive effects of tobacco smoke. In the present study, the relatively small number of subjects in each smoking sub-group, and the high levels of plaque observed in all cases, may have prevented any effect of smoking on gingival bleeding from being readily apparent.

At all time points, greater full-mouth mean PD was recorded in smokers compared to non- and ex-smokers. Test site PD was also significantly greater in smokers than non-smokers at month 6 and significantly greater than those in ex-smokers at months 6, 10, and 13. These findings are consistent with previously published data.[49] Non-, ex-, and current smokers all demonstrated clinically and statistically significant reductions in mean full-mouth PD and mean test site PD as a result of treatment. This contrasts with data which suggest that smokers respond less favorably to treatment compared to non-smokers,[53] with the implication that smokers constitute a subset of the population whose disease is more resistant to conventional therapy. Indeed, smokers have been shown to comprise up to 90% of a population clinically diagnosed with refractory periodontitis.[54] The present study, however, provides reassurance that a high standard of interventional therapy coupled with frequent maintenance visits can achieve a similar degree of resolution of probing depths in smokers and non-smokers, at least in the short-term, following treatment. Furthermore, a recently published study of 54 non-smokers and 33 smokers with moderate to advanced CAPD who received SRP and were then monitored for 9 months showed that smokers and non-smokers respond equally well to non-surgical treatment.[55] In that study, probing depth reductions of 0.60 mm and 0.65 mm were reported at test sites for non-smokers and current smokers, respectively. These changes are smaller than the PD reductions observed at test sites in the present study, which may further underline the importance of regular and frequent supportive periodontal therapy. Subjects in the Pucher et al. study[55] received no maintenance recalls, while those in our study were seen once every month in the post-treatment period. In another recent study of patients with untreated advanced CAPD, statistically significant PD reductions of 1.9 mm and 2.5 mm were recorded in smokers and non-smokers, respectively, following SRP.[56] Thus, smoking status does not appear to preclude a good soft tissue response to non-surgical treatment, providing such treatment is of a high standard.

CAL was also greater at all time points in smokers than in non- and ex-smokers, although not significantly so (data not shown). This observation supports the concept that smokers have significantly greater periodontal breakdown than non-smokers,[40] even in subjects with minimal periodontal disease,[57] and suggests that smoking does not result in merely a pro-inflammatory effect in the periodontal tissues, but leads to irreversible LOA. The biological effects of cigarette smoke on the periodontal tissues include a vasoconstrictive effect on the gingival microvasculature,[58] impairment of peripheral blood and oral neutrophil chemotactic and phagocytic functions,[59] reduced antibody production,[60] alteration of peripheral blood T cell subset ratios,[61] cytotoxic effects due to nicotine and cotinine (its major metabolite), and impaired healing and fibroblast function.[48] It would seem appropriate, therefore, that longitudinal clinical trial populations should be balanced for smoking status.

No statistically significant differences were detected between the smoking subgroups in bone height and mass changes, but these parameters also demonstrated considerable variability during the course of the study. Smokers have been shown to exhibit greater radiographic bone loss than non-smokers,[40] although this study monitored untreated periodontitis patients longitudinally for 1 year. In our study, the relatively

short pretreatment phase (6 months) may be insufficient to detect emerging differences between smokers and non-smokers in alveolar bone status.

No differences were seen between non-, ex-, and current smokers in GCF PGE_2 concentrations. An investigation of the pathogenesis of smoking-related periodontal disease demonstrated that nicotine upregulates lipopolysaccharide (LPS)-mediated secretion of PGE_2 by monocytes.[62] Nicotine alone had no effect on monocyte production of PGE_2. However, PGE_2 production was potentiated more than 3-fold by *Porphyromonas gingivalis (Pg)* LPS and nicotine, relative to *Pg* LPS alone. These authors suggested that high plasma and oral cavity nicotine concentrations in smokers, coupled with the presence of *Pg* in the subgingival microflora, could lead to elevated PGE_2 levels in smokers and resultant hard and soft tissue destruction. Previous studies have shown that smoking and subgingival infection with *Pg* are significant risk factors for attachment loss in the adult population.[48] The finding that nicotine potentiates PGE_2 secretion by *Pg* LPS-stimulated monocytes[62] could provide a unifying theory for the increased risk of attachment loss seen in smokers when compared to non-smokers, as mediated by PGE_2. No evidence of increased GCF PGE_2 concentrations in smokers was detected in the present study.

At all time points in our study, there were no clinically or statistically significant differences in any of the clinical, radiographic, and biochemical parameters between non- and ex-smokers. Previous studies have shown that the periodontal status of ex-smokers is intermediate to that of current and non-smokers.[63,64] These studies indicate that smoking cessation is of benefit to periodontal health, which is confirmed by the findings of the present study. There is evidence to suggest that approximately 1 year following smoking cessation, the gingiva loses the fibrotic appearance associated with smoking and assumes a normal anatomy.[49] In our study, all ex-smokers had given up smoking at least 2 years prior to the study, and the results confirm that ex-smokers are very similar to non-smokers with regards to short-term disease progression and response to treatment.

In summary, in a cohort of subjects with a history of chronic periodontitis, mean clinical measurements failed to identify disease progression over a 6-month period. DSR revealed small but statistically significant alveolar bone loss, while GCF PGE_2 levels gradually increased from month 0 to 6. Following non-surgical therapy, statistically and clinically significant reductions were observed in mean full-mouth PI, BOP, and PD, and also in test site PD and BOP. A statistically significant, but clinically non-significant, reduction in test site CAL was noted. The trend towards progressive bone loss was halted, and a statistically significant decrease in GCF PGE_2 concentrations was detected. In the post-treatment period, the improvements in clinical parameters were maintained as a result of regular and frequent maintenance visits. Alveolar bone height and mass gain were observed, such that bone status at month 13 approximated that at commencement of the study. GCF PGE_2 concentrations remained relatively stable. There were no significant differences between non-smokers, ex-smokers and current smokers in PI, BOP, CAL, alveolar bone height and mass changes, and GCF PGE_2 concentrations at any time point. Probing depths were significantly greater in current smokers than in non- and ex-smokers and were reduced significantly and comparably in all three smoking subgroups as a result of treatment.

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