KEY WORDS
Periodontal probing, Gingival pocket depth, Dental plaque, Bleeding on probing, Gingival recession

Randomized controlled trial

CAN PERIODONTAL PROBING ALTER CLINICAL VARIABLES IN UNTREATED PERIODONTAL PATIENTS? A BLIND-EXAMINER SPLIT-MOUTH RANDOMIZED CONTROLLED TRIAL

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PURPOSE. To assess any variations in periodontal indices (dental plaque, bleeding on probing, gingival recession, pocket depth) after periodontal probing, without carrying out any therapy, in subjects suffering from periodontal disease.

MATERIALS AND METHODS. A blind-examiner split-mouth randomized trial with one-week follow-up was conducted. Adult periodontal patients were recruited from a private dental practice. At baseline (T0), an examiner measured the presence of plaque, bleeding on probing (BoP), probe depth (PD) and gingival recession in one upper and one lower quadrant of participant's dentition. After 10 minutes (T1), another blind examiner recorded the same variables in both quadrants, one probed and one unprobed, in the same arch. The opposite quadrants were examined after one week (T2) by another blind examiner. Then, sites previously probed and not were compared at T1 and T2 using multilevel models.

RESULTS. Data from thirty patients were analysed, and no drop-outs or adverse effects were recorded during the study. At T1, plaque scores (present/absent) were lower at previously probed sites (OR = 0.61; 95% CI from 0.50 to 0.73; P <0.0001) while at T2 there was no difference in plaque score between probed and unprobed sites (P = 0.5050). Conversely, PD was higher at previously probed sites at T1 (estimated difference of 0.19 mm; 95% CI from 0.06 to 0.31; P = 0.0027) but there was no difference at T2 (P = 0.6495). BoP did not differ between probed and unprobed sites at either T1 (P = 0.6741) or T2 (P = 0.5190), but, while there was no difference in recession between probed and unprobed sites at T1 (P = 0.6682), recession at probed sites was slightly greater at T2 (estimated difference of 0.08 mm; 95% CI from 0.002 to 0.16; P = 0.0455).

CONCLUSIONS. Routine probing manoeuvres were found to be slightly invasive, removing some bacterial plaque and immediately causing a temporary increase in PD, which appeared to lead to a slight increase in recession after one week.

CONFLICT OF INTEREST STATEMENT
The authors declare that they have completely self-funded the study, and that there are no conflicts of interest.

INTRODUCTION

Periodontal probing is one of the most useful diagnostic tools for detecting the presence and severity of periodontal lesions[1,2]. Periodontal probing is also used for classification and screening purposes, or to evaluate periodontal changes after therapy[3,4]. However, other clinical factors may help give an indication of the extent and severity of periodontal disease, such as gingival

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recession, plaque accumulation, bleeding on probing, clinical attachment level, furcation involvement, and tooth mobility and migration[4]. A comprehensive assessment of a patient's periodontal status involves compilation of a six-point periodontal chart, recording pocket depth, bleeding on probing, mobility, furcation, etc.[3], many of which rely on probing to measure.

Numerous studies have reported on repeated periodontal probing, usually to establish the intra- or inter-rater agreement between assessors or examiner calibration in clinical trials[5-8]. Repeated periodontal probing (at the same sites at baseline and follow-up) is also carried out to assess the effect of a therapy in the short or long term. This assumes that the diagnostic procedure itself is not able to change the clinical course of periodontal disease, and indeed it is generally accepted that the periodontal probing procedure has no effect on periodontal tissues. However, the probing procedure is potentially invasive, and could therefore theoretically modify the levels of plaque, bleeding, gingival recession, and probe depth itself[6], especially if repeated.

Recently, a case series has been reported in which variations in periodontal indices (plaque, bleeding, recession, and probe depths) after periodontal probing, without any therapy being given, were recorded in subjects suffering from periodontal disease[9]. In that case series, dental plaque was reduced, and bleeding on probing increased after one hour[9]. After two weeks, a decrease in inflammation was noted, characterized by reduced bleeding on probing, a slight decrease in probe depth, and a slight increase in gingival recession[9]. Although these results are noteworthy, it is important to note that several factors could have influenced the observed decrease in inflammation.

In particular, a Hawthorne effect may have affected measurements taken in the first few days after probing; due to their awareness of being observed, participants may have brushed their teeth more carefully during the first few days following the first appointment[10]. In addition, participants may have sought out periodontal therapy during a time of relatively pronounced gingivitis, accompanied by a high FMBS, which naturally decreased over time. Furthermore, the examiner may have unwittingly introduced bias by probing participants with less force at two weeks[9].

A randomized controlled trial (RCT) would be necessary to eliminate these sources of bias, and determine whether probing does, in fact, have an effect on periodontal indices. Unfortunately, designing an RCT on this issue would be problematic, because the "therapy" under investigation would be the probing itself, with the outcome variables being the resulting measures, and it would be impossible to quantify pocket depth in the unprobed arm without performing probing. For this reason, we designed a split-mouth randomized controlled trial assuming mean equal periodontal values in contralateral quadrants of the same subject to record any variations in periodontal indices (plaque, bleeding, recession, and probe depths) due to periodontal probing in subjects suffering from periodontal disease, without administering any therapy.

MATERIALS AND METHODS

Trial design

This was a single-centre, blind-examiner randomized clinical trial, with balanced randomization and split-mouth design. At baseline, the presence or absence of plaque, bleeding on probing (BoP), probe depth (PD), and gingival recession in one upper and one lower quadrant were to be recorded with the aid of a periodontal probe. After 10 minutes, the same variables were to be measured in both quadrants of the same arch were compared. After one week, the same variables were compared in both quadrants in the opposing arch.

This study is reported in accordance with the 2010 CONSORT guidelines for reporting parallel-group randomized controlled trials[11], and the design, analysis and presentation conform to their extension checklist for reporting within-person randomized trials[12].

Eligibility criteria for participants

The investigation was carried out from June to October 2014. During this period, 30 subjects seeking professional cleaning at a private office located in Campi Bisenzio, Florence, Italy were consecutively recruited. The inclusion criteria used for selection were:

- age greater than 18 years;
- presence of at least 20 teeth or implant-supported crowns (both were included in the study);
- need for periodontal therapy.

The exclusion criteria were:

- subjects with manual inability to perform dental care;
- patients with neurological disorders;
- pregnant women;
- periodontal chart compiled in the preceding three months.

Informed written consent was obtained from all participants.

Interventions

At baseline (T0), one examiner (UP) measured plaque (presence/absence), bleeding on probing (BoP), probe depth (PD), and gingival recession in one upper and one lower quadrant (either 1 and 3 or 2 and 4) according to the indication provided in the first randomization envelope assigned to the participant in question. After that, participants were asked to wait in the waiting room for 10 minutes, after which (T1) another examiner (MC) recorded the same variables in both quadrants in one arch (either quadrants 1 and 2 or quadrants 3 and 4), one having been previously probed and one not, according to the instruction in the second envelope assigned to the patient. Participants were discharged without any dental care instructions or encouragement. After one week (T2), the patients were recalled and a third examiner (MG) recorded the same variables in the quadrants not analysed at T1 (either quadrants 1 and 2 or quadrants 3 and 4), following the indications in the third envelope assigned to the patient. Only once data had been collected were patients given periodontal-related therapy.

Outcome measures

The primary outcome measure of the study was the dichotomous plaque score (present/absent)[13] on six sites per tooth or implant (buccal, lingual, mesiobuccal, mesiolingual, distobuccal, and distolingual). Bleeding on probing (BoP) was also measured in a dichotomous manner[14] at the same six sites per tooth using a PCP-UNC 15 periodontal probe, as were pocket depth (PD) in mm and gingival recession (Rec) in mm.

The three examiners were blinded to the quadrants measured by the other examiners, and each underwent a calibration session before the start of the study, measuring PD and Rec at 72 sites in two patients; the inter-rater agreement was good (the intraclass correlation coefficient was 0.88 for Rec and 0.61 for PD).

Sample size

Considering the results of a previous study[9], to detect an odds ratio of 0.60 for presence of dental plaque with a two-side 5% significance level, a power of 95%, 30 sites per quadrant, and an intra-patient correlation of 0.40, a sample size of 30 patients was necessary, given an anticipated drop-out rate of 25%.

Random sequence

Two computer-generated lists of random numbers were used for quadrant allocation for each participant. The first list contained the quadrants to be measured at baseline (T0), either quadrants 1 and 3 or quadrants 2 and 4. The second list contained the quadrants to be measured at T1, either quadrants 1 and 2 or quadrants 3 and 4. At T2, variables were measured at the quadrants not assessed at T1 (either quadrants 1 and 2 or quadrants 3 and 4). A third list of random numbers was not necessary, because at T2 the quadrants assessed were those not measured at T1.

Allocation concealment and blinding

One investigator (MN) concealed the allocation sequence. The quadrants to be measured at T0, T1 and T2 were sealed and stapled in three sequentially numbered (1T0, 1T1, 1T2 for the first participant, 2T0, 2T1, 2T2 for the second participant, and so on) opaque envelopes, which were opened just before the corresponding assessment. The three examiners were blinded to the quadrants measured by the other examiners.

Statistical methods

Descriptive statistics were calculated (by MN) using mean and standard deviation for quantitative data and frequency and percentage for qualitative data. Multilevel models with two levels (participant and site) were constructed to compare "treatments" (probing *versus* not) at both T1 and T2. Specifically, logistic multilevel models were applied to variables plaque and BoP, while linear multilevel models were used for variables Rec and PD.

As a sensitive inferential analysis at quadrant level, the difference between previously probed and unprobed quadrants at T1 and T2 were tested for mean PD, mean Rec, quadrant plaque score (QPS), and quadrant bleeding score (QBS) using the paired t-test. QPS and QBS are essentially the same as full-mouth plaque score (FMPS) and full-mouth bleeding score (FMBS), but measured at quadrant level rather than patient level. The analysis at quadrant level was undertaken because, even though it is less powerful than multilevel analysis, it is more frequently used in the dental literature.

The significant level was set at 0.05. The statistical software was MLwiN v. 3.05 (Centre for multilevel modelling, University of Bristol).

RESULTS

Participant flow and recruitment

Thirty patients were consecutively enrolled in the trial, and their quadrants were randomized in a split-mouth design. No patient dropped out, and all the patients were assessed as per the study protocol.

Baseline data

There were 18 females (60%) and 5 smokers (17%). Mean age was 51.3±10.4 years (range 27 to 76). At baseline, two quadrants per participant were assessed, making a total 2454 sites (a mean of 81.8 sites per participant) on 409 teeth or implant-supported crowns.

Site-level statistics at T0 are presented in **TABLE 1**.

Outcomes and estimation

Site-level statistics at T1 are presented in **TABLE 2**. Fewer probed sites showed the presence of plaque, and the odd's ratio (OR) for PI was significant (OR = 0.61; 95% CI from 0.50 to 0.73; P <0.0001). There was very little difference between the number of probed and unprobed sites

showing bleeding on probing (BoP) at T1 (OR= 0.96; 95% CI from 0.78 to 1.18; P = 0.6741), but PD was significantly higher in previously probed sites (difference 0.19 mm; 95% CI from 0.06 to 0.31; P = 0.0027). There was no significant difference between probed and unprobed sites in terms of Rec (difference -0.02 mm; 95% CI from -0.12 to 0.08; P = 0.6682).

Site-level statistics at T2 are presented in **TABLE 3**. The numbers of probed and unprobed sites with plaque present were almost the same, and the odd's ratio for PI was not significant (OR = 1.06; 95% CI from 0.89 to 1.28; P = 0.5050). Similarly, there was a negligible difference between the number of probed and unprobed sites displaying bleeding on probing (BoP) (OR = 1.09; 95% CI from 0.83 to 1.43; P = 0.5190), and no significant difference between the two in PD

TABLE 1 SITE-LEVEL STATISTICS AT BASELINE (T0)

	T0 N = 2454
Number of sites with PI (%)	925 (38%)
Number of sites with BoP (%)	289 (12%)
PD mm (SD)	2.18 (1.05)
Rec mm (SD)	0.85 (1.20)

PI: Plaque; BoP: Bleeding on probing; PD: Probe depth; Rec: Gingival recession

TABLE 2 SITE-LEVEL STATISTICS AFTER 10 MINUTES (T1)

	PPS N = 1243	UPS N = 1283	Estimate*	95%CI	P-value
Number of sites with PI (%)	278 (22%)	397 (31%)	0.61	0.50; 0.73	<0.0001
Number of sites with BoP (%)	257 (21%)	277 (22%)	0.96	0.78; 1.18	0.6741
PD mm (SD)	2.42 (1.76)	2.26 (1.58)	0.19	0.06; 0.31	0.0027
Rec mm (SD)	1.09 (1.42)	1.14 (1.52)	-0.02	-0.12; 0.08	0.6682

PPS: Previously probed sites; UPS: Unprobed sites; PI: Plaque; BoP: Bleeding on probing; PD: Probe depth; Rec: Gingival recession
˙Difference for PD and Rec; Odd's ratio for PI and BoP

TABLE 3 SITE-LEVEL STATISTICS AFTER 1 WEEK (T2)

	PPS N = 1206	UPS N = 1230	Estimate*	95%CI	P-value
Number of sites with PI (%)	392 (33%)	390 (32%)	1.06	0.89; 1.28	0.5050
Number of sites with BoP (%)	132 (11%)	126 (10%)	1.09	0.83; 1.43	0.5190
PD mm (SD)	2.51 (1.14)	2.54 (1.17)	-0.02	-0.11; 0.07	0.6495
Rec mm (SD)	0.93 (1.35)	0.86 (1.30)	0.08	0.002; 0.16	0.0455

PPS: Previously probed sites; UPS: Unprobed sites; PI: Plaque; BoP: Bleeding on probing; PD: Probe depth; Rec: Gingival recession
˙Difference for PD and Rec; Odd's ratio for PI and BoP

(difference -0.02 mm; 95% CI from -0.11 to 0.07; P = 0.6495). However, recession was significantly greater at sites that had been previously probed (difference 0.08 mm; 95% CI from 0.002 to 0.16; P = 0.0455).

Participant-level sensitivity analyses are presented in **TABLES 4-6**. **TABLE 4** shows the descriptive statistics (two probed quadrants), and **TABLE 5** the differences at T1-the estimated differences were similar to those from the main analysis, but the difference in PD was found to be not significant. **TABLE 6** illustrates the differences at T2-the estimated differences are similar to those from the main analysis, but the difference in recession was found to be not significant.

TABLE 4 PATIENT-LEVEL STATISTICS AT BASELINE (T0)

	T0 N = 30
QPS (SD)	30.8 (15.4)
QBS (SD)	9.6 (6.5)
PD mm (SD)	2.18 (0.33)
Rec mm (SD)	0.90 (0.77)

Two quadrants in each patient were assessed. QPS: Quadrant plaque score; QBS: Quadrant bleeding score; PD: Probe depth; Rec: Gingival recession.

TABLE 5 QUADRANT-LEVEL STATISTICS AFTER 10 MINUTES (T1)

	PPQ N = 30	UPQ N = 30	Estimated difference	95%CI	P-value
QPS (SD)	22.3 (15.9)	30.7 (18.2)	-8.4	-4.8; -12.1	<0.0001
QBS (SD)	21.5 (20.0)	21.4 (17.0)	0.1	-6.2; 6.4	0.9720
PD mm (SD)	2.49 (0.89)	2.29 (0.71)	0.20	-0.03; 0.43	0.0864
Rec mm (SD)	1.15 (0.88)	1.17 (0.83)	-0.02	-0.14; 0.10	0.7212

PPQ: Previously probed quadrant; UPQ: Unprobed quadrant
QPS: Quadrant plaque score; QBS: Quadrant bleeding score; PD: Probe depth; Rec: Gingival recession

TABLE 6 QUADRANT-LEVEL STATISTICS AFTER 1 WEEK (T2)

	PPQ N = 30	UPQ N = 30	Estimated difference	95%CI	P-value
QPS (SD)	32.7 (18.9)	31.5 (16.9)	1.3	-2.6; 5.1	0.5120
QBS (SD)	10.9 (11.4)	10.2 (8.3)	0.6	-3.3; 4.6	0.7364
PD mm (SD)	2.52 (0.48)	2.54 (0.43)	-0.01	-0.13; 0.11	0.8183
Rec mm (SD)	0.97 (0.90)	0.89 (0.91)	0.08	-0.02; 0.19	0.1139

PPQ: Previously probed quadrant; UPQ: Unprobed quadrant
QPS: Quadrant plaque score; QBS: Quadrant bleeding score; PD: Probe depth; Rec: Gingival recession

DISCUSSION

The objective of the present randomized split-mouth study was to determine whether probing procedures can induce alterations in dental plaque, bleeding on probing, probe depth and gingival recession in the short term. As such, the thirty patients needing periodontal therapy included in this trial received neither instruction or motivation to oral hygiene nor periodontal therapy until the measurements had been completed. Although no therapy was administered, several significant changes in the measured variables were observed. In particular, 10 minutes after the first measurement session, dichotomous plaque score decreased and probe depth increased from baseline, whereas gingival recession increased after one week. Sensitivity analysis at quadrant level confirmed a significant drop in plaque after 10 minutes, but did not confirm the previously noted significant increase in probe depth. This was likely due to the lower power of the quadrant-level analysis as compared to multilevel analysis, because the estimated difference was the same as that at site level.

The observation that plaque decreased significantly in the probed quadrant after 10 minutes but returned to baseline after one week indicates that manipulating tooth surfaces and soft tissues with the periodontal probe can disturb the structure of the bacterial biofilm, eliminating a certain amount of plaque in the immediate term. In fact, after 10 minutes, the quadrant plaque score (QPS) decreased by 8.4%. Ten minutes is not enough time for the deposition of new plaque, while after one week sufficient plaque can be deposited at previously probed sites, explaining the comparable plaque scores at probed *versus* unprobed sites at T2. These results are similar to those reported in a previous case series study, in which plaque was found to be significantly reduced one hour after probing, but was unchanged with respect to baseline after two weeks[9].

There was no change in bleeding on probing observed at either probed or unprobed sites after 10 minutes or one week. This contrasts with findings from the previously cited case series[9], in which bleeding on probing increased significantly after one hour but decreased significantly after 2 weeks[9]. This discrepancy may be explained by the fact that the patients in the case series had more inflammation at baseline, with a BoP rate of approximately 60%, while in this RCT the BoP rate was approximately 20%.

Pocket depth was significantly increased, by approximately 0.2 mm, at probed sites after 10 minutes, but not after one week. This increase was not observed in the case series study, likely because in that that case the second probing was performed after one hour as opposed to 10 minutes[9]. Indeed, probing the same site at 10-minute intervals is likely to cause greater trauma to the periodontal epithelial-connective tissue, and could therefore explain the slight increase in PD.

Conversely, gingival recession was unchanged after 10 minutes, but increased significantly, by 0.08 mm, after one week. This value was truly negligible from a clinical standpoint, and was in any case not confirmed by the participant-level analysis, probably because the analysis was underpowered for this variable. That being said, a similar difference was observed in the case series study[9], and could be due to a small reduction in inflammation-related gingival oedema. As above, probing may have disturbed the architecture of the bacterial biofilm, which may have been sufficient to reduce inflammation over the course of a week.

Regardless of the phenomenon that generated the differences between probed and unprobed sites, it is clear in any case that this phenomenon is not attributable to any therapy, given that none was administered. Moreover, considering that this was a split-mouth randomized clinical trial, neither the placebo nor the Hawthorne effect[10] could be responsible. Hence, it was more than likely that the probing itself generated the small changes observed, at least in the short term.

This is important to note when designing or interpreting the results of any study relying on probing as a measurement tool. Indeed, if probing were used in the measurement of outcomes of a particular treatment administered to a case series, the effect seen here would be erroneously attributed to the treatment. Hence to establish the efficacy of a therapy that includes probe depth and other periodontal indices as outcome variables, it would be necessary to use a control group for comparison; only by comparing the difference in efficacy between two or more treatments in such an RCT can we obtain meaningful results unaffected by measurement error, the Hawthorne effect, the placebo effect, and the effect of probing itself. As these effects are produced in the same way in different arms of a randomized controlled trial, they are cancelled out, and therefore do not affect the differences in outcome between the arms.

The principal limitation of this RCT is that we could not measure the differences between probed and unprobed sites in plaque, BoP, PD or recession directly, because this would have involved probing all sites. As probing was the "therapy" under investigation, and the resulting measures are the outcome variables, it is very difficult to design an RCT to explore the issue, which is why we opted for a split-mouth design, assuming equal baseline values at both quadrants in the same arch.

CONCLUSIONS

Routinely used periodontal variables such as dental plaque, pocket depth, and gingival recession can change over time with probing alone, without any therapy being administered. Merely measuring these variables with the aid of a periodontal probe can reduce plaque and increase pocket depth after 10 minutes, and slightly increase gingival recession after one week, which should be borne in mind when designing studies reliant on probing.

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