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Effect of nonsurgical periodontal treatment on clinical periodontal variables and salivary resistin levels in obese Asians

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Abstract: This study investigated changes in periodontal outcomes after nonsurgical periodontal treatment (NSPT) and evaluated associations of change in salivary resistin level with periodontal outcomes in obese Malaysians with chronic periodontitis. Sixty-two obese adults with chronic periodontitis were randomly divided into a test group (*n* = 31), which received NSPT, and a control group (*n* = 31), which received no treatment. Plaque score (PS), gingival bleeding index (GBI), probing pocket depth (PPD), and clinical attachment loss (CAL) were measured at baseline and at 6 and 12 weeks after NSPT. Salivary resistin levels were evaluated by using an enzyme-linked immunosorbent assay. PS was significantly lower in patients who received NSPT than in the control group at 6 and 12 weeks (*P* < 0.05). In the NSPT group the percentages of sites with shallow and moderate pockets decreased significantly, but there was no significant change in deep pockets. Resistin levels significantly decreased after NSPT (*P* < 0.05). Change in salivary resistin level was not significantly associated with periodontal outcomes. In obese Malaysians, NSPT significantly improved PS and GBI, and improved PPD and CAL for shallow and moderately deep pockets but not for deep pockets. Salivary resistin level was not associated with improvement in either periodontal variable.

Keywords: obesity; chronic periodontitis; resistin; dental scaling; root planing; clinical trial.

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Introduction

Recent systematic reviews (1-4) have reported that obesity is a risk factor for development of chronic periodontitis (CP). Although the pathophysiological mechanisms for this association are unclear, it has been suggested that, through secretion of a variety of interleukins and other bioactive mediators, called adipokines, adipose tissue may be important in the pathogenesis of periodontal disease (5).

Resistin is a 12.5-kilodalton (kD) adipokine from the cysteine-rich protein family and is elevated in obese individuals (6). In humans, resistin is largely expressed in bone marrow and may also be present in trophoblastic cells of placenta, pancreas, primary cell leukemia, synovial fluid, synovial tissue, and circulating blood (7,8). Monocytes and macrophages are the primary cells for resistin expression in white adipose tissue (7,9). Elevated levels of proinflammatory cytokines (such as interleukin [IL]-6, IL-1β, and tumor necrosis factor [TNF]-α) were reported to be associated with periodontal disease progression (10-12). Among these cytokines, IL-6 and TNF-α regulate resistin expression in peripheral blood mononuclear cells (13). A significant positive correlation between levels of resistin and pro-inflammatory markers has also been reported (8). Although the role of resistin in the human inflammatory pathway is unclear, some studies have shed light on its role in inflammation through the nuclear factor-κB pathway.

Several studies have shown that resistin levels range from 9.9 to 12.34 ng/mL in, presumably non-obese, individuals with CP, which is higher than levels observed in people (14,15) without CP. A few clinical studies reported no change in resistin levels after periodontal treatment for CP (16-19). Saliva is used as a diagnostic tool to evaluate various biomarkers associated with periodontal disease (20), and previous studies collected saliva to monitor inflammatory cytokines such as resistin (21,22). Salivary resistin level is a potential local inflammatory marker for periodontitis in obese persons. Although periodontal disease can be site-specific, saliva may be useful in identifying periodontal destruction and can thus form the basis of a salivary diagnostic test for periodontal disease (20).

Several studies (23-27) have examined periodontal outcomes after nonsurgical periodontal treatment (NSPT) for CP in obese patients. Although some studies reported that obesity did not affect clinical attachment gain or probing pocket depth (PPD) reduction after periodontal treatment (25,26,28), other studies showed that patients with a higher body mass index (BMI) had a poorer response to NSPT (19,24). It remains to be determined whether NSPT improves periodontal outcomes in obese patients (4). These contradictory findings suggest a need to examine the association between obesity and CP and to determine whether periodontal treatment can regulate the systemic inflammatory burden in obese patients. In this study, we investigated changes in periodontal outcomes after NSPT in obese Malaysians with CP and evaluated the association of post-NSPT change in salivary resistin level with periodontal outcomes.

Materials and Methods

Trial design and study settings

This study was a 12-week, parallel-arm, randomized controlled clinical trial that was designed, conducted, and reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) statement and registered at www.clinicaltrials.gov (number NCT02508415). This study was conducted at the Postgraduate Clinic, Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia and was approved by the Medical Ethics Committee of the Faculty of Dentistry, University Malaya (DF RD 13080/0080(P)).

Sample size calculation

The sample size was calculated based on an expected mean difference of 2 mm in the reduction of PPD between the control and test groups (28). At least 26 patients would be needed in each group to detect a difference with 80% power at a significance level of 0.05. With an anticipated 20% drop-out, 66 eligible participants were recruited: 33 patients per group.

Inclusion and exclusion criteria

The inclusion criteria for participant recruitment were age 30-66 years; ability to give informed consent; a BMI ≥ 27.5 kg/m^2 (29); presence of at least 12 teeth, excluding third molars; and a diagnosis of CP (30) (i.e., ≥2 interproximal sites with clinical attachment loss [CAL] ≥ 4 mm [not for the same tooth] or ≥2 interproximal sites with PPD ≥ 5 mm [not for the same tooth] at baseline). Patients were excluded if they were pregnant or lactating mothers, if they had any medical condition requiring prophylactic antibiotic administration before dental treatment, if they had received periodontal treatment during the previous 6 months, if they had an intellectual disability that might interfere with oral hygiene procedures, or if they were not Malaysian. Patients were also excluded if they reported the presence of systemic conditions that could affect progression of periodontitis, or weight gain/loss (e.g., diabetes mellitus, autoimmune diseases, immunological disorders, hormonal disorders, osteoporosis) or other inflammatory conditions.

Population

Participants were recruited from Bilik Rawatan Utama (Primary Dental Care Unit) Clinic, Faculty of Dentistry, University of Malaya, Malaysia from September 2013 to April 2014. Eligible participants were informed verbally and in writing of the purpose and procedures of the study in the local language (Malay, Chinese, or Tamil) or English, after which written informed consent

was obtained. Medical and dental histories, including smoking status, were recorded on a questionnaire. Block randomization was then used to assign participants to test and control groups. The participants were divided into blocks of 10 participants. Numbers were written on a piece of paper and shuffled in a box. The first five randomly selected numbers were assigned to the test group and the next five to the control group. The control group received no treatment or oral hygiene instructions. The test group received NSPT comprising scaling and root planing with oral hygiene instructions.

Anthropometric measurements

Anthropometric measurements were performed in the morning by the same trained examiner (Z.A.). The measurements included weight, height (m), waist (cm), and hip circumference (cm). BMI was calculated as body weight divided by the square of the height (m). The waist-hip ratio (WHR) was calculated as waist circumference (cm) divided by hip circumference (cm). Obesity was classified by using World Health Organization guidelines for Asians, namely, a BMI $\geq$ 27.5 kg/m^2 (29), a WHR $\geq$ 0.85 for women, and a WHR $\geq$ 0.90 for men (31).

Saliva sampling

Unstimulated whole saliva was sampled (5 mL) from each patient on the same day before clinical examination. Participants were comfortably seated on the chair with their head bent slightly forward. Patients were instructed to collect saliva in the mouth without oral movement and to expectorate the saliva into a Falcon tube (Fisher Scientific, Chicago, IL, USA). The patients were also instructed to refrain from any muscle movement during saliva collection. All samples were stored at −80°C until analysis.

Periodontal examination

Full-mouth plaque score (PS) (32), gingival bleeding index (GBI) (32) (presence or absence), and CAL were measured by a calibrated examiner (Z.A.) using a UNC-15 periodontal probe (Hu-Friedy, Chicago, IL, USA) at six sites per tooth (mesio-facial, mid-facial, disto-facial, mesio-lingual, mid-lingual, and disto-lingual). Sites were divided into three subcategories according to PPD and CAL, which were categorized as shallow (0-3 mm), moderate (4-6 mm), and deep (>6 mm). Periodontal examinations were performed at baseline and 6 and 12 weeks after therapy.

NSPT

Participants in the test group were given instruction on oral hygiene care, including toothbrushing with the modified Bass technique (33) and interdental cleaning, and underwent scaling and root planing (SRP), which was performed under local anesthesia by a trained dentist (Z.A.) using an ultrasonic scaler (SATELEC P5 Newtron XS, Cambridge, UK) and Gracey curettes (Hu-Friedy) at a single appointment. SRP was performed until the root surfaces were satisfactorily smooth. Participants were instructed to rinse with 0.12% chlorhexidine gluconate mouthwash (Hexipro Evapharm, Kuala Lumpur, Malaysia) 3 times a day for 2 weeks after completion of SRP (34). No antibiotics were prescribed after the treatment. Professional prophylaxis, including re-motivation and personally tailored oral hygiene instruction, was given to the test group at each recall visit (6 and 12 weeks after SRP).

Analysis of resistin in saliva

An enzyme-linked immunosorbent assay kit (Resistin [RETN] Human ELISA kit; Abcam, Cambridge, UK) was used to measure resistin concentration in accordance with the manufacturer's instructions. Fifty microliters of the respective saliva samples was dispensed, in duplicate, into wells coated with a resistin-specific antibody. The plates were then incubated at room temperature for 120 min, after which they were washed 3 times manually with wash buffer. Fifty µL of biotinylated resistin antibody was then added to the wells and allowed to incubate for 120 min. Conjugate solution (50 µL) was then added, and the plates were incubated at room temperature for another 30 min. The wells were washed once again 3 times with a wash solution, after which 50 µL of chromogen substrate solution was added. The plates were incubated for 12 min at room temperature until the optimal blue color density was achieved. Then, 50 µL of stop solution was added to terminate color development. Absorbance was determined by reading the plate at a wavelength of 450 nm on a microplate reader (VersaMax ELISA Microplate Reader, Cambridge, UK). The procedure was repeated with different concentrations of standard resistin, and the absorbance of the different resistin concentrations was then used to plot the standard curve, which was then utilized to determine the resistin concentration in samples. Resistin concentration was expressed in ng/mL.

Statistical analysis

Statistical analyses were performed using statistical software (SPSS, v.20.0 for Windows, IBM, Chicago, IL, USA). For all tests, the level of significance was set at 0.05. Data were expressed as means and standard deviations. Normality of distribution of the variables was

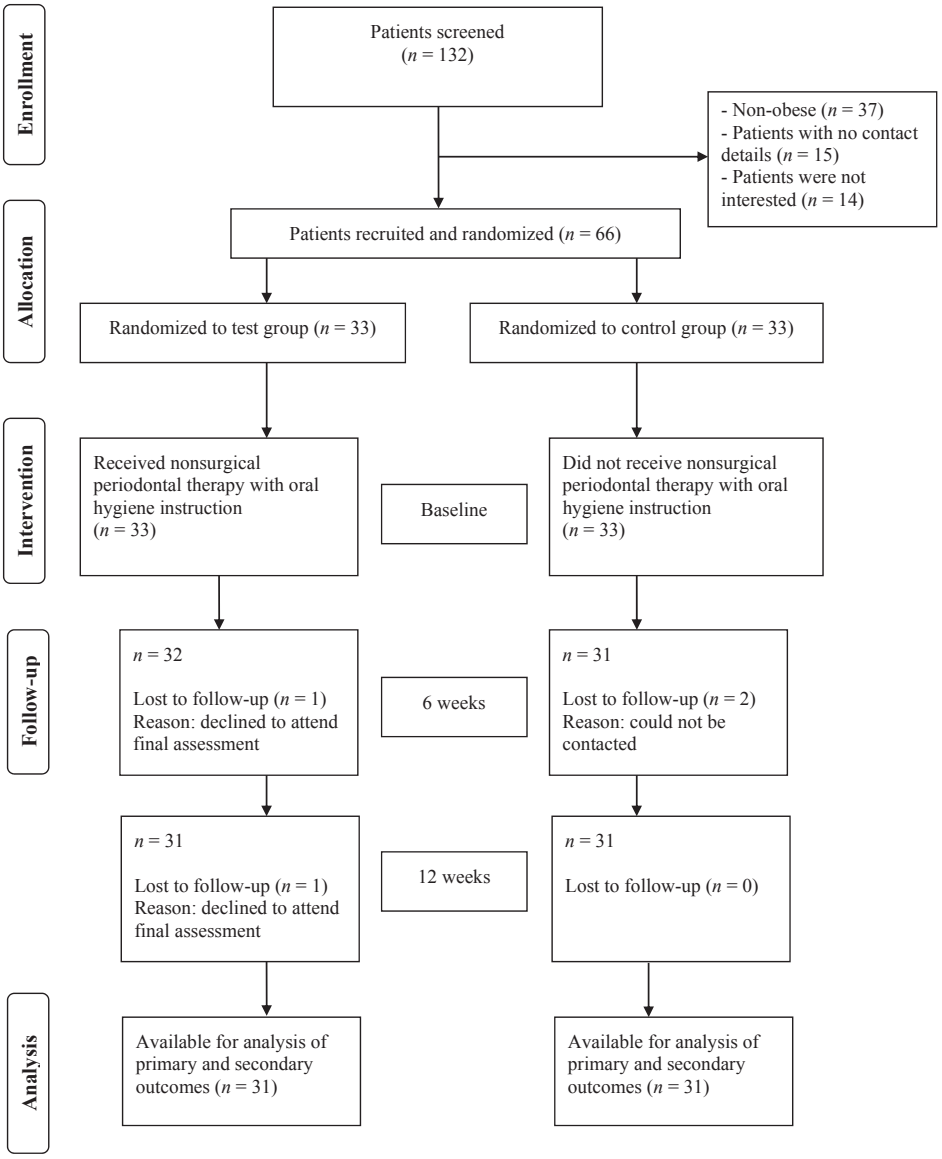

Fig. 1 Study flow diagram.

tested with the Kolmogorov-Smirnov and Shapiro-Wilk tests and confirmed with Q-Q plots. Changes in clinical variables (PS, GBI, PD, CAL) within groups before and after periodontal treatment were assessed with the Friedman test. Comparison of changes in resistin levels within groups was performed using the Wilcoxon signed rank test. The Mann-Whitney *U* test was used to compare measurements between groups. The Bonferroni post-hoc adjustment test was used for multiple comparisons. The Spearman rank correlation coefficient test was used to evaluate correlations between change in salivary resistin level and periodontal outcomes. Stepwise logistic regression analysis was used to identify explanatory variables for reduction in PPD and CAL, after controlling for the effects of other covariates. Odds ratios and 95% confidence intervals were used to assess the direction and strength of associations.

Results

Retention

Sixty-six obese adults with a diagnosis of CP entered the study at baseline. In the test group, one participant at the 6-week examination and one participant at the 12-week examination declined to attend the final assessment. Two patients from the control group did not return for the 6-week follow-up examination because they could not be contacted. These four patients were therefore excluded from the analysis. Figure 1 shows the flow diagram of the study.

Demographic and anthropometric characteristics

Demographic data are presented in Table 1. Sex, age,

Table 1 Demographic characteristics, smoking status, anthropometric variables, and number of teeth for study participants

Characteristic	Control group (n = 31)	Test group (n = 31)	P value
Age (years, mean ± SD)	44.84 ± 9.02	44.68 ± 10.63	0.79[a]
Sex, *n* (%)			
Male	9 (32.3)	8 (25.8)	0.78[b]
Female	22 (67.7)	23 (74.2)	
Race, *n* (%)			
Malay	24 (77.4)	20 (64.5)	
Chinese	4 (12.9)	2 (6.5)	0.132[b]
Indian	3 (9.7)	9 (29.0)	
Education level, *n* (%)			
Primary	2 (6.5)	4 (15.6)	
Secondary	16 (51.6)	14 (43.8)	0.327[b]
Tertiary	13 (41.9)	13 (40.6)	
Smoking, *n* (%)			
Smoker	6 (19.4)	5 (16.1)	0.89[b]
Nonsmoker	25 (80.6)	26 (83.9)	
WHR (mean ± SD)	0.92 ± 0.09	0.88 ± 0.08	0.11[c]
BMI (kg/m², mean ± SD)	35.83 ± 5.75	32.98 ± 2.89	0.12[a]
Number of teeth (mean ± SD)	26.24 ± 4.3	25.79 ± 5.1	0.46[a]

SD, standard deviation; WHR, waist-hip ratio; BMI, body mass index; [a]Mann-Whitney *U* test; [b]Pearson chi-square test; [c]Paired-sample *t*-test.

Table 2 Plaque score and gingival bleeding index at baseline, 6 weeks, and 12 weeks

Variables	Test group (n = 31) (mean ± SD)	Control group (n = 31) (mean ± SD)	P value
Plaque Score			
Baseline	0.81 ± 0.22	0.81 ± 0.20	0.89
6 weeks	0.22 ± 0.22	0.41 ± 0.25	0.002*
12 weeks	0.11 ± 0.16	0.27 ± 0.25	0.001*
P_1	<0.05*	<0.05*	
P_2	<0.05*	<0.05*	
P_3	<0.05*	<0.05*	
P_4	<0.05*	<0.05*	
Δ_1	0.59 ± 0.29	0.41 ± 0.32	$P^{\S}$ = 0.02*
Δ_2	0.70 ± 0.23	0.54 ± 0.31	$P^{\dagger}$ = 0.01*
Gingival Bleeding Index			
Baseline	0.77 ± 0.25	0.83 ± 0.16	0.25
6 weeks	0.35 ± 0.28	0.51 ± 0.25	0.007*
12 weeks	0.21 ± 0.23	0.34 ± 0.29	0.03*
P_1	<0.05*	<0.05*	
P_2	<0.05*	<0.05*	
P_3	<0.05*	<0.05*	
P_4	<0.05*	<0.05*	
Δ_1	0.42 ± 0.33	0.32 ± 0.28	$P^{\S}$ = 0.27
Δ_2	0.56 ± 0.24	0.49 ± 0.33	$P^{\dagger}$ = 0.50

*significant, $P < 0.05$. P_1, P value for comparison between baseline and 6-week time points; P_2, P value for comparison between baseline and 12-week time points; P_3, P value for comparison between 6-week and 12-week time points; P_4, P value for comparison between baseline, 6-week and 12-week time points; Δ_1, mean difference from baseline to 6 weeks; Δ_2, mean difference from baseline to 12 weeks; $P^{\S}$, P value for mean differences between groups from baseline and 6-week time points; $P^{\dagger}$, P value for mean differences between groups from baseline and 12-week time points.

race, and education level did not significantly differ between the test and control groups ($P > 0.05$). There was no significant difference between groups in smoking status, BMI, or WHR at baseline ($P > 0.05$).

Clinical variables

The baseline findings for PS, GBI, PPD, and CAL were similar ($P > 0.05$) in the groups (Tables 2, 3). All patients had localized PPD ≥ 5 mm. At 6 weeks, mean PS had decreased from 0.81 to 0.22 ($P < 0.05$) in the test group and from 0.81 to 0.41 ($P < 0.05$) in the control group. The change in PS and mean difference in PS between groups over 6 weeks was statistically significant ($P < 0.05$). Similarly, GBI at 6 weeks had decreased from

Table 3 Probing pocket depths (PPD) and clinical attachment loss (CAL) at baseline, 6 weeks, and 12 weeks in the test and control groups

Variable	Test group (n = 31)		Control group (n = 31)		P^+	P^{++}
	Mean PPD (mean ± SD)	Mean CAL (mean ± SD)	Mean PPD (mean ± SD)	Mean CAL (mean ± SD)		
Baseline	2.33 ± 0.44	2.89 ± 0.61	2.40 ± 0.37	3.06 ± 0.66	0.34	0.18
6 weeks	1.93 ± 0.44	2.45 ± 0.63	2.08 ± 0.50	2.61 ± 0.69	0.27	0.39
12 weeks	1.96 ± 0.47	2.45 ± 0.59	2.11 ± 0.41	2.63 ± 0.67	0.17	0.29
P_1	<0.05*	<0.05*	<0.05*	<0.05*		
P_2	<0.05*	<0.05*	<0.05*	<0.05*		
P_3	0.66	0.97	0.78	0.98		
P_4	<0.05*	<0.05*	<0.05*	<0.05*		
Δ_1	0.41 ± 0.36	0.44 ± 0.42	0.31 ± 0.42	0.43 ± 0.55	$P^§$ = 0.42	$P^§$ = 0.91
Δ_2	0.38 ± 0.30	0.44 ± 0.35	0.28 ± 0.39	0.41 ± 0.47	$P^†$ = 0.10	$P^†$ = 0.98
	Sites with PPD ≤ 3mm (% ± SD)	Sites with CAL ≤ 3mm (% ± SD)	Sites with PPD ≤ 3mm (% ± SD)	Sites with CAL ≤ 3mm (% ± SD)		
Baseline	90.67 ± 9.76	78.49 ± 16.97	87.41 ± 9.97	73.27 ± 16.41	0.44	0.21
6 weeks	96.28 ± 7.26	89.97 ± 16.74	93.42 ± 10.24	84.88 ± 18.80	0.21	0.27
12 weeks	96.34 ± 7.18	85.96 ± 16.39	93.08 ± 8.85	80.40 ± 18.12	0.12	0.18
P_1	0.000	0.000	0.002	0.000		
P_2	0.000	0.000	0.034	0.000		
P_3	1.000	1.000	1.000	1.000		
	Sites with PPD 4-6 mm (% ± SD)	Sites with CAL 4-6 mm (% ± SD)	Sites with PPD 4-6 mm (% ± SD)	Sites with CAL 4-6 mm (% ± SD)		
Baseline	8.91 ± 8.90	24.46 ± 14.53	12.28 ± 9.82	28.90 ± 13.31	0.35	0.20
6 weeks	2.74 ± 6.60	13.97 ± 13.68	6.17 ± 8.20	19.24 ± 15.01	0.07	0.15
12 weeks	3.44 ± 6.57	12.94 ± 13.77	6.50 ± 7.94	18.27 ± 15.68	0.10	0.16
P_1	0.000	0.000	0.000	0.000		
P_2	0.000	0.000	0.002	0.000		
P_3	1.000	1.000	1.000	1.000		
	Sites with PPD > 6mm (% ± SD)	Sites with CAL > 6mm (% ± SD)	Sites with PPD > 6mm (% ± SD)	Sites with CAL > 6mm (% ± SD)		
Baseline	0.40 ± 1.24	18.41 ± 22.06	0.35 ± 0.78	13.34 ± 18.26	0.18	0.31
6 weeks	0.58 ± 1.10	19.09 ± 21.25	0.48 ± 1.55	12.09 ± 16.99	0.13	0.16
12 weeks	0.22 ± 0.70	1.09 ± 3.09	0.43 ± 1.30	1.33 ± 3.25	0.43	0.77
P_1	1.000	1.000	1.000	0.544		
P_2	1.000	1.000	0.231	1.000		
P_3	1.000	1.000	0.368	1.000		

Intergroup P values for PPD (%) determined using independent sample t-test. Intragroup P values for mean PPD determined using Bonferroni adjustment. *Significant at $P < 0.05$; P_1, P value for comparison between baseline and 6-week time points; P_2, P value for comparison between baseline and 12-week time points; P_3, P value e for comparison between 6-week and 12-week time points; P_4, P value for comparison between baseline, 6-week, and 12-week time points; Δ_1, mean difference from baseline to 6 weeks; Δ_2, mean difference from baseline to 12 weeks; P^+, intergroup P value for PPD; P^{++}, intergroup P value for CAL; $P^§$, P value for mean differences between groups from baseline and the 6-week time points for PPD; $P^†$, P value for mean differences between groups from baseline and the 12-week time points for CAL.

0.77 to 0.35 ($P < 0.05$) in the test group and from 0.83 to 0.51 ($P < 0.05$) in the control group. Change in GBI over 6 weeks significantly differed between the test and control groups ($P < 0.05$) (Table 2). The intragroup mean decrease in PPD between baseline and 6 weeks was significant in both groups. Mean PPD decreased from 2.33 mm to 1.93 mm in the test group and from 2.40 mm to 2.08 mm in the control group. When PPD sites were further assessed in relation to initial PPD, the percentage of sites with a PPD of ≤3 mm increased in the test group, the percentage of sites with a PPD of 4-6 mm decreased in both groups, and the percentage of sites with a PPD of >6 mm slightly increased in both groups. There was no significant difference between groups over time (Table 3). Similarly, mean CAL decreased from 2.89 mm to 2.45 mm in the test group and from 3.06 mm to 2.61 mm in the control group. The percentage of sites with shallow pockets increased in both groups and the percentage of sites with moderate pockets decreased in both groups. However, the percentage of sites with deep pockets did not significantly change in either group. There was no significant difference between groups over time (Table 3).

At 12 weeks, mean PS further decreased, from 0.22 to 0.11 ($P < 0.05$) in the test group and from 0.41 to 0.27 ($P < 0.05$) in the control group. Change in PS over 12 weeks significantly differed between the test and control groups ($P < 0.05$). Similarly, GBI at 12 weeks further

Table 4 Change in salivary resistin level (ng/mL) after nonsurgical periodontal therapy

Variable resistin (ng/mL)	Test group (n =31) (mean ± SD)	Control group (n = 31) (mean ± SD)	P value
Baseline	12.26 ± 1.24	14.25 ± 4.58	0.001*[b]
12 weeks	11.62 ± 0.90	13.47 ± 5.20	0.055[b]
P_1	0.002*[a]	0.48[a]	
Δ	0.65 ± 1.24	0.78 ± 4.08	$P^{\dagger}$ = 0.418[b]

*Significant at $P < 0.05$. [a]Wilcoxon signed ranks test; [b]Mann-Whitney U test; P_1, P value for comparison between baseline and 12-week time points; $P^{\dagger}$, P value for mean difference between groups.

Table 5 Correlations of change from baseline to 12 weeks in salivary resistin level with periodontal variables

Correlation coefficient	Δ Resistin	
	S[a]	P
PD	0.02	0.93
CAL	−0.16	0.40
PS	0.14	0.44
GI	−0.009	0.96

[a]Spearman rank correlation coefficient.

Table 6 Results of logistic regression analysis of associations of selected variables with periodontal variables

	Odds ratio	95% CI	P value
Mean PPD (≥0.5 mm)			
Smoking	1.03	0.80-13.20	0.98
Resistin level	0.82	0.39-1.72	0.60
Pseudo-R^2 = 0.015			
Mean CAL (≥0.5 mm)			
Smoking	0.64	0.06-7.55	0.72
Resistin level	0.60	0.28-1.29	0.19
Pseudo-R^2 = 0.125			

CI, confidence interval.

decreased, from 0.35 to 0.21 ($P < 0.05$) in the test group and from 0.51 to 0.34 ($P < 0.05$) in the control group. Change in GBI over 12 weeks significantly differed between the test and control groups ($P < 0.05$), but the mean differences over time were not significant (Table 2). The intragroup mean decrease in PPD between baseline and 12 weeks was significant in both groups. When PPD sites were further analyzed in relation to initial PPD, the percentages of sites with shallow, moderate, and deep pockets did not change in either group from 6 weeks to 12 weeks, although the change from baseline to 12 weeks was significant for shallow and moderate pockets in both groups. There was no significant difference between groups over time (Table 3). Similarly, intragroup mean CAL significantly decreased in both groups. The percentages of sites with shallow, moderate, and deep pockets did not change in either group from 6 to 12 weeks, although the change from baseline to 12 weeks was significant for shallow and moderate pockets in both groups. There was no significant difference between groups over time (Table 3).

Salivary resistin

Changes in salivary resistin level at 12 weeks after periodontal treatment are shown in Table 4. At baseline, the mean resistin level was 12.26 ± 1.24 ng/mL in the test group and 14.25 ± 4.58 ng/mL in the control group ($P < 0.05$). Resistin level significantly decreased after periodontal therapy ($P < 0.05$) in the test group but not in the control group (mean difference: 0.65 ± 1.24 ng/mL and 0.78 ± 4.08 ng/mL, respectively). Change in resistin level at 12 weeks did not significantly differ between groups.

None of the clinical periodontal variables were correlated with change in salivary resistin level ($P > 0.05$) (Table 5). Logistic regression analysis revealed that change in salivary resistin level was not significantly associated with improvement in PPD or CAL, even after controlling for smoking ($P > 0.05$) (Table 6).

Discussion

The present results indicate that NSPT significantly improved PS and GBI in obese adults with CP. The significant differences between groups in PS at 12 weeks could be attributable to the repeated professional debridement provided and to the absence of plaque-retentive factors in the test group, which simplified plaque control and reduced local inflammation. In our study, the significant changes in the clinical periodontal variables in the control group may have been the result of the Hawthorne effect (35). In other words, although the control group did not receive any form of periodontal treatment, PS and GBI may have decreased because of self-improved oral hygiene in response to participant awareness of being observed while participating in the trial. The assumed favorable effect of this awareness was also seen for PPD and CAL. However, the observed improvements were limited to shallow and moderately deep pockets. Other studies have reported similar clinical outcomes, namely, improvement in moderate pocket depth with oral hygiene alone (36,37).

To our knowledge, this is the first randomized clinical trial that included a control group that did not receive any form of periodontal treatment (negative control), which allowed us to estimate the real magnitude of the effect of NSPT in obese CP patients. Previous studies compared the effects of periodontal treatment in obese and non-obese participants with CP and found improved clinical periodontal characteristics at follow-up in both groups after full-mouth scaling and root planing (19,25-28). In the present study, participants in the control group were assured that they would be given periodontal treatment at the end of the trial. Suspending treatment for 12 weeks was not considered unethical, because of the low risk of disease progression over that period of time (38). After completion of the trial, participants in the control group immediately received individualized periodontal treatment.

The clinical data showed significant improvement in PS and GBI in obese participants with CP. There were significant changes in mild (≤3 mm) and moderate (4-6 mm) PPD and CAL in both groups, whereas severe (>6 mm) PPD and CAL did not exhibit significant improvement at any time-point in either group. These findings are consistent with those of recent studies, which reported persistent residual periodontal pockets of >6 mm, even after NSPT, in obese participants (27,39). These data suggest that obesity compromises periodontal healing, especially in deep periodontal pockets. This hypothesis requires confirmation in larger-scale studies of patients with generalized advanced periodontitis.

Improvements in clinical periodontal variables in the test group were associated with a significant decrease in mean salivary resistin level. Mean salivary resistin level also decreased in the control group but the change was not statistically significant. These improvements might result from any form of periodontal intervention, even toothbrushing, because the outcome was reduction of inflammation. This indicates that NSPT decreased salivary resistin levels. The present test and control groups did not differ in change in salivary resistin level at 12 weeks. However, it should be noted that the levels in the two groups were significantly different at baseline, which makes comparison difficult. Resistin plays an important role in inflammation, and resistin levels are elevated in obese persons (15,40) and individuals with CP (14,41). The presence of inflammatory chronic diseases such as diabetes mellitus, obesity, and rheumatoid arthritis in a person with CP might further increase the level of systemic resistin (20,42).

Our results show that changes in periodontal variables did not correlate with change in salivary resistin level after NSPT. The reason for this finding is that only shallow and moderately deep, but not deep, periodontal pockets exhibited improvement. Other studies have investigated salivary resistin levels but only in patients with generalized and advanced disease (43). Saliva might not be sensitive enough to detect changes in cytokine levels caused by localized inflammatory conditions. To increase sensitivity, use of gingival crevicular fluid would be a better choice.

Although the present sample size was calculated to have sufficient power to detect a 2-mm difference, a meaningful change between groups was not detected because of the presence of localized advanced disease among participants and unfavorable periodontal outcomes for deep periodontal pockets. The control and test groups were appropriate for the use of resistin testing in assessing whether NSPT could reduce the total systemic inflammatory burden in obese participants with CP. However, to evaluate the effects of periodontal therapy on CP, studies should include non-obese, medically fit participants with CP. The inclusion of smokers is an important potential confounder (44,45). However, smoking did not significantly affect periodontal outcomes (change in mean PPD and CAL, ≥0.5 mm; $P > 0.05$).

Periodontal assessment and treatment were performed by a single trained dentist, which may have introduced bias to the evaluations. Because the criterion PPD or CAL rather than PPD and CAL was used to determine periodontal disease, some of the present participants may have presented with shallow pockets on a reduced periodontium rather than true pockets. Moreover, BOP was not considered together with PPD and CAL; therefore, sites with no active disease may have been included. The criteria outlined by Armitage (46) would have been a better choice to identify generalized periodontal disease, as they call for defining diseased sites with a given PPD, CAL, and positive BOP.

Resistin levels were higher among participants in the control group than in the test group at baseline ($P < 0.05$). This may have introduced bias to the findings, which prevented us from comparing change in resistin level between the test and control groups. Matching at baseline was not performed because randomization was done before saliva collection and resistin levels were not measured until later. The difference in resistin levels at baseline was unexpected, as all participants were matched for periodontal disease and BMI criteria. A point worth noting in the present study was our use of the World Health Organization obesity classification for Asians, which defines obesity as a BMI of ≥27.5 kg/m^2 (29). Moreover, assessment of inflammatory cytokines

in gingival crevicular fluid rather than saliva might be a more sensitive method for detecting changes in cytokine levels—and thus periodontal inflammation—particularly in localized disease. These limitations suggest that our findings should be interpreted with caution.

NSPT significantly improved PS and GBI in obese Malaysians with chronic periodontitis and improved PPD and CAL in shallow and moderately deep pockets but not deep pockets. Change in salivary resistin level was not associated with improvement in either periodontal variable.

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Conflict of interest

The authors have no conflict of interest to declare.

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