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Nonsurgical Periodontal Therapy Impacts Salivary and Serum Bone Turnover Markers in Periodontitis

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Abstract

Objectives To assess nonsurgical periodontal therapy's (NSPT's) effect on bone turnover markers (BTMs) in periodontitis patients and compare the efficacy of saliva and serum samples in evaluating periodontal health.

Materials and Methods Sixty-five females aged 18 to 45 years were divided into control ($n = 20$) and test ($n = 45$) groups. Periodontitis patients underwent NSPT. Full mouth clinical periodontal parameters and salivary/serum BTMs (C-terminal telopeptides of type I collagen [CTX], bone-specific alkaline phosphatase [BALP], osteoprotegerin [OPG]) were recorded at baseline, 3 weeks, and 3 months post-NSPT.

Statistical Analysis The study measured test group readings at three points: baseline, 3 weeks, and 3 months after NSPT. Normality was assessed using the Shapiro–Wilk test, showing a nonnormal distribution ($p < 0.05$). To test for significance among groups, the Kruskal–Wallis test was applied. The Friedman test, along with pairwise comparisons, was used to compare test group readings across time points. A 5% significance level was maintained. Pearson correlation measured relationships between the control and test groups and within the test group at different intervals. Multiple linear regression was conducted across time points, with plaque index (PI), gingival index (GI), probing pocket depth (PPD), and clinical attachment level (CAL) set as dependent variables, and salivary and serum BALP, CTX, and OPG as independent variables. All analyses were performed using IBM SPSS version 20 and R version 3.5.2.

Results Following NSPT, significant changes were observed in all measured salivary/serum BTMs except serum BALP ($p > 0.05$). No significant differences noted between BTM measurements taken at 3 weeks and 3 months post-NSPT ($p > 0.05$). Correlations between BTMs and periodontal measurements were weak to moderate

Keywords
- bone turnover markers
- periodontal disease
- nonsurgical periodontal therapy
- saliva
- serum

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after NSPT. A multiple linear regression model explained 46.7% of the total variability for PI, 50.1% for PPD, 54.1% for CAL, and 29.3% for GI signifying association with BTMs among groups.

Conclusion NSPT results in significant changes in salivary/serum BTMs. Following NSPT, BTMs show associations with periodontal parameters, highlighting modulation of host responses. BTMs in salivary/serum samples effectively reflected periodontal health and disease status and improvements after therapy.

Introduction

Periodontal disease is a dynamic process including phases of stability, remission, or progression.[1,2] One of the most common causes of tooth loss among adults is an immune-inflammatory multifactorial disease of supporting tissues of the teeth. Its sequel is bleeding on probing, resorption of alveolar bone, and deepened periodontal pockets.[3–5] As it advances, collagen fibers and cemental surface attachment are lost, along with pocket epithelium migrating apically.[6] Because of its widespread prevalence of 51%[7] and impact on other systemic conditions such as diabetes and insulin resistance, cardiovascular disease, gastrointestinal and colorectal cancer, Alzheimer's disease, respiratory tract infections, adverse pregnancy outcomes, etc., it has become a disease of concern.[8]

A constant remodeling process occurs in bones called the coupling mechanism, which balances osteoclast-mediated resorption and osteoblast-induced deposition of bone. These two processes determine the bone turnover rate. An increased bone turnover rate is associated with increased bone loss. As a result, changes in serum and salivary levels of bone turnover biological markers such as osteoprotegerin (OPG), C-terminal telopeptide region of type I collagen (CTX), receptor activator for nuclear factor k-B ligand (RANKL), bone-specific alkaline phosphatase (BALP), osteocalcin (OC), and osteonectin (ON) are seen.[5] Investigation of salivary OPG, RANKL, OC, and ON has been performed in periodontitis patients with contradictory results.[9] In several studies, there were increased OPG levels in the saliva of some periodontitis patients,[10,11] whereas, in other studies, it was not elevated[12,13] and in a study by Frodge et al,[14] it was undetectable. The reasons for such contradictions in the results are related to either the biomarkers' half-life and clearance or different assay methods and sample storage conditions.[9] Bone turnover markers (BTMs) can be classified as bone resorptive and formative, reflecting osteoclastic and osteoblastic activities, respectively. They have been used as noninvasive and inexpensive tools for studying bone metabolism. However, monitoring treatment response remains their primary usage.

Significant associations between BTMs (serum OC, urinary deoxypyridinoline, and serum BALP) and periodontitis were observed in a study on elderly Japanese people.[15] The use of urine and serum biomarkers to evaluate different aspects of periodontitis has also been demonstrated in some studies.[16] Few studies have demonstrated a reduction in biomarkers after nonsurgical periodontal therapy (NSPT), resulting in better periodontal health.[17] Saliva is often considered a preferred specimen as it is noninvasive, rapid, generally readily abundant, and easily collected.[17] However, very few studies have monitored salivary biomarker profiles following NSPT longitudinally to confirm their accuracy in determining periodontal status.

Identifying these biomarkers is crucial for determining how effective NSPT is in patients with periodontitis. In this light, we hypothesized that NSPT would affect BTM concentration in patients with periodontitis. The objectives of the present study were (1) to compare the impact of NSPT on salivary and serum BTMs in patients with periodontitis and (2) to identify if saliva or serum sample is better for assessing periodontal status. To our knowledge, this is the first longitudinal study comparing salivary and serum BTMs' effectiveness in assessing periodontal treatment outcome.

Materials and Methods

Study Population

This longitudinal interventional prospective study enrolled 65 female patients after screening 500 females of the 18 to 45 age groups. This study continues with a prior study by Zia et al.[18] The study was approved by the Human Subject Ethics Board Institutional Ethics Committee, JNMCH (330/FM/IEC), was conducted in accordance with the Helsinki Declaration of 1975, agreed to the STROBE guidelines for observational studies, and was registered with the Clinical Trial Registry-India (CTRI/2019/09/021214). Patients coming to the outpatient Department of Periodontics and Community Dentistry, Dr. Ziauddin Ahmed Dental College, Aligarh, Uttar Pradesh, India, between November 2019 and January 2021 were recruited for the study. The patients were divided into two groups: the control group ($n = 20$) included subjects who were periodontally and systemically healthy, and the test group ($n = 45$) included subjects with periodontitis (►**Fig. 1**). The test group subjects were followed up to 3 months after treatment.

Periodontitis was diagnosed using the criteria defined by the European Federation of Periodontology and the American Academy of Periodontology.[19] Patients included in the study belonged to both stage I/II/III/IV and grade A periodontitis only. The patients excluded from the study were those who received periodontal therapy in the last 6 months, had a history of smoking, were pregnant and lactating females, and had an intake of alcohol, antibiotics, anti-inflammatory drugs, and any systemic disease that could interfere with

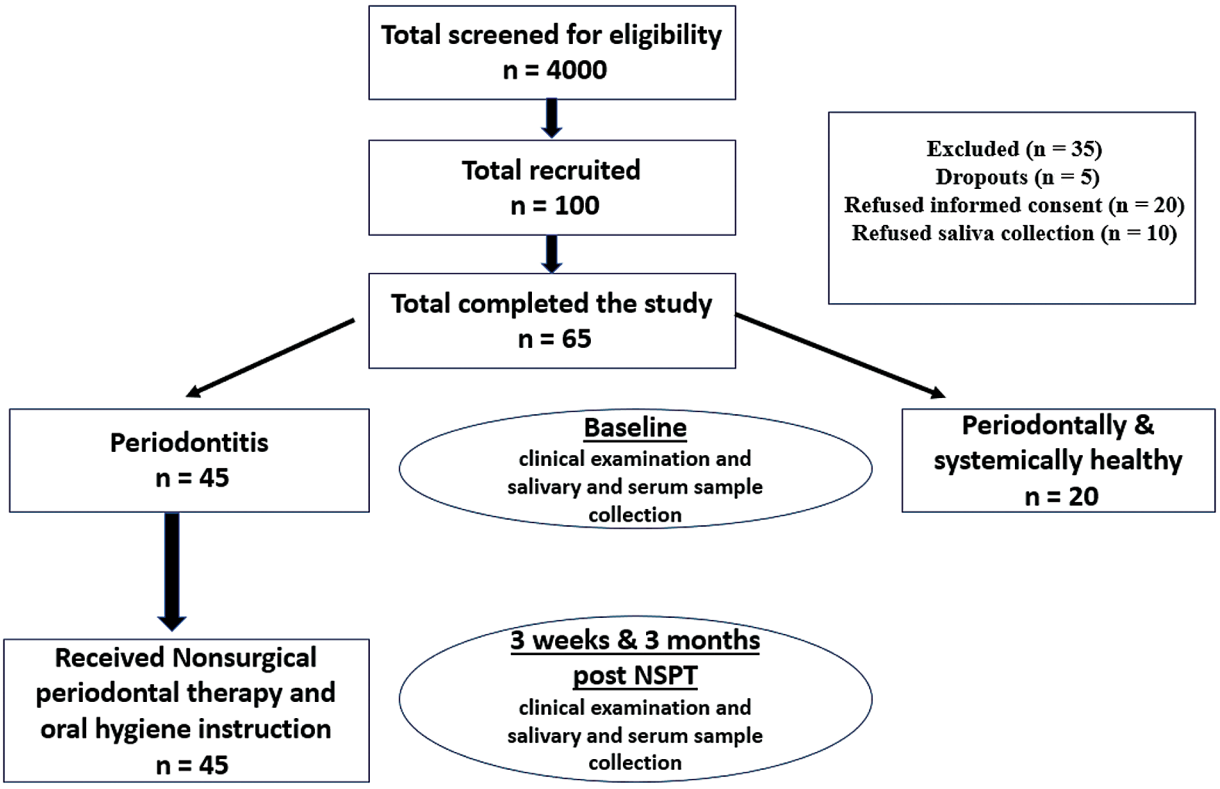

Fig. 1 Study flowchart.

clinical response to periodontal treatment. All subjects participating in the study signed an informed consent document after explaining the study protocol to them. At baseline, a questionnaire was given to each patient concerning age, marital status, religion, education, income, body weight and height, oral hygiene practices, and frequency of dental check-ups. A trained clinician also recorded the gingival color, contour, consistency, surface texture, and position.

Clinical Procedures

After taking the patient's medical and dental history, the following clinical outcome measures were recorded at baseline using a manual periodontal probe (UNC-15) at six sites for each tooth: plaque index (PI), gingival index (GI), probing pocket depth (PPD) and clinical attachment level (CAL). NSPT was provided to all participants in the test group by a trained clinician. This procedure was completed in four visits within 15 days following the baseline visit. Both manual and ultrasonic scaling instruments were utilized for scaling and root planing. Oral hygiene instructions included proper brushing techniques and inter-dental cleaning aids. Patients were reviewed 3 weeks and 3 months after the last NSPT visit. All clinical periodontal parameters were again recorded at recall visits. A single calibrated examiner performed all the recordings. The intra-examiner reproducibility was 70% for PPD and CAL.

Sample Collection

Unstimulated whole saliva from each patient was collected in the morning. It was done before periodontal probing to forestall saliva contamination with the blood or gingival crevicular fluid (GCF). Patients were asked to rinse their mouths for 30 seconds, and then expectorate into a sterile vial of 5 mL. The vial was then sealed, labeled, placed in an ice-filled Styrofoam box, and sent to the Department of Biotechnology for storage until further analysis (at −80°C). A standard venipuncture method was used to draw venous blood (5 mL) in serum-separating collection tubes and centrifuged for 10 minutes. Then, serum samples were separated, placed in plain tubes, labeled, and stored in a deep freezer (−80°C) in plastic containers for later analysis. The saliva and serum were recollected at 3 weeks and 3 months following NSPT.

Measurement of CTX, BALP, and OPG

Bone biomarkers, namely CTX, BALP, and OPG, were measured using the commercially available enzyme-linked immunosorbent assay kits for humans (Sinogeneclon, China) in the stored salivary and serum samples. Manufacturers' instructions were used to estimate the bone marker levels.

Statistical Analysis

Test group readings were measured at three time points: baseline, 3 weeks, and 3 months following NSPT. The normality of control and test groups was checked through the Shapiro–Wilk test, and the data did not follow a normal distribution (p-value < 0.05). For the test of significance among the groups, the Kruskal–Wallis test was used. Since the data were not following a normal distribution, comparison of test group readings between the three time points was performed using the Friedman test and pairwise

comparisons. The level of significance was set at 5%. The Pearson correlation was used to measure correlations between the control and test group and also within the test group at different time points. Multiple linear regression was applied to the control and test groups at different time points combined. PI, GI, PPD, and CAL were individually set as the dependent variables for each model, and salivary and serum BALP, CTX, and OPG were set as the independent variables. IBM SPSS version 20 and R version 3.5.2 software packages were used for all analyses and computations.

Results

Out of 100 patients recruited, 45 periodontitis and 20 healthy subjects completed the study. Twenty patients refused to submit informed consent. Ten patients refused salivary collection. Five patients did not report for baseline examination. Hence, the data of 65 patients were analyzed. The demographic characteristics of the study population are shown in ►**Table 1**. The mean age of periodontitis patients was 39.29 ± 7.78 years, with the percentage of those using horizontal brushing technique being 68.9% and interdental floss 100%. The mean and standard deviation of clinical periodontal parameters and BTMs of the control and test groups are shown in ►**Table 2**. Pairwise comparisons within the test group using Friedman analysis are outlined in ►**Table 3**.

Table 1 Socio-demographic characteristics of patients with periodontitis

Variables	Descriptive statistics
Age (years)	39.29 ± 7.78
BMI (kg/m^2)	26.17 ± 3.63
Education	
Illiterate	01 (2.2%)
Primary	03 (6.7%)
High school	08 (17.8%)
Intermediate	10 (22.2%)
Bachelors	18 (40.0%)
Post graduate	05 (11.1%)
Income	
0–50,000	28 (62.2%)
50,000–1.5 lac	10 (22.2%)
1.5–2.5 lac	04 (8.9%)
2.5–5 lac	02 (4.4%)
5–10 lac	01 (2.2%)
$>$15 lac	00 (0.0%)
Brushing	
Yes	45 (100.0%)
No	00 (0.0%)
Brush technique	
Scrub	02 (4.4%)
Horizontal	31 (68.9%)
Vertical	02 (4.4%)
None	00 (0.0%)
Vibratory	00 (0.0%)
Scrub and horizontal	08 (17.8%)
Horizontal and vertical	02 (4.4%)
Brushing frequency	
Once daily	34 (75.6%)
Twice daily	11 (24.4%)
Use finger	00 (0.0%)
Brush replacement	
Monthly	03 (6.7%)
3 monthly	33 (73.3%)
6 monthly	08 (17.8%)
Yearly	01 (2.2%)
Oral rinse	
After meals with water	45 (100.0%)
After meals with mouthwash	00 (0.0%)
Mouthwash	
Never	45 (100.0%)
Monthly	00 (0.0%)
3 Monthly	00 (0.0%)
6 Monthly	00 (0.0%)
Yearly	00 (0.0%)
Interdental floss	
Never	10 (22.0%)
Once daily	30 (68.0%)
Twice daily	05 (10.0%)
Dental checkup	
Never	39 (86.7%)
Monthly	00 (0.0%)
3 monthly	00 (0.0%)
6 monthly	00 (0.0%)
Yearly	00 (0.0%)
Only when in pain	06 (13.3%)
Gingival color	
Pale pink	09 (20.0%)
Pink	08 (17.8%)
Bright red	09 (20.0%)
Bluish red	19 (42.2%)
Gingival contour	
Scalloped	15 (33.3%)
Loss	30 (66.7%)
Gingival consistency	
Firm and leathery	13 (28.9%)
Spongy and edematous	32 (71.1%)
Gingival surface texture	
Stippling	12 (26.7%)
Loss	33 (73.3%)
Gingival position	
At CEJ	33 (73.3%)
Above CEJ	07 (15.6%)
Apical CEJ	05 (11.1%)
Gingival bleeding	
On probing	25 (55.6%)
Absent	20 (44.4%)
Gingival ulceration	
Present	00 (0.0%)
Absent	45 (100.0%)

Abbreviations: BMI, body mass index; CEJ, cemento-enamel junction.

Table 2 Mean and SD of clinical periodontal parameters and BTMs in healthy and periodontitis patients

Variable		Healthy	Periodontitis			*p*-Value
			Baseline	At 3 weeks post-NSPT	At 3 months post-NSPT	
Clinical periodontal parameters	**PI**	0.11 ± 0.23	1.29 ± 0.34	0.56 ± 0.23	0.61 ± 0.32	<0.001
	GI	0.03 ± 0.05	1.39 ± 0.44	0.53 ± 0.19	0.99 ± 0.63	<0.001
	PPD	1.15 ± 0.13	2.76 ± 0.59	1.67 ± 0.43	1.61 ± 0.36	<0.001
	CAL	0.66 ± 0.31	2.68 ± 0.67	0.98 ± 0.31	1.08 ± 0.49	<0.001
Salivary bone markers	**BALP**	26.78 ± 15.21	8.61 ± 14.71	11.16 ± 5.59	7.93 ± 3.41	<0.001
	CTX	0.01 ± 0.01	0.44 ± 0.88	0.01 ± 0.01	0.01 ± 0.01	<0.001
	OPG	0.69 ± 0.16	0.06 ± 0.04	0.04 ± 0.06	0.04 ± 0.06	<0.001
Serum bone markers	**BALP**	32.79 ± 27.93	44.22 ± 57.77	11.12 ± 7.01	17.13 ± 18.81	0.023[a]
	CTX	0.01 ± 0.01	241.76 ± 1128.52	0.28 ± 1.33	0.07 ± 0.19	<0.001
	OPG	0.68 ± 0.15	0.04 ± 0.03	0.38 ± 0.33	0.34 ± 0.31	<0.001

Abbreviations: BALP, bone alkaline phosphatase; BTMs, bone turnover markers; CAL, clinical attachment level; CTX, C-terminal telopeptides of type I collagen; GI, gingival index; NSPT, nonsurgical periodontal therapy; OPG, osteoprotegerin; PI, plaque index; PPD, probing pocket depth.
[a]Showing nonsignificant findings.

Table 3 Pairwise comparison of clinical periodontal parameters and BTMs in healthy and periodontitis patients

Variable		Healthy vs. baseline	Periodontitis		
			Baseline vs. 3 weeks post-NSPT	Baseline vs. 3 months post-NSPT	3 weeks vs. 3 months post-NSPT
Clinical periodontal parameters	**PI**	<0.001	<0.001	<0.001	1.000[a]
	GI	<0.001	<0.001	<0.05	<0.05
	PPD	<0.001	<0.001	<0.001	1.000[a]
	CAL	<0.001	<0.001	<0.001	1.000[a]
Salivary bone markers	**BALP**	<0.001	<0.001	0.127[a]	0.129[a]
	CTX	<0.001	<0.001	<0.001	1.000[a]
	OPG	<0.001	<0.05	<0.05	1.000[a]
Serum bone markers	**BALP**	0.085[a]	1.000[a]	1.000[a]	1.000[a]
	CTX	<0.001	<0.001	<0.001	1.000[a]
	OPG	<0.001	<0.05	<0.05	1.000[a]

Abbreviations: BALP, bone alkaline phosphatase; BTMs, bone turnover markers; CAL, clinical attachment level; CTX, C-terminal telopeptides of type I collagen; GI, gingival index; NSPT, nonsurgical periodontal therapy; OPG, osteoprotegerin; PI, plaque index; PPD, probing pocket depth.
[a]Showing nonsignificant findings.

Effect of NSPT on Clinical Periodontal Parameters

All clinical periodontal measures at baseline in the test group showed significant statistical differences compared with the control ($p < 0.001$, ►**Table 2**). Following NSPT in the test group, significant improvement was observed in clinical periodontal measures at 3 weeks and 3 months compared with baseline ($p < 0.001$, for all; ►**Table 3**). However, no significant differential change was seen in PI, PPD, and CAL at 3 weeks compared with 3 months post-NSPT ($p > 0.001$, ►**Table 3**). Nevertheless, GI was significantly improved at 3 months compared with 3 weeks post-NSPT ($p < 0.05$).

Effect of NSPT on BTMs

At baseline, there was a significant difference in salivary BALP, CTX, and OPG and serum CTX and OPG between the control and test groups ($p < 0.001$, for all; ►**Table 3**). However, serum BALP did not significantly change between the control and test group at baseline ($p > 0.05$). On comparing within the test group at three time points, all the measured salivary and serum BTMs showed significant change except the serum BALP, which did not depict significant change ($p > 0.05$). However, all the estimated BTMs showed significant differences at 3 weeks after NSPT compared with baseline, except serum BALP ($p > 0.05$). The salivary and serum BALP did not show a significant difference at 3 months after NSPT when compared with baseline. All the measured BTMs showed no significant change ($p > 0.05$ for all) when compared at 3 weeks and 3 months after NSPT.

►**Table 4** shows the correlation of salivary and serum BTMs with clinical periodontal measures in the test group at

Table 4 Correlation of BTMs in periodontitis patients with clinical periodontal parameters

At baseline		PI	GI	PPD	CAL
Salivary bone markers	BALP	0.142	0.162	−0.213	−0.203
	CTX	−0.210	0.024	0.107	0.110
	OPG	0.299	0.177	−0.011	−0.064
Serum bone markers	BALP	0.014	−0.073	−0.131	−0.149
	CTX	0.164	0.056	0.116	0.090
	OPG	−0.091	−0.116	−0.087	0.075
Three weeks post-NSPT		PI	GI	PPD	CAL
Salivary bone markers	BALP	−0.335	0.251	0.013	0.074
	CTX	−0.029	0.113	0.009	−0.063
	OPG	0.117	−0.140	0.116	0.036
Serum bone markers	BALP	−0.148	0.080	−0.112	−0.115
	CTX	0.138	−0.228	−0.226	−0.146
	OPG	−0.023	−0.034	−0.085	−0.218
Three months post-NSPT		PI	GI	PPD	CAL
Salivary bone markers	BALP	0.240	0.252	0.155	0.035
	CTX	0.222	0.101	0.236	0.133
	OPG	−0.129	−0.099	−0.155	−0.118
Serum bone markers	BALP	0.243	0.069	0.105	0.115
	CTX	0.036	−0.028	−0.012	0.080
	OPG	−0.070	0.112	0.024	0.196

Abbreviations: BALP, bone alkaline phosphatase; BTMs, bone turnover markers; CAL, clinical attachment level; CTX, C-terminal telopeptides of type I collagen; GI, gingival index; NSPT, nonsurgical periodontal therapy; OPG, osteoprotegerin; PI, plaque index; PPD, probing pocket depth.

follow-up visits. Salivary and serum BTMs showed a weak positive correlation with clinical periodontal parameters at baseline. Three weeks post-NSPT, salivary BALP showed a moderate negative with PI ($r = -0.335$), salivary CTX showed a very weak negative correlation with PI ($r = -0.029$) and CAL ($r = -0.063$), and salivary OPG showed a weak positive correlation with PI ($r = 0.117$) and PPD ($r = 0.116$). Serum CTX showed a moderate negative correlation with GI ($r = -0.228$) and PPD ($r = -0.226$). Three months following NSPT, salivary OPG depicted a weak negative correlation with PI ($r = -0.129$), GI ($r = -0.099$), PPD ($r = -0.155$), and CAL ($r = -0.118$).

►**Table 5** shows the multiple linear regression method considering the clinical periodontal parameter as the dependent variable and BTMs as the independent variable. The assumption of independence checked by running ANOVA (analysis of variance) was insignificant (p-value > 0.05) for

Table 5 Multiple regression analysis where PI, GI, PPD, and CAL are set as the dependent variable, and BTMs and group variables were set as the independent variables

PI is set as the dependent variable								
Variables		Regression coefficients	Standard error	95% confidence interval		p-Value	ANOVA p-value	Adjusted R^2
				Lower bound	Upper bound			
	Constant	1.597[a]	0.153	1.294	1.900	≤0.01	≤0.001	0.467
Salivary bone markers	BALP	−0.003	0.003	−0.009	0.002	0.218		
	CTX	0.024	0.062	−0.099	0.147	0.698		
	OPG	−1.301[a]	0.199	−1.694	−0.909	≤0.01		
Serum bone markers	BALP	0.002	0.001	0.000	0.003	0.058		
	CTX	0.001[a]	0.000	0.000	0.000	≤0.05		
	OPG	−0.341[a]	0.109	−0.556	−0.126	≤0.01		
	Group	−0.222[a]	0.044	−0.309	−0.135	≤0.01		

(*Continued*)

Table 5 (*Continued*) Multiple regression analysis where PI, GI, PPD, and CAL are set as the dependent variable, and BTMs and group variables were set as the independent variables

GI is set as the dependent variable								
Variables		Regression coefficients	Standard error	95% confidence interval		*p*-Value	ANOVA *p*-value	Adjusted R^2
				Lower bound	Upper bound			
	Constant	1.111[a]	0.224	0.668	1.553	≤0.01	≤0.001	0.293
Salivary bone markers	**BALP**	0.001	0.004	−0.008	0.008	0.915		
	CTX	0.177	0.091	−0.003	0.356	0.054		
	OPG	−1.145[a]	0.290	−1.718	−0.572	≤0.01		
Serum bone markers	**BALP**	0.002	0.001	0.000	0.005	0.063		
	CTX	0.000	0.001	0.001	0.000	0.144		
	OPG	−0.339[a]	0.159	−0.653	−0.026	≤0.05		
	Group	−0.034	0.064	−0.161	0.092	0.593		
PPD is set as the dependent variable								
Variables		Regression coefficients	Standard error	95% confidence interval		*p*-Value	ANOVA *p*-value	Adjusted R^2
				Lower bound	Upper bound			
	Constant	3.425[a]	0.219	2.992	3.858	≤0.01	≤0.001	0.501
Salivary bone markers	**BALP**	−0.011[a]	0.004	−.019	−0.003	0.008		
	CTX	0.176	0.089	.000	0.351	0.050		
	OPG	−1.783[a]	0.283	−2.343	−1.223	≤0.01		
Serum bone markers	**BALP**	0.001	0.001	−.001	0.004	0.246		
	CTX	0.001	0.001	0.0001	0.001	0.058		
	OPG	−0.410[a]	0.155	−0.717	−0.104	0.009		
	Group	−0.406[a]	0.063	−0.530	−.282	≤0.01		
CAL is set as the dependent variable								
Variables		Regression coefficients	Standard error	95% confidence interval		*p*-Value	ANOVA *p*-value	Adjusted R^2
				Lower bound	Upper bound			
	Constant	3.515[a]	0.271	2.980	4.050	≤0.01	≤0.001	0.541
Salivary bone markers	**BALP**	−0.011[a]	0.005	−0.021	−0.002	≤0.05		
	CTX	0.274[a]	0.110	0.057	0.491	≤0.05		
	OPG	−2.347[a]	0.351	−3.040	−1.655	≤0.01		
Serum bone markers	**BALP**	0.003[a]	0.001	0.001	0.006	≤0.05		
	CTX	0.001[a]	0.001	0.001	0.002	≤0.05		
	OPG	−0.470[a]	0.192	−0.849	−0.091	≤0.05		
	Group	−0.583[a]	0.078	−0.737	−0.430	≤0.01		

Abbreviations: BALP, bone alkaline phosphatase; BTMs, bone turnover markers; CAL, clinical attachment level; CTX, C-terminal telopeptides of type I collagen; GI, gingival index; OPG, osteoprotegerin; PI, plaque index; PPD, probing pocket depth.

any models. Also, the adjusted R^2 values were shallow. This indicated that multiple linear regression could not be applied to each group separately. ANOVA test resulted in a significant value of $p \leq 0.001$ for the groups. A prediction equation was obtained. The adjusted R^2 value was attained as 0.467 for PI, 0.501 for PPD, and 0.541 for CAL. This indicated that the model explained 46.7% of the total variability for PI, 50.1% for PPD, and 54.1% for CAL. The constructed model is an intermediate fitting model for predicting the combined groups' PI, PPD, and CAL. However, the adjusted R^2 value was attained as 0.293 for GI. This indicated that the model explains 29.3% of the total variability for GI. The constructed model is unsuitable for predicting GI for the combined groups.

Discussion

The primary aim of this study was to assess the effect of NSPT on BTM levels in patients with periodontitis. We also

investigated if saliva or serum fluid samples better predicted the underlying periodontal status. We found significant improvement in all measured clinical periodontal parameters at 3 weeks following NSPT, but no change at 3 months except for GI. This long-term trend suggests that NSPT provides initial periodontal health and inflammation control benefits. Maintenance and continuous oral hygiene efforts are necessary to sustain these improvements over time. Patients should adhere to a rigorous oral hygiene regimen and attend regular follow-up appointments to ensure the long-term success of NSPT. A systematic review conducted by Shanbhag et al[20] showed the importance of NSPT in improving oral health-related quality of life. In line with our study findings, Kardesler et al[21] reported a significant reduction in clinical periodontal parameters (PI, GI, and PPD) 1 and 3 months after NSPT.

In the current study, the salivary BALP levels in periodontitis patients decreased significantly compared with healthy controls. Following NSPT, its level increased significantly at 3 weeks compared with baseline. Alarmingly, 3 months after NSPT, no change in BALP levels was observed. BALP reflects anabolic activity in bone and degrades pyrophosphate, a natural mineralization inhibitor.[22] Therefore, increased BALP levels following NSPT reflect a shift from bone destruction to bone formation. A weak correlation of salivary BALP with periodontal clinical parameters was observed at 3 weeks and 3 months following NSPT. Interestingly, we reported a nonsignificant decrease in serum BALP 3 weeks post-NSPT compared with baseline. This finding may not be unusual as several local and systemic factors, as well as lifestyle and dietary habits, may contribute to the constant bone remodeling process. In line with our finding, studies have reported increased levels of salivary BALP than serum, owing to increased local fibroblast production in the oral cavity.[23–25]

In accordance with our findings, salivary CTX levels increased in patients with periodontitis compared with healthy controls.[26] CTX is a reference bone resorption marker and is a specific product of cathepsin K-mediated bone resorption and a fragment of type 1 collagen.[27] Its release is at a rate equivalent to the bone resorption activity.[28] Both salivary and serum CTX levels decreased significantly at 3 weeks following NSPT. This reduction suggests that NSPT effectively mitigated periodontitis-associated bone resorption. However, 3 months after NSPT, there was a nonsignificant decrease in CTX levels compared with 3 weeks after NSPT. Hence, there is an indication of increased bone remodeling immediately after periodontal treatment. However, the impact of NSPT on BTMs is then limited. The sensitivity of the tests in detecting a change in CTX levels may possibly be one reason. Our study depicted a weak positive correlation of salivary and serum CTX levels with periodontal parameters, which is in line with the findings by Schulze-Späte et al.[2] Contrastingly, a study by Reckelkamm et al[29] showed no solid association.

Several studies evaluated OPG as a potential biomarker in the saliva and serum of periodontitis patients.[30–34] OPG belongs to the tumor necrosis factor receptor superfamily and structurally resembles RANK. This allows it to prevent the interaction of RANK to its ligand, thereby suppressing all molecular events that enable osteoclast differentiation and bone resorption.[35] Our study depicts a significant decrease in salivary and serum OPG levels compared with healthy controls. Consistent with our findings, studies by Ramseier et al,[36] Costa et al,[37] Tobón-Arroyave et al,[13] and Aldahlawi[38] indicate that the OPG level is decreased in periodontitis compared with healthy subjects; contrarily, few studies have demonstrated the higher concentration of salivary OPG levels in periodontitis compared with healthy controls.[10,11,17,39,40] Following NSPT at 3 weeks, a further decrease in salivary OPG levels was observed, but the serum OPG levels increased significantly. However, at 3 months after NSPT, salivary and serum OPG levels showed no change. This difference in salivary and serum OPG levels suggests serum OPG being a more sensitive marker predicting alveolar bone loss. Contrary to our results, Sexton et al[17] reported diminished OPG levels at 16 and 28 weeks after therapy. We reported weak negative salivary and serum OPG correlation with clinical periodontal parameters. This is in contrast with the study[17] which demonstrated statistically significant correlations with respect to change in all clinical measures.

Saliva as a study specimen has several advantages over serum. Its collection is easy and noninvasive and can be used to detect locally putative biomarkers of periodontal disease. Yet, the method of salivary collection and varied salivary flow may influence the analysis of its biomarkers.[41] Conversely, serum biomarkers too reflect the periodontal disease status, based on the evidence that periodontal inflammation induces chronic inflammatory status. Our study demonstrated the correlation of salivary and serum bone biomarkers with the clinical periodontal measures, verifying that local disease sites influence systemic circulation. However, the question is whether saliva or serum bone biomarkers better reflect the periodontal disease and treatment outcome. The available studies have demonstrated significant heterogeneity when analyzing markers responding to periodontal treatment.[42] We, too, found variation after periodontal treatment, showing improvements after 3 weeks and then detecting no change at 3 months. When interpreting biomarker values, it is vital to consider the specific biomarker being studied and the individual patient's clinical and demographic factors. It is also essential to consider the limitations of the specific biomarker, such as the accuracy and reliability of the assay, as well as the potential for confounding factors that may impact the interpretation of the results. Discrepancies may be attributed to potential blood contamination or BTM presence from GCF in salivary samples[24] and different sampling kits for saliva and serum. The discrepancies between salivary and serum biomarker levels emphasize the importance of selecting the appropriate fluid sample for BTM assessment. Clinicians must recognize that the choice of sample can yield different results, and this awareness can guide more accurate clinical decision-making. Nevertheless, previous studies and ours conclude that salivary and serum biomarkers equally determine bone health in periodontal disease.

Despite certain limitations associated with the study, the findings are still novel. This is the first longitudinal study determining the impact of periodontal health on salivary and serum BTMs. Meticulous inclusion and exclusion criteria, control of confounding effects of age, and full mouth periodontal assessment are the study's strengths. First, only young adult females were included in the study is a significant limitation. Second, more bone markers should have been assessed to predict associations, as periodontal disease is a multifactorial disease involving a complex array of biomarkers. Third, a small sample size does not allow for the generalizability of the results. Fourth, multiple samples should be considered for the diurnal variation of biomarkers. Fifth, a more extended follow-up period would determine whether NSPT results in long-term maintenance of successful outcomes without risk of disease relapse.

In conclusion, our study demonstrated a considerable association of salivary and serum BALP, CTX, and OPG with periodontal parameters in patients with periodontitis and improvements following NSPT at 3 weeks and no change at 3 months. Furthermore, we also found that BTMs in salivary and serum samples effectively reflected the periodontal health and disease status and improvements after therapy, thereby offering an understanding of the dynamics of bone remodeling. Future studies should pave the way for assessing bone remodeling and determining the health and disease limits of BTMs with longer follow-ups and larger sample sizes, consequently enabling health care providers to make more informed decisions and deliver more effective treatment.

Clinical Relevance

This study emphasizes the significance of regular periodontal evaluation and the use of nonsurgical periodontal therapy to manage periodontitis and prevent further bone loss. It also highlights the value of using either saliva or serum samples as a diagnostic tool to monitor bone health in periodontitis patients.

Registration Information

The trial is registered with the Indian Clinical Trials Registry (CTRI/2019/09/021214).

Ethical Approval and Consent to Participate

Institutional Ethics Committee: JNMCH (330/FM/IEC). Written informed consent was obtained from study participants.

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Conflict of Interest

None declared.

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References

1 Faddy MJ, Cullinan MP, Palmer JE, Westerman B, Seymour GJ. Ante-dependence modeling in a longitudinal study of periodontal disease: the effect of age, gender, and smoking status. J Periodontol 2000;71(03):454–459
2 Schulze-Späte U, Turner R, Wang Y, et al. Relationship of bone metabolism biomarkers and periodontal disease: the Osteoporotic Fractures in Men (MrOS) study. J Clin Endocrinol Metab 2015;100(06):2425–2433
3 Betsy J, Ahmed JM, Mohasin AK, Mohammed A, Nabeeh A A. Diagnostic accuracy of salivary biomarkers of bone turnover in identifying patients with periodontitis in a Saudi Arabian population. J Dent Sci 2019;14(03):269–276
4 Papapanou PN, Susin C. Periodontitis epidemiology: is periodontitis under-recognized, over-diagnosed, or both? Periodontol 2000 2017;75(01):45–51
5 Shazam H, Shaikh F, Hussain Z. Bone turnover markers in chronic periodontitis: a literature review. Cureus 2020;12(01):e6699
6 Armitage GC. Periodontal diagnoses and classification of periodontal diseases. Periodontol 2000 2004;34:9–21
7 Janakiram C, Mehta A, Venkitachalam R. Prevalence of periodontal disease among adults in India: a systematic review and meta-analysis. J Oral Biol Craniofac Res 2020;10(04):800–806
8 Martínez-García M, Hernández-Lemus E. Periodontal inflammation and systemic diseases: an overview. Front Physiol 2021; 12:709438
9 Gursoy UK, Liukkonen J, Jula A, et al. Associations between salivary bone metabolism markers and periodontal breakdown. J Periodontol 2016;87(04):367–375
10 Al-Sabbagh M, Alladah A, Lin Y, et al. Bone remodeling-associated salivary biomarker MIP-1α distinguishes periodontal disease from health. J Periodontal Res 2012;47(03):389–395
11 Miller CS, King CP Jr, Langub MC, Kryscio RJ, Thomas MV. Salivary biomarkers of existing periodontal disease: a cross-sectional study. J Am Dent Assoc 2006;137(03):322–329
12 Buduneli N, Biyikoğlu B, Sherrabeh S, Lappin DF. Saliva concentrations of RANKL and osteoprotegerin in smoker versus non-smoker chronic periodontitis patients. J Clin Periodontol 2008;35 (10):846–852
13 Tobón-Arroyave SI, Isaza-Guzmán DM, Restrepo-Cadavid EM, Zapata-Molina SM, Martínez-Pabón MC. Association of salivary levels of the bone remodelling regulators sRANKL and OPG with periodontal clinical status. J Clin Periodontol 2012;39(12): 1132–1140
14 Frodge BD, Ebersole JL, Kryscio RJ, Thomas MV, Miller CS. Bone remodeling biomarkers of periodontal disease in saliva. J Periodontol 2008;79(10):1913–1919
15 Yoshihara A, Deguchi T, Hanada N, Miyazaki H. Relation of bone turnover markers to periodontal disease and jaw bone morphology in elderly Japanese subjects. Oral Dis 2009;15(02):176–181
16 Gibert P, Tramini P, Sieso V, Piva MT. Alkaline phosphatase isozyme activity in serum from patients with chronic periodontitis. J Periodontal Res 2003;38(04):362–365
17 Sexton WM, Lin Y, Kryscio RJ, Dawson DR III, Ebersole JL, Miller CS. Salivary biomarkers of periodontal disease in response to treatment. J Clin Periodontol 2011;38(05):434–441
18 Zia A, Hakim S, Khan AU, et al. Bone markers and bone mineral density associates with periodontitis in females with poly-cystic ovarian syndrome. J Bone Miner Metab 2022;40(03): 487–497

19 Caton JG, Armitage G, Berglundh T, et al. A new classification scheme for periodontal and peri-implant diseases and conditions - introduction and key changes from the 1999 classification. J Periodontol 2018;89(Suppl 1):S1–S8
20 Shanbhag S, Dahiya M, Croucher R. The impact of periodontal therapy on oral health-related quality of life in adults: a systematic review. J Clin Periodontol 2012;39(08):725–735
21 Kardeşler L, Buduneli N, Cetinkalp S, Kinane DF. Adipokines and inflammatory mediators after initial periodontal treatment in patients with type 2 diabetes and chronic periodontitis. J Periodontol 2010;81(01):24–33
22 Greenblatt MB, Tsai JN, Wein MN. Bone turnover markers in the diagnosis and monitoring of metabolic bone disease. Clin Chem 2017;63(02):464–474
23 Lian JB, Stein GS, Aubin JE. Bone formation: maturation and functional activities ofosteoblast lineage cells. In: Favus MJ, ed. Primer on the Metabolic Bone Diseases and Disorder of Mineral Metabolism. 5th edn Washington, DC: American Society for Boneand Mineral Research; 2003:13–28
24 Mogi M, Otogoto J, Ota N, Togari A. Differential expression of RANKL and osteoprotegerin in gingival crevicular fluid of patients with periodontitis. J Dent Res 2004;83(02):166–169
25 Pellegrini GG, Gonzales CM, Somoza JC, Friedman SM, Zeni SN. Correlation between salivary and serum markers of bone turnover in osteopenic rats. J Periodontol 2008;79(01):158–165
26 Joseph B, Javali MA, Khader MA, AlQahtani SM, Amanullah M. Salivary osteocalcin as potential diagnostic marker of periodontal bone destruction among smokers. Biomolecules 2020;10 (03):380
27 Vasikaran S, Cooper C, Eastell R, et al. International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine position on bone marker standards in osteoporosis. Clin Chem Lab Med 2011;49(08):1271–1274
28 Garnero P, Ferreras M, Karsdal MA, et al. The type I collagen fragments ICTP and CTX reveal distinct enzymatic pathways of bone collagen degradation. J Bone Miner Res 2003;18(05): 859–867
29 Reckelkamm SL, Hannemann A, Kocher T, et al. Association between bone turnover markers and periodontitis: a population-based cross-sectional study. J Clin Periodontol 2022;49(07): 633–641
30 Asquino N, Vigil G, Prado VP, Rossy LAB, Molina RB. Bone resorption in periodontal disease: the role of RANK, RANKL and OPG. A literature review. Odontoestomatología 2022;24 (40):e316
31 Bostanci N, Ilgenli T, Emingil G, et al. Differential expression of receptor activator of nuclear factor-kappaB ligand and osteoprotegerin mRNA in periodontal diseases. J Periodontal Res 2007;42 (04):287–293
32 Lu HK, Chen YL, Chang HC, Li CL, Kuo MYP. Identification of the osteoprotegerin/receptor activator of nuclear factor-kappa B ligand system in gingival crevicular fluid and tissue of patients with chronic periodontitis. J Periodontal Res 2006;41(04): 354–360
33 Szulc P, Hofbauer LC, Heufelder AE, Roth S, Delmas PD. Osteoprotegerin serum levels in men: correlation with age, estrogen, and testosterone status. J Clin Endocrinol Metab 2001;86(07): 3162–3165
34 Yang L, Grey V. Pediatric reference intervals for bone markers. Clin Biochem 2006;39(06):561–568
35 Belibasakis GN, Bostanci N. The RANKL-OPG system in clinical periodontology. J Clin Periodontol 2012;39(03):239–248
36 Ramseier CA, Kinney JS, Herr AE, et al. Identification of pathogen and host-response markers correlated with periodontal disease. J Periodontol 2009;80(03):436–446
37 Costa PP, Trevisan GL, Macedo GO, et al. Salivary interleukin-6, matrix metalloproteinase-8, and osteoprotegerin in patients with periodontitis and diabetes. J Periodontol 2010;81(03):384–391
38 Aldahlawi S. Evaluation of serum RANKL and OPG concentrations in patients with periodontitis in Saudi Arabia. IJPRAS 2019;8(01): 45–51
39 Browner WS, Lui LY, Cummings SR. Associations of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures, and mortality in elderly women. J Clin Endocrinol Metab 2001;86 (02):631–637
40 Kinney JS, Morelli T, Braun T, et al. Saliva/pathogen biomarker signatures and periodontal disease progression. J Dent Res 2011; 90(06):752–758
41 Ozçaka O, Nalbantsoy A, Buduneli N. Salivary osteocalcin levels are decreased in smoker chronic periodontitis patients. Oral Dis 2011;17(02):200–205
42 Zekeridou A, Mombelli A, Cancela J, Courvoisier D, Giannopoulou C. Systemic inflammatory burden and local inflammation in periodontitis: what is the link between inflammatory biomarkers in serum and gingival crevicular fluid? Clin Exp Dent Res 2019;5 (02):128–135

