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Comparison of nonsurgical periodontal therapy with oral hygiene instruction alone for chronic periodontitis

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Abstract: We evaluated changes in clinical variables and microbiological profiles of periodontopathogens among 56 patients with moderate to severe CP who were randomly assigned to oral hygiene instruction (OHI; *n* = 28) or nonsurgical periodontal treatment (NSPT; *n* = 28). Periodontal variables were assessed and subgingival plaque samples were obtained from deep pockets (≥5 mm) at baseline and 3 months after treatment. Real-time polymerase chain reaction was used to quantify *Actinobacillus actinomycetemcomitans, Tannerella forsythia, Porphyromonas gingivalis*, and *Prevotella intermedia*. All clinical variables significantly improved in both groups. Improvements in gingival bleeding index (GBI), probing pocket depth (PPD), and periodontal attachment loss (PAL) were significantly greater at 3 months after treatment in the NSPT group. At baseline, the prevalences of all pathogens were high. Significant reductions in microbial count were observed for *A. actinomycetemcomitans* and *T. forsythia* ($P \leq 0.05$) in the NSPT group. None of the improvements in clinical variables was associated with changes in microbiological profiles. At 3 months after treatment, NSPT was associated with significantly greater improvements in GBI, PPD, and PAL as compared with OHI. *A. actinomycetemcomitans* and *T. forsythia* counts were significantly lower in the NSPT group.

Keywords: chronic periodontitis; periodontal pathogens; nonsurgical periodontal therapy; oral hygiene instructions; qPCR.

Introduction

Periodontitis is a chronic inflammatory disease of the supporting tissues surrounding teeth and can result in irreversible destruction of the periodontal ligament, connective tissues, cementum, and alveolar bone (1). The prevalence of chronic periodontitis among Asians is around 15 to 20% (2), and about 5 to 15% of adults worldwide have severe periodontitis (3).

Chronic periodontitis is a multifactorial disease. Dental biofilm is the initiator, and chronic periodontitis is thus characterized as a microbial dental biofilm-based infectious disease (4). A number of bacterial hypotheses have been proposed regarding the pathogenesis and progression of periodontal disease. These range from specific to nonspecific plaque theories, the ecological plaque hypothesis, and, more recently, the "polymicrobial synergy and dysbiosis" model (5). Most of these theories identify bacteria as the main cause of pathogenesis.

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More than 700 species of bacteria have been found in subgingival dental biofilm, among *which Actinobacillus actinomycetemcomitans, Tannerella forsythia, Porphyromonas gingivalis,* and *Prevotella intermedia* have been implicated in periodontitis pathogenesis (6).

Chronic periodontitis is managed by nonsurgical periodontal therapy (NSPT), which includes oral hygiene instruction (OHI), scaling, and root planing. NSPT improves clinical variables by enhancing attachment level, reducing gingival bleeding, decreasing probing pocket depths, and eliminating or reducing periodontal pathogens by destroying the subgingival biofilm that protects resident bacteria from the host immune system (7). Treatment success is gauged by improvements in clinical variables and reductions in subgingival microbial counts (8). The presence and concentration of specific periodontal pathogens are important in disease initiation and progression, and numerous studies have thus investigated these pathogen characteristics. Studies comparing pre- and post-treatment prevalence, percentage, and frequency of detection of pathogenic species at healthy and non-healthy sites have yielded varying results (9,10).

This study compared the effects of NSPT and OHI alone on the periodontal status and microbiological profile of *P. gingivalis, A. actinomycetemcomitans, T. forsythia,* and *P. intermedia* and evaluated changes in clinical variables associated with microbiological changes in systemically healthy participants with periodontitis. Because of ethical concerns, the control group in this study received oral hygiene instruction, which is the most important service a dental professional can provide and the cornerstone of good dental health. To our knowledge, no previous study has compared the effects of NSPT and OHI alone on the clinical characteristics and microbiological profile of systemically healthy persons with chronic periodontitis.

Materials and Methods

Participants with moderate to severe chronic periodontitis, as determined using the case definition proposed by Armitage (11), were selected for this longitudinal randomized clinical trial. Ethical clearance for the study was granted by the Dental Ethics Committee of the University of Malaya (MEC No. DF PE1002/0045(P)). The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000. The CONSORT guidelines for clinical trials were followed. The ClinicalTrials.gov registration number for this study is NCT02208739. All patients received a detailed explanation of the treatment and gave their written informed consent.

The inclusion criteria for this study were 1) male or female patients aged 30-70 years, 2) a minimum of 12 teeth present with at least five pockets that had both a probing pocket depth (PPD) of 5 mm or more and probing attachment loss (PAL) of 4 mm or more in at least two quadrants that bled on probing. Patients were excluded if they had a history of stroke or cardiovascular conditions that required prophylactic antibiotic administration before treatment. We also excluded immunocompromised patients, pregnant women, smokers, and participants who had received antibiotics during the previous 4 months or scaling during the previous 6 months. Patients with type 2 diabetes were excluded if their glycated hemoglobin (HbA1c) level was greater than 7% (which suggests uncontrolled type 2 diabetes) (12).

For sample size calculation, mean PAL values before and after treatment (13) were used to calculate sample size. According to the Cohen d formula, at least 28 patients would be needed in each group to detect a difference with 80% power and an α of <0.05. Thus, 174 potential patients were screened, and 56 patients who satisfied the inclusion criteria were recruited at the Periodontology Clinic at the Faculty of Dentistry, University of Malaya. Using block randomization, we assigned patients to the NSPT (n = 28) or OHI (controls; n = 28) group before the baseline examination (Fig. 1). All examination, treatment, and assessment of participants were performed by two calibrated examiners (W.N.A.W.A. and R.P.C.R.). To determine intra- and interexaminer reproducibility, three adults who were not study participants underwent repeated measurements (separated by a 3-h interval). Analysis of intraclass correlation showed good agreement (>0.7) for intra- and interoperator reproducibility in all recorded clinical variables. Microbiological analyses were performed by one of the authors (M.A.).

OHI was performed with a soft-bristled toothbrush, a compact-tuft toothbrush, interdental brushes, and dental floss (TePe oral hygiene education set, Malmo, Sweden), using the modified Bass technique. Patients in the NSPT group received full-mouth debridement comprising OHI, single-visit scaling, and root planing under local anesthesia, using an ultrasonic scaler (Satelec P5 Newtron XS, Acteon, Mt Laurel, NJ, USA) and Gracey curettes (Hu-Friedy, Chicago, IL, USA). This was followed by thrice daily rinsing with 0.12% chlorhexidine (Hexipro, Evapharm, Kuala Lumpur, Malaysia) mouthwash (15 mL each rinse) for a period of 14 days. At each recall visit, only NSPT group participants received professional prophylaxis comprising scaling and polishing.

Patients in the OHI group received OHI only, with no advice on mouthwash use. At our clinic, patients without acute symptoms who are referred for periodontal

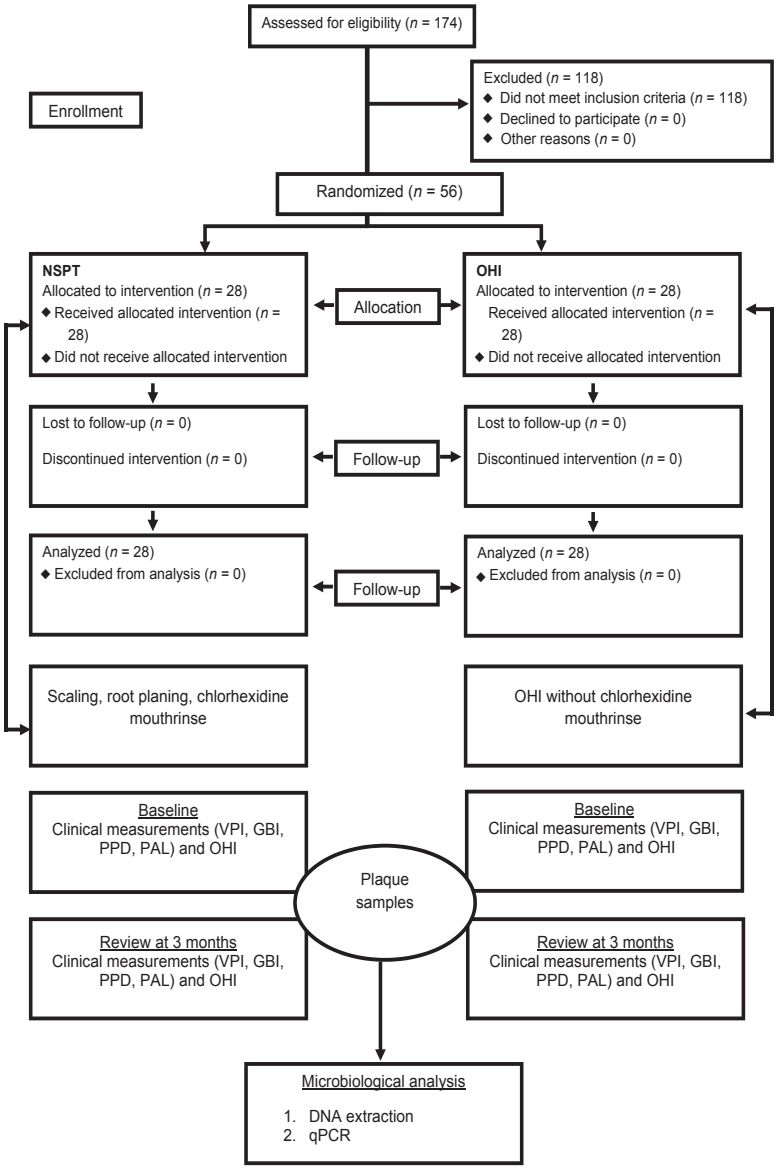

Fig. 1 Flow chart of the study protocol.

treatment are placed on a waiting list before receiving treatment from postgraduate or undergraduate students. The waiting period for periodontal treatment is usually longer than 3 months. Therefore, it was considered ethical to expose our controls to 3 months without scaling or root planing, as these participants would receive OHI and would later undergo NSPT immediately after the study was completed.

At monthly recall visits, participants in both groups were examined and encouraged, but professional supragingival prophylaxis was provided only to the NSPT group. PPD, PAL, gingival bleeding index (GBI), and visible plaque index (VPI) (14) were determined for all present teeth, except third molars, at baseline and 3 months after treatment. Using an electronic constant force probe (Florida Probe), we evaluated four sites for VPI and GBI (mesial, distal, buccal, lingual) and six sites for PPD and PAL (distobuccal, midbuccal, mesiobuccal, mesiolingual, midlingual, and distolingual).

At baseline and 3 months after treatment, sterile curettes were used to collect subgingival plaque samples from the deepest sites with a PPD $\geq$ 5 mm at five or more sites with a tendency for bleeding. These samples were then pooled together. Before sampling, isolation was maintained with cotton rolls, and supragingival plaque was removed with cotton pellets. Plaque scrapings were resuspended in 1.5 mL of phosphate-buffered saline and stored at −80°C in a freezer before DNA extraction. A 100-µL plaque sample was used for automated DNA extraction with a Qiacube device (Qiagen Biotechnology, Kuala Lumpur, Malaysia). To obtain pellets the tubes containing the plaque samples were centrifuged at 5,000

Table 1 Demographic characteristics of patients, by treatment type

Characteristic	OHI	NSPT	*P* value
Sex, *n* (%)			
Male	13 (46.0)	16 (57.0)	0.422
Female	15 (53.6)	12 (42.9)	
Ethnicity, *n* (%)			
Malay	12 (42.9)	9 (32.1)	0.706
Chinese	9 (32.1)	11 (39.3)	
Indian	7 (25.0)	8 (28.6)	
Systemic diseases			
Type 2 diabetes, *n* (%)	13 (46.4)	11 (39.3)	0.589
HbA1c (mmol/mol ± SD)	6.6 ± 0.30	6.7 ± 0.30	0.929
Age (mean ± SD)	50.7 (8.5)	51.5 (10.1)	0.777
No. of teeth (mean ± SD)	23.53 ± 4.46	23.07 ± 4.46	0.70

The independent-sample *t*-test used for analysis of age and HbA1c levels; the chi-square test was used for analysis of sex, ethnicity, and type 2 diabetes status. NSPT: nonsurgical periodontal therapy, OHI: oral hygiene instruction.

× g for 10 min on a tabletop centrifuge. The pellets were then used for automated DNA extraction with the Qiacube machine. The concentration and purity of extracted DNA were determined with a spectrophotometer (Nanodrop 2000, Thermo Scientific, Wilmington, DE, USA). The eluted DNA was stored at −20°C before bacterial detection using qPCR (Applied Biosystems, Carlsbad, CA, USA).

The reference bacterial strains used in this study were *P. gingivalis* (W83) (15), *A. actinomycetemcomitans* (NCTC9710) (16), *P. intermedia* (ATCC25611) (16), and *T. forsythia* (CCUG 21028A^T) (16). The protocol used by Boutaga et al. (16) was followed for the qPCR procedure. A total reaction mixture of 20 µL was used for amplification, and contained 2 µL of template DNA from the plaque sample/reference strain, 10 µL of 2× TaqMan Fast Advanced Master Mix (Applied Biosystems), 1 µL of 20× gene expression assay, and 7 µL nuclease-free water. The cycling conditions used were as follows: 50°C for 2 min and 95°C for 10 min followed by 45 cycles at 95°C for 15 s and 60°C for 1 min.

Dilutions of known amounts of reference strain DNA were used to generate standard curves with a correlation coefficient (R^2: 0.99). For quantification/copy number determination, the results from unknown samples were projected on the standard curves obtained from the pure cultures, using the following formula: Quantity DNA = $10^{(ct-b)/m}$ (17).

The paired-sample *t*-test and independent-sample *t*-test were used for intragroup and intergroup comparisons, respectively. The McNemar test was used to analyze intragroup changes in the frequency of microbial detection. The correlations of changes in clinical variables with microbiologic profiles were examined by Pearson correlation analysis. Multivariate analysis using a general linear model procedure with Bonferroni correction was used to assess treatment outcomes while controlling for type 2 diabetes status. A *P* value of ≤0.05 was considered to indicate statistical significance. All effect size values within groups at different time points are based on the results of paired-sample *t*-tests.

Results

The groups did not significantly differ in any demographic characteristic (Table 1). All 56 patients (n = 28 per group) completed the study. The OHI group had a higher proportion of women (53.6%), while men were predominant in the NSPT group (57%). Mean age was 50.7 ± 8.5 years for the OHI group and 51.5 ± 10.1 years for the NSPT group. There were more Malays in the OHI group and more Chinese in the NSPT group; Indians were approximately equally represented between groups. The number of teeth present was 23.5 ± 4.4 in the OHI group and 23.0 ± 4.4 in the NSPT group. Thirteen participants in the OHI group and 11 participants in the NSPT group had well-controlled type 2 diabetes (Table 1).

At 3 months after treatment (Tables 2, 3), VPI, GBI, PPD, and PAL had significantly decreased ($P \leq 0.05$) in both the OHI and NSPT groups. These statistically significant improvements in the clinical variables from baseline to 3 months were associated with a clinically significant effect size (>1).

All participants had localized moderate to severe chronic periodontitis at baseline, but the percentage of deep sites (>6 mm) was very low in both groups. A PPD > 6 mm was found in 4% of sites in the NSPT group and in 3% of sites in the OHI group, while a PPD of 4-6 mm was found in 20% of sites in the NSPT group and in 15% of sites in the OHI group. The findings were similar for PAL in both groups at baseline. At 3 months after treat-

Table 2 Visible plaque index (VPI) and gingival bleeding index (GBI) at baseline and 3 months after treatment in the OHI and NSPT groups

	VPI (mean ± SD)			GBI (mean ± SD)		
	OHI group	NSPT group	*P***	OHI group	NSPT group	*P***
Baseline	66.91 (16.19)	65.53 (23.51)	0.80	55.97 (21.80)	58.19 (22.08)	0.70
3 months	26.83 (14.70)	23.16 (20.53)	0.44	29.08 (20.61)	16.76 (12.13)	0.09
Change	40.07 (17.33)	42.37 (29.39)	0.72	26.89 (20.95)	41.43 (25.25)	0.02
*P**	0.01	0.01		0.01	0.01	
Effect size[1]	2.31	1.44		1.28	1.64	

n = 28. *P**: intragroup (paired-sample *t*-test), *P***: intergroup *P* value for VPI and GBI (independent-sample *t*-test), Effect size[1]: effect size of baseline as compared with 3 months after treatment, NSPT: nonsurgical periodontal therapy, OHI: oral hygiene instruction.

Table 3 Probing pocket depth (PPD) and probing attachment loss (PAL) at baseline and 3 months after treatment in the OHI and NSPT groups

	PPD (mean ± SD)			PAL (mean ± SD)		
	NSPT group	OHI group	*P***	NSPT group	OHI group	*P***
Baseline	2.77 (0.54)	2.44 (0.61)	0.05	3.30 (0.69)	2.88 (0.58)	0.05
3 months	1.96 (0.34)	2.10 (0.62)	0.32	2.68 (0.60)	2.55 (0.85)	0.53
Change	0.78 (0.41)	0.34 (0.22)	0.01	0.62 (0.40)	0.32 (0.24)	0.01
*P** value	0.01	0.01		0.01	0.01	
	Sites with PPD <4 mm (mean ± SD)			Sites with PAL <4 mm (mean ± SD)		
Baseline	75.5 ± 13.3	81.9 ± 13.6	0.73	62.9 ± 16.9	72.3 ± 19.3	0.22
3 months	90.6 ± 7.1	87.5 ± 13.9	0.27	76.8 ± 15.1	78.5 ± 19.3	0.06
Change	17.4 ± 11.7	5.5 ± 4.8	0.77	13.8 ± 11.6	6.1 ± 9.4	0.12
*P** value	<0.05	<0.05		<0.05	<0.05	
	Sites with PPD 4-6 mm (mean ± SD)			Sites with PAL 4-6 mm (mean ± SD)		
Baseline	20.4 ± 10.8	15.0 ± 9.9	0.03	29.6 ± 13.2	22.2 ± 12.3	0.28
3 months	4.7 ± 4.9	9.8 ± 9.9	0.02	19.2 ± 13.4	18.1 ± 13.4	0.81
Change	15.7 ± 9.9	5.2 ± 5.1	0.06	10.4 ± 10.1	4.1 ± 8.8	0.22
*P** value	<0.05	<0.05		<0.05	<0.05	
	Sites with PPD >6 mm (mean ± SD)			Sites with PAL >6 mm (mean ± SD)		
Baseline	3.9 ± 3.8	2.7 ± 4.6	0.84	6.8 ± 6.4	5.5 ± 8.1	0.50
3 months	1.2 ± 2.6	2.8 ± 6.5	0.91	3.4 ± 4.2	3.3 ± 7.0	0.37
Change	2.7 ± 2.9	0.3 ± 2.6	0.98	3.3 ± 4.0	2.1 ± 2.6	0.95
*P** value	>0.05	>0.05		<0.05	<0.05	

n = 28. *P**: intragroup (paired-sample *t*-test), *P***: intergroup (independent-sample *t*-test), NSPT: nonsurgical periodontal therapy, OHI: oral hygiene instruction.

ment, at the site level, PAL was significantly reduced at sites with deep, moderate, and shallow pockets in both the OHI and NSPT groups. However, for PPD, only the number of sites with a PPD of <4 mm or 4-6 mm significantly decreased in both groups.

In a comparison of the two treatment groups, the changes were significant for GBI, PPD, and PAL, although the magnitude of the reductions was greater in the NSPT group ($P \leq 0.05$; Tables 2, 3). However, VPI did not significantly differ between groups ($P = 0.72$). When assessed at the site level, changes in PPD and PAL did not significantly differ between groups. Multilinear regression analysis was used to adjust for the significant difference in baseline PPD and PAL values between groups. The findings confirmed significant intergroup p-values for changes in PPD and PAL at 3 months.

The prevalences of microbial pathogens were high at baseline and only slightly lower at 3 months in both groups. The prevalence for *T. forsythia* in the OHI group was actually higher at 3 months after treatment ($P > 0.05$). The prevalence of *A. actinomycetemcomitans* was lower at 3 months after treatment: the change was 10.71% ($P = 0.54$) in the OHI group and 3.57% ($P = 1.00$) in the NSPT group (Table 4).

Microbial counts had decreased for all pathogens at 3 months after treatment, except for *A. actinomycetemcomitans* and *P. gingivalis* in the OHI group, which increased ($P > 0.05$). In the NSPT group, the microbial count was significantly lower for *A. actinomycetemcomitans* and *T. forsythia* at 3 months after treatment. The reductions observed for *P. intermedia* and *P. gingivalis* were not significant ($P > 0.05$). The reductions in microbial counts were greater for the NSPT group than for the OHI group. However, there was no significant difference in the change

Table 4 Changes in detection frequency and mean counts of microbes in the OHI and NSPT groups 3 months after treatment

Periodontal pathogen	Frequency *n* (%)		*P***	Mean count (Copy no.)		*P***
	OHI group	NSPT group		OHI group	NSPT group	
Actinobacillus actinomycetemcomitans						
Baseline	22 (78.6)	15 (53.6)	0.05	9.2×10^7	37.0×10^7	0.41
3 months	19 (67.9)	14 (50.0)	0.18	1.0×10^7	0.4×10^7	0.13
Changes	10.71%	3.57%	0.56	1.6×10^7	37.0×10^7	0.27
*P**	0.54	1.00		0.28	0.05	
Tannerella forsythia						
Baseline	25 (89.3)	27 (96.4)	0.30	0.35×10^7	0.37×10^7	0.73
3 months	26 (92.9)	27 (96.4)	0.56	0.30×10^7	0.23×10^7	0.43
Changes	3.57%	0	0.32	0.048×10^7	0.14×10^7	0.41
*P**	1.00	–		0.60	0.03	
Prevotella intermedia						
Baseline	28 (100)	27 (96.4)	0.32	0.36×10^7	0.30×10^7	0.31
3 months	28 (100)	27 (96.4)	0.32	0.29×10^7	0.28×10^7	0.99
Changes	0	0	–	0.067×10^7	0.45×10^7	0.44
*P**	–	–		0.31	0.94	
Porphyromonas gingivalis						
Baseline	27 (96.4)	27 (100)	0.33	1.0×10^7	0.40×10^7	0.21
3 months	27 (96.4)	27 (100)	0.32	2.3×10^7	0.35×10^7	0.15
Changes	0	0	–	0.13×10^7	2.5×10^7	0.36
*P**	–	–		0.73	0.35	

n = 28. *P**: intragroup (paired-sample *t*-test), frequency of detection (McNemar test); *P***: intergroup (independent-sample *t*-test); NSPT: nonsurgical periodontal therapy; OHI: oral hygiene instruction.

Table 5 Changes in microbial profile and clinical variables in the NSPT and OHI groups

Clinical variables	Treatment	*Actinobacillus actinomycetemcomitans*		*Tanarella forsythia*		*Prevotella intermedia*		*Porphyromonas gingivalis*	
		r	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value
VPI	OHI	−0.074	0.70	0.258	0.18	0.353	0.06	0.197	0.31
	NSPT	0.240	0.21	−0.090	0.64	−0.219	0.26	−0.131	0.50
GBI	OHI	0.183	0.35	0.069	0.72	0.054	0.78	−0.390	0.84
	NSPT	0.408	0.06	−0.191	0.33	−0.224	0.25	−0.144	0.46
PPD	OHI	−0.276	0.15	0.541	0.06	0.350	0.06	0.027	0.89
	NSPT	−0.013	0.90	−0.062	0.75	−0.100	0.61	−0.013	0.94
PAL	OHI	−0.231	0.23	0.241	0.21	0.157	0.42	0.053	0.78
	NSPT	0.027	0.89	0.165	0.40	0.121	0.54	0.154	0.43

r: Pearson correlation coefficient, VPI: visible plaque index, GBI: gingival bleeding index, PPD: probing pocket depth, PAL: periodontal attachment loss. NSPT: nonsurgical periodontal therapy; OHI: oral hygiene instruction.

in any periodontal pathogen (*A. actinomycetemcomitans, P. gingivalis, P. intermedia,* and *T. forsythia*) between the OHI and NSPT groups ($P > 0.05$) (Table 4). No clinical variable (VPI, GBI, PPD, and PAL) was associated with microbiological profile ($P > 0.05$) (Table 5).

Multivariate analysis using a general linear model showed no difference in results related to changes in clinical variables or microbiological profile when participants with type 2 diabetes were excluded (Table 6).

Discussion

NSPT is the most common treatment for chronic periodontitis. It eliminates or reduces the microbial counts of subgingival flora after improving the clinical characteristics and oral health of patients (10). The present findings show that, as compared with OHI alone, NSPT significantly improved all clinical variables (GBI, PPD, and PAL) except VPI at 3 months after treatment. Although microbial counts of *A. actinomycetemcomitans* and *T. forsythia* significantly decreased after NSPT, comparisons of the NSPT and OHI groups showed no significant difference between groups at 3 months after treatment.

The treatment for the NSPT group comprised oral hygiene instruction, single-visit scaling, and root planing, followed by chlorhexidine mouthwash. Professional prophylaxis was performed at recall visits. This protocol was similar to a previously reported protocol comprising single-visit, full-mouth disinfection and adjunctive

Table 6 Multivariate analysis of clinical variables and microbiological profiles using a general linear model procedure to control for type 2 diabetes

Clinical variables	Treatment	Mean count	Standard deviation	*P* value
VPI	OHI	39.24	17.58	
	NSPT	26.78	24.96	0.11
GBI	OHI	16.38	15.89	
	NSPT	27.91	15.43	0.04
PPD	OHI	0.37	0.25	
	NSPT	0.72	0.37	0.05
PAL	OHI	0.38	0.27	
	NSPT	0.61	0.37	0.05
Periodontal pathogens				
Actinobacillus actinomycetemcomitans	OHI	18.0×10^7	47.0×17^7	
	NSPT	5.7×10^7	23.0×10^7	0.09
Tanarella forsythia	OHI	0.024×10^7	0.59×10^7	
	NSPT	0.19×10^7	0.35×10^7	0.14
Prevotella intermedia	OHI	0.02×10^7	0.37×10^7	
	NSPT	0.062×10^7	0.36×10^7	0.36
Porphyromonas gingivalis	OHI	2.3×10^7	1.0×10^7	
	NSPT	0.006×10^7	0.42×10^7	0.11

VPI: visible plaque index, GBI: gingival bleeding index, PPD: probing pocket depth, PAL: periodontal attachment loss, NSPT: nonsurgical periodontal therapy, OHI: oral hygiene instructions.

use of chlorhexidine mouthrinse (18). Several studies reported that the combination of NSPT with chlorhexidine significantly reduced PPD and PAL levels (18,19). Because of ethical concerns, rather than not receiving any treatment, the controls in this study received oral hygiene instruction, which is the most important service a dental professional can provide and the cornerstone of good dental health. As soon as the controls completed the study protocol after 3 months, they received individualized periodontal treatment.

Patients with uncontrolled diabetes were excluded from the current study because studies reported an association between type 2 diabetes and periodontitis (20). In the present study, participants with type 2 diabetes and an HbA1c level less than 7% were included. The response to periodontal treatment was similar in patients with and without well-controlled type 2 diabetes (12).

Improvement in VPI was similar in the two groups because both groups received comparable OHI. Individualized OHI—including toothbrushing and individualized interdental cleaning—motivation, and oral hygiene reinforcement were provided by trained postgraduate students (W.N.A.W.A. and R.P.C.R.) and was beneficial in removing supragingival dental biofilm in both groups during the 3-month study period, even though supragingival deposits were present in the OHI group. However, when the NSPT and OHI groups were compared, improvements in GBI, PPD, and PAL were significantly greater for the NSPT group, which confirmed that cause-related therapies such as NSPT are more effective than OHI alone.

Our findings confirm those of previous studies, which showed that individually, NSPT (21) and OHI (22) improved all clinical variables. A previous study found that subgingival root brushing eliminated nearly all bleeding on probing at tested sites and reduced PPD by a mean of 1.8 mm. In addition, deep periodontal pockets were clinically improved by rigorous OHI even in the absence of subgingival debridement (NSPT) (23). Although we found a significant improvement in PPD and PAL in the OHI group at 3 months after treatment, site-level assessment showed that only shallow and moderately deep pockets significantly improved. The number of sites with deep pockets (>6 mm) was not significantly lower after 3 months. Improvements in supragingival plaque control brought about by OHI might result in partial shrinkage of the gingiva and reduced probing depths at periodontally involved shallow sites but not deeper sites (24). In the NSPT group, although there was a decrease in the number of deep sites at 3 months, the change was not significant, perhaps because of the very small number of sites with deep pockets in both groups.

At the site level, there was a significant reduction in PAL in deep sites in both groups, even though PPD did not significantly decrease at these sites (25,26). This significant gain in attachment level might have been due to healing at very deep sites after NSPT or OHI (27). Longitudinal studies indicate that NSPT results in significant attachment gain but unpredictable PPD reduction, as pocket formation starts to recur after treatment (25).

A site-level comparison of the two treatments showed no significant difference in GBI, PPD, or PAL, in contrast to findings at the group level. The participants in this study had localized disease with a low prevalence of deep and moderate pockets at baseline. This may be a limitation of the present study, and a future study should examine patients with more generalized severe disease, to determine if changes in periodontal pathogens in the generalized and more severe forms of the disease are associated with improvement in clinical characteristics.

In the current study, the baseline prevalences of tested pathogens were higher than in previous studies (10,28), perhaps because of differences in ethnicity (29) and the microbial diagnostic techniques used (30). At 3 months after treatment, the prevalences of pathogens generally declined, but the changes were not significant. It should be mentioned that the presence of these pathogens does not necessarily indicate that disease is or will be present, as these pathogens are also present in healthy individuals (31). However, Darby et al. reported that clinical variables improved with significant reductions in the prevalence of *A. actinomycetemcomitans, P. gingivalis, P. intermedia,* and *T. forsythia* after NSPT (32).

In contrast to our data on prevalence, we observed significant decreases in microbial counts of *A. actinomycetemcomitans* and *T. forsythia* after NSPT. However, in the OHI group, the microbial counts of *A. actinomycetemcomitans* and *P. gingivalis* increased, although the change was not significant. These findings are consistent with those of previous studies, which found that reductions in *A. actinomycetemcomitan*s and *T. forsythia* were associated with improved clinical findings after periodontal therapy (33,34). Although OHI brought about a significant reduction in VPI, this was not enough to change the subgingival microbiota profile. This finding contradicts a previous hypothesis that a decrease in gingival inflammation and gingival crevicular fluid flow will result in reduced nutrition for subgingival plaque after supragingival plaque control (35,36).

A comparison of the two treatment groups showed no significant change in any of the tested pathogens, perhaps because of the small sample size in this study. The sample size calculation was performed using the primary outcome variable, post-treatment change in PAL, to achieve 80% power. However, although the 28 participants in each group may have been sufficient for analysis of the primary outcome, the study might have been underpowered for assessing microbial changes. In contrast, studies with larger samples (>100 participants) and 6 to 12 months of follow-up yielded more significant results (7). In addition, because of ethical considerations, we were unable to extend the study period. A longer study might have yielded findings similar to those reported by Knöfler et al. (37) and Liu et al. (38). Thus, the small sample size and short duration of 3 months are limitations of this study.

None of the changes in clinical characteristics was associated with changes in the periodontal pathogens studied. These findings corroborate those of previous findings, which also found no association between clinical variables and periodontal pathogens such as *A. actinomycetemcomitans* and *P. intermedia* (10). However, the present findings contradict those of studies reporting a positive correlation of number of *P. gingivalis* with PPD and PAL (39,40). There are a number of possible explanations for this inconsistency, including differences in disease status at the sampled site and the method used to detect and estimate the copy numbers of pathogens present (41). Loomer reported that analysis of the overall oral microbiota profile of a patient is more representative when a greater number of sites is sampled (42). Thus, in this study, samples from at least five sites with the deepest pockets were collected and pooled together, which was essential in accurately characterizing subgingival microbiota in its natural niche in the deepest pockets.

NSPT was more effective than OHI in improving clinical variables and reducing pathogen counts. The combined improvement in clinical characteristics and reduction in microbiological counts contribute to long-term treatment stability. However, the value of OHI alone should not be overlooked in regions where periodontal treatment is not available, as it significantly improved the clinical characteristics of shallow and moderate pockets at 3 months after treatment. This may have implications for public health services, particularly in rural populations.

Within the limitations of this study, we conclude that improvements in GBI, PPD, and PAL at 3 months after treatment were significantly greater in the NSPT group than in the OHI group. In the NSPT group, microbial counts for *A. actinomycetemcomitans* and *T. forsythia* were significantly lower at 3 months after treatment. None of the clinical variables was associated with microbiological variables.

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Conflict of interest

None declared.

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