

Effect of scaling and root planing on the expression of neuregulin-4 levels and its receptor ErbB4 in GCF in patients with stage III periodontitis with and without type 2 diabetes mellitus: An interventional comparative study

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Abstract. The present study evaluated and associated the effects of scaling and root planing (SRP) on gingival crevicular fluid (GCF) levels of neuregulin-4 (NRG4) and its receptor ErbB4 in patients with stage III periodontitis, with and without type 2 diabetes mellitus (T2DM). A total of 45 subjects were divided into three groups, as follows: Periodontally healthy individuals, patients with stage III periodontitis and patients with stage III periodontitis with well-controlled T2DM. Clinical parameters, including gingival index, plaque index, bleeding on probing, probing pocket depth and clinical attachment level, were recorded at baseline and 3 months following non-surgical periodontal therapy (NSPT). GCF samples were collected from the deepest periodontal site 24-72 h following SRP at baseline and at 3 months post-therapy, and analyzed for NRG4 and ErbB4 levels using enzyme-linked immunosorbent assay. At baseline, the GCF levels of NRG4 and ErbB4 were significantly higher in patients with periodontitis compared to healthy individuals, with the highest levels observed in patients with T2DM. Following NSPT, all clinical parameters exhibited a significant improvement, accompanied by a statistically significant reduction in biomarker levels at 3 months. Significant positive correlations were observed between the GCF NRG4 and ErbB4 levels and periodontal clinical parameters at baseline in groups 2 and 3. These findings indicated that GCF NRG4 and ErbB4 levels were associated with periodontal clinical parameters in this cohort and changed following NSPT. However, their independent clinical utility as

biomarkers of disease activity or treatment response cannot be established from the present study. Further larger, longitudinal, and mechanistic studies are required to validate their clinical significance.

Introduction

Periodontitis is a chronic inflammatory disease affecting the supporting tissues of the teeth, and can result in the progressive and irreversible destruction of hard and soft tissues. Periodontitis is a multifactorial disease associated with dysbiotic dental biofilms, and is characterized by clinical attachment loss (CAL), radiographic bone loss and gingival recession (1).

A well-established bidirectional association exists between diabetes mellitus and periodontitis (2,3). Diabetes may impair host immune responses, including neutrophil chemotaxis and phagocytosis, compromising the defense against periodontal pathogens. Persistent hyperglycemia also promotes the production of pro-inflammatory cytokines, such as IL-1, IL-6 and TNF- α , thereby aggravating periodontal inflammation. Diabetes may additionally impair wound healing and tissue repair, and favor pathogenic bacterial growth due to elevated glucose levels in gingival crevicular fluid (GCF). Conversely, periodontal inflammation can contribute to the systemic dissemination of inflammatory mediators, promoting insulin resistance and poor glycemic control (2,3).

Adipokines are bioactive molecules secreted by adipose tissue that regulate metabolism, inflammation and insulin sensitivity. Increasing evidence suggests their involvement in chronic inflammatory conditions, such as type 2 diabetes mellitus (T2DM) and periodontitis, indicating a possible shared pathophysiological link (3,4).

Neuregulin-4 (NRG4), a member of the epidermal growth factor family, is a novel adipokine predominantly secreted by brown adipose tissue. NRG4 exerts its biological effects mainly through the receptor tyrosine kinase ErbB4. In addition to its metabolic functions, NRG4 has anti-inflammatory and cytoprotective properties, and may regulate macrophage polarization and pro-inflammatory cytokine production via ErbB4-mediated signaling (4).

Previous studies have demonstrated NRG4 and ErbB4 expression in periodontal tissues and GCF, with levels being

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associated with the severity of periodontal disease and reported to decrease following periodontal therapy. The NRG4-ErbB4 axis activates downstream pathways, such as the PI3K/AKT and STAT pathways, which are involved in cell survival, epithelial integrity and tissue repair (5-7). NRG4 has also been implicated in obesity, insulin resistance, dyslipidemia, oxidative stress, non-alcoholic fatty liver disease and cardiovascular diseases, further supporting its possible role in the association between metabolic dysfunction and chronic inflammation (1).

Scaling and root planing (SRP) remains the gold standard non-surgical periodontal therapy (NSPT) to reduce the microbial burden and control inflammation. Previous meta-analyses have also demonstrated improvements in glycemic control following NSPT in patients with diabetes (6,3,8).

Therefore, the present study aimed to evaluate the effects of SRP on the GCF levels of NRG4 and its receptor ErbB4 in patients with stage III periodontitis with and without T2DM.

Patients and methods

Study design and population. The present interventional comparative study was conducted at the Department of Periodontology and Oral Implantology, Vydehi Institute of Dental Sciences and Research Centre, Bangalore, India. The study was conducted over a period of 1 year, from April 15, 2024 to April 15, 2025. A total of 89 participants were assessed for eligibility. Following the application of the predefined inclusion and exclusion criteria (described below), 44 participants were excluded and 45 eligible participants were included and categorized into three groups (n=15 per group). Participant recruitment, allocation, follow-up and analysis are summarized in Fig. 1. The patient groups were as follows: i) Group 1 (healthy individuals): Systemically healthy individuals with a clinically healthy periodontium, defined by bleeding on probing (BOP) <10% and no CAL. ii) Group 2 (stage III periodontitis without T2DM): Systemically healthy patients diagnosed with stage III periodontitis according to the 2017 classification, characterized by CAL $\geq$ 5 mm at $\geq$ 2 non-adjacent teeth and/or radiographic bone loss extending to the middle third of the root, with complexity factors, such as probing pocket depth (PPD) $\geq$ 6 mm, vertical bone loss $\geq$ 3 mm and/or class II or III furcation involvement, were included in group 2 (9). These patients had no diagnosis of T2DM (10). Group 3 (stage III periodontitis with well-controlled T2DM): Patients diagnosed with stage III periodontitis and T2DM for $\geq$ 1 year were included in group 3. An HbA1c <7% at baseline was considered indicative of well-controlled diabetes (10).

Ethics approval was obtained from the Institutional Review Board of Vydehi Institute of Dental Sciences and Research Centre (approval no. VIDS-IEC/UG/AAP/2024/136; Bengaluru, India). Written informed consent was obtained from all participants prior to the commencement of the study.

Inclusion and exclusion criteria. Participants aged 35-60 years with $\geq$ 16 natural teeth were eligible for inclusion. The exclusion criteria included systemic diseases other than T2DM, type 1 diabetes mellitus, pregnancy or lactation, current or former smoking, periodontal therapy within the previous 6 months, antibiotic or anti-inflammatory drug use within the previous 3 months, medications other than antidiabetic drugs, and intraoral lesions.

Clinical protocol. Clinical parameters were recorded at baseline and at 3 months following NSPT. The healthy group received scaling and oral hygiene instructions, whereas the patients in groups 2 and 3 received SRP at baseline along with oral hygiene instructions. All participants were reassessed at 3 months. There was no loss to follow-up, and all 45 participants completed the study.

Demographic and metabolic characteristics, antidiabetic medication and dietary habits. Well-controlled T2DM was defined as HbA1c <7%. The demographic and metabolic characteristics of the patients are presented in Table I. The patients with T2DM received antidiabetic medication (group 3, n=15). The type, dose and duration of medication were recorded at baseline and are presented in Table II. Dietary habits and physical activity were also recorded at baseline and are presented in Table III. The antidiabetic medication regimen remained unaltered throughout the 3-month follow-up period, and no clinically significant hypoglycemic episodes were reported.

Clinical parameters. The plaque index (PI; Silness and Loe, 1964) (11), gingival index (GI; Loe and Silness, 1963) (12), BOP, PPD and clinical attachment level (CAL) were recorded at six sites per tooth using a UNC-15 periodontal probe. All measurements were performed by a single calibrated examiner to minimize intra-examiner variability.

GCF collection and biomarker estimation. GCF samples were collected from the deepest periodontal site 24-72 h after baseline SRP and again at 3 months post-therapy. The site was isolated with cotton rolls and gently air-dried, and supragingival plaque was removed prior to collection.

GCF (~1 μ l) was collected extracrevicularly using calibrated volumetric microcapillary pipettes (MilliporeSigma). Samples were immediately transferred into sterile Eppendorf tubes containing 199 μ l phosphate-buffered saline (PBS), pH 7.4 (HiMedia Laboratories Pvt. Ltd.), resulting in a final volume of 200 μ l, and stored at -80°C until analysis. The GCF concentrations of NRG4 and ErbB4 were quantified using ELISA kits specific for human NRG4 (cat. no. RE1494H) and human ErbB4 (cat. no. RE4226H), respectively (Reed Biotech Ltd.), according to the manufacturer's instructions (<https://www.reedbiotech.com/upload/file/ELISA/RE1494H-2.pdf>). The assays employed a sandwich ELISA technique using pre-coated microplate wells containing monoclonal antibodies specific for human NRG4 and ErbB4. The kit-provided standards and GCF samples were added to the respective pre-coated wells, followed by the addition of the kit-provided biotinylated detection antibodies and streptavidin-horseradish peroxidase (HRP) conjugate. Following incubation at 37°C for 60 min, the wells were washed four times with 1X wash buffer. The kit-provided 3,3',5,5'-tetramethylbenzidine (TMB) substrate was then added and incubated at 37°C for 10 min. The reaction was terminated using the kit-provided stop solution, and absorbance was measured at 450 nm using a Tecan ELISA microplate reader (Tecan Group Ltd.). All samples were analyzed in duplicate, and concentrations were calculated using the respective standard calibration curves.

Table I. Demographic and metabolic characteristics of the patients in group 3.

Variable	Group 3 (patients with type 2 diabetes mellitus), n=15
Age, years	49.3±6.2
Male, n (%)	9 (60.0%)
Female, n (%)	6 (40.0%)
Body mass index, kg/m ²	27.4±2.8
Duration of diabetes, years	6.1±2.7
Fasting blood glucose, mg/dl	124.6±12.8
HbA1c, %	6.4±0.3

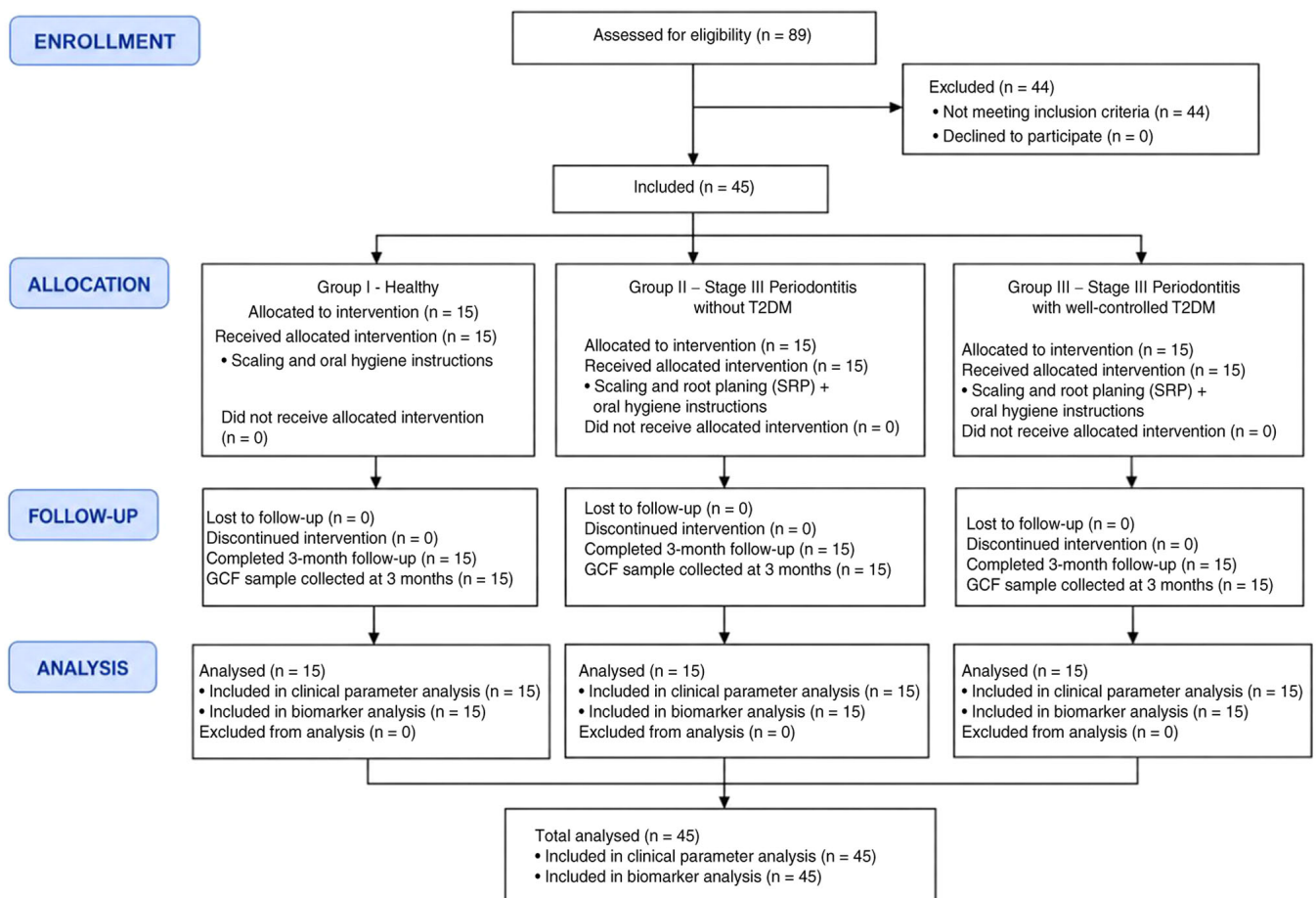


Figure 1. CONSORT-style flow diagram demonstrating participant recruitment, group allocation, follow-up and analysis.

Statistical analysis. Statistical analysis was performed using SPSS version 22.0 (IBM Corp.). The normality of continuous variables was assessed using the Shapiro-Wilk test. Normally distributed continuous variables are presented as the mean ± SD, whereas non-normally distributed variables are presented as the median and interquartile range (IQR). Categorical variables are presented as frequency and percentage. One-way ANOVA followed by Tukey's post hoc test was used for normally distributed intergroup data, while the Kruskal-Wallis test followed by Dunn's post hoc test was used for non-normally distributed intergroup data. Intragroup comparisons were performed using a paired t-test for normally distributed data or the Wilcoxon

signed-rank test for non-normally distributed data, as appropriate. Spearman's rank correlation coefficient was used to assess associations between clinical parameters and biomarker levels. A value of $P < 0.05$ was considered to indicate a statistically significant difference.

Results

A total of 45 participants completed the study and were included in the final analysis, with 15 participants in each group: Group 1, periodontally healthy; group 2, stage III periodontitis without T2DM; and group 3, stage III periodontitis with well-controlled

Table II. Antidiabetic medication regimen among participants with T2DM (group 3, n=15.)

Antidiabetic medication	No. of patients, n (%)	Dose	Duration of therapy
Metformin	8 (53.3)	500 mg once daily	2-4 years
Metformin	2 (13.3)	500 mg twice daily	1-3 years
Metformin	2 (13.3)	1,000 mg twice daily	4-7 years
Metformin + Glimepiride	3 (20.0)	Metformin 500 mg once daily + glimepiride 1 mg once daily	3-5 years
Total	15 (100)	-	-

The type, dose and duration of antidiabetic medication were recorded at baseline. T2DM, type 2 diabetes mellitus.

Table III. Dietary habits and physical activity recorded at baseline.

Dietary habits	No. of patients (%)
Mixed diet	10 (66.7)
Vegetarian	5 (33.3)
Physical activity	
Regular	6 (40.0)
Irregular	9 (60.0)

Table IV. Comparison of mean values of clinical parameters between 3 groups at baseline.

Parameters	Groups	No. of patients	Mean	SD	P-value ^a	Sig. Diff	P-value ^b
PI	Group 1	15	0.86	0.42	<0.001	1 vs. 2	<0.001
	Group 2	15	2.48	0.27		1 vs. 3	<0.001
	Group 3	15	2.42	0.30		2 vs. 3	0.88
GI	Group 1	15	0.74	0.42	<0.001	1 vs. 2	<0.001
	Group 2	15	2.41	0.24		1 vs. 3	<0.001
	Group 3	15	2.40	0.41		2 vs. 3	0.99
BOP	Group 1	15	9.06	1.04	<0.001	1 vs. 2	<0.001
	Group 2	15	94.52	3.87		1 vs. 3	<0.001
	Group 3	15	95.35	2.19		2 vs. 3	0.67
PD	Group 1	15	1.70	0.37	<0.001	1 vs. 2	<0.001
	Group 2	15	6.01	0.75		1 vs. 3	<0.001
	Group 3	15	6.10	0.25		2 vs. 3	0.87
CAL	Group 1	15	0.00	0.00	<0.001	1 vs. 2	<0.001
	Group 2	15	5.22	0.59		1 vs. 3	<0.001
	Group 3	15	5.44	0.29		2 vs. 3	0.26

Data were analyzed using ^aone-way ANOVA test with ^bTukey's post hoc test. Values in bold font indicate statically significant differences. PI, plaque index; GI, gingival index; BOP, bleeding on probing; PD, probing depth; CAL, clinical attachment level; SD, standard deviation.

T2DM. The participant recruitment, group allocation, follow-up and final analysis are presented in Fig. 1.

At baseline, PI, GI, BOP, PPD and CAL differed significantly among the three groups ($P<0.001$; one-way ANOVA). Groups 2 and 3 exhibited significantly higher values than group 1 ($P<0.001$), while no significant differences were observed between groups 2 and 3 (Table IV). The baseline GCF NRG4

and ErbB4 levels also differed significantly among the groups ($P<0.001$; Kruskal-Wallis test). Dunn's post hoc analysis demonstrated significant differences between the respective group pairs for both biomarkers (Table V).

At 3 months following SRP, all clinical parameters continued to differ significantly among the groups ($P<0.001$). Groups 2 and 3 had significantly higher values than group

Table V. Comparison of GCF NRG4 and ErbB4 levels (in ng/ml) between the three groups at baseline.

Parameters	Groups	No. of patients	Median	IQR	P-value ^a	Sig. Diff	P-value ^b
GCF NRG-4	Group 1	15	6.49	1.39	<0.001	1 vs. 2	0.009
	Group 2	15	8.51	1.25		1 vs. 3	<0.001
	Group 3	15	12.01	3.18		2 vs. 3	<0.001
GCF ErbB4	Group 1	15	0.77	0.16	<0.001	1 vs. 2	<0.001
	Group 2	15	1.80	0.61		1 vs. 3	<0.001
	Group 3	15	3.04	0.68		2 vs. 3	<0.001

Data were analyzed using the ^aKruskal-Walst test ^bDunn's post hoc test. Values in bold font indicate statically significant differences. GCF, gingival crevicular fluid; NRG4, neuregulin-4.

Table VI. Comparison of mean values of clinical parameters between the three groups at 3 months.

Parameters	Groups	No. of patients	Mean	SD	P-value ^a	Sig. Diff	P-value ^b
PI	Group 1	15	0.81	0.41	<0.001	1 vs. 2	<0.001
	Group 2	15	1.26	0.13		1 vs. 3	<0.001
	Group 3	15	1.39	0.15		2 vs. 3	0.43
GI	Group 1	15	0.71	0.43	<0.001	1 vs. 2	<0.001
	Group 2	15	1.19	0.11		1 vs. 3	<0.001
	Group 3	15	1.41	0.34		2 vs. 3	0.16
BOP	Group 1	15	8.99	1.05	<0.001	1 vs. 2	<0.001
	Group 2	15	51.15	2.69		1 vs. 3	<0.001
	Group 3	15	53.46	1.28		2 vs. 3	0.13
PD	Group 1	15	1.59	0.41	<0.001	1 vs. 2	<0.001
	Group 2	15	4.38	0.62		1 vs. 3	<0.001
	Group 3	15	4.84	0.58		2 vs. 3	0.09
CAL	Group 1	15	0.00	0.00	<0.001	1 vs. 2	<0.001
	Group 2	15	4.22	0.57		1 vs. 3	<0.001
	Group 3	15	4.63	0.28		2 vs. 3	0.07

Data were analyzed using ^aone-way ANOVA test with ^bTukey's post hoc test. Values in bold font indicate statically significant differences. PI, plaque index; GI, gingival index; BOP, bleeding on probing; PD, probing depth; CAL, clinical attachment level; SD, standard deviation.

1, while the differences between groups 2 and 3 remained non-significant (Table VI).

At 3 months following SRP, GCF NRG4 levels remained significantly different among the groups ($P<0.001$), with the highest levels in group 3, followed by group 2 and group 1. The GCF ErbB4 levels exhibited a similar pattern ($P<0.001$; Table VII).

All groups exhibited reductions in clinical parameters from baseline to 3 months following SRP. The reductions in PI, GI, BOP, PPD and CAL were highly significant in groups 2 and 3 ($P<0.001$), while group 1 exhibited smaller, yet statistically significant improvements in selected parameters (Table VIII).

The GCF NRG4 and ErbB4 levels also decreased significantly from baseline to 3 months following SRP in all groups ($P<0.001$; Wilcoxon signed-rank test). Since the baseline biomarker concentrations differed among the groups, absolute reductions were not interpreted as evidence of a greater treatment response. Therefore, the percentage change from

baseline to 3 months following SRP was additionally calculated to compare relative changes between groups (Table IX).

At baseline, Spearman's rank correlation analysis revealed no significant correlations between GCF NRG4 levels and the periodontal clinical parameters in group 1. Similarly, the GCF ErbB4 levels exhibited no significant correlations with PI, GI, BOP or PD in group 1. In group 2, GCF NRG4 demonstrated significant positive correlations with PI ($\rho=0.63$, $P=0.011$), GI ($\rho=0.63$, $P=0.011$), BOP ($\rho=0.63$, $P=0.011$), PD ($\rho=0.68$, $P=0.005$) and CAL ($\rho=0.68$, $P=0.005$). GCF ErbB4 also exhibited significant positive correlations with PI ($\rho=0.64$, $P=0.010$), GI ($\rho=0.64$, $P=0.010$), BOP ($\rho=0.64$, $P=0.010$), PD ($\rho=0.69$, $P=0.004$) and CAL ($\rho=0.70$, $P=0.004$). In Group 3, GCF NRG4 exhibited significant positive correlations with PI ($\rho=0.72$, $P=0.002$), GI ($\rho=0.72$, $P=0.002$), BOP ($\rho=0.72$, $P=0.002$), PD ($\rho=0.77$, $P<0.001$) and CAL ($\rho=0.77$, $P<0.001$). Similarly, GCF ErbB4 demonstrated significant positive correlations with PI ($\rho=0.74$, $P=0.002$), GI ($\rho=0.74$, $P=0.002$), BOP ($\rho=0.74$,

Table VII. Comparison of GCF NRG4 and ErbB4 levels (in ng/ml) between the three groups at 3 months.

Parameters	Groups	No. of patients	Median	IQR	P-value ^a	Sig. Diff	P-value ^b
GCF NRG4	Group 1	15	6.23	1.40	<0.001	1 vs. 2	0.002
	Group 2	15	7.89	1.10		1 vs. 3	<0.001
	Group 3	15	10.94	4.39		2 vs. 3	<0.001
GCF ErbB4	Group 1	15	0.57	0.17	<0.001	1 vs. 2	<0.001
	Group 2	15	1.57	0.60		1 vs. 3	<0.001
	Group 3	15	2.82	0.60		2 vs. 3	<0.001

Data were analyzed using the ^aKruskal-Walish test ^bDunn's post hoc test. Values in bold font indicate statically significant differences. GCF, gingival crevicular fluid; NRG4, neuregulin-4.

Table VIII. Comparison of mean values of clinical parameters between pre- and post-operation period in each group.

Parameter	Groups	Time	No. of patients	Mean	SD	Mean Diff	P-value
PI	Group 1	Baseline	15	0.86	0.42	0.05	0.02
		3 Months	15	0.81	0.41		
	Group 2	Baseline	15	2.48	0.27	1.21	<0.001
		3 Months	15	1.26	0.13		
	Group 3	Baseline	15	2.42	0.30	1.03	<0.001
		3 Months	15	1.39	0.15		
GI	Group 1	Baseline	15	0.74	0.42	0.03	0.02
		3 Months	15	0.71	0.43		
	Group 2	Baseline	15	2.41	0.24	1.22	<0.001
		3 Months	15	1.19	0.11		
	Group 3	Baseline	15	2.40	0.41	0.99	<0.001
		3 Months	15	1.41	0.34		
BOP	Group 1	Baseline	15	9.06	1.04	0.07	<0.001
		3 Months	15	8.99	1.05		
	Group 2	Baseline	15	94.52	3.87	43.38	<0.001
		3 Months	15	51.15	2.69		
	Group 3	Baseline	15	95.35	2.19	41.89	<0.001
		3 Months	15	53.46	1.28		
PD	Group 1	Baseline	15	1.70	0.37	0.11	0.04
		3 Months	15	1.59	0.41		
	Group 2	Baseline	15	6.01	0.75	1.63	<0.001
		3 Months	15	4.38	0.62		
	Group 3	Baseline	15	6.10	0.25	1.27	<0.001
		3 Months	15	4.84	0.58		
CAL	Group 1	Baseline	15	0.00	0.00	0.00	..
		3 Months	15	0.00	0.00		
	Group 2	Baseline	15	5.22	0.59	1.00	<0.001
		3 Months	15	4.22	0.57		
	Group 3	Baseline	15	5.44	0.29	0.82	<0.001
		3 Months	15	4.63	0.28		

Data were analyzed using the paired t-test. Values in bold font indicate statically significant differences. PI, plaque index; GI, gingival index; BOP, bleeding on probing; PD, probing depth; CAL, clinical attachment level; SD, standard deviation.

P=0.002), PD ($\rho=0.78$, $P<0.001$) and CAL ($\rho=0.79$, $P<0.001$). Additionally, a very strong positive correlation was observed

between the GCF NRG4 and ErbB4 levels in group 2 ($\rho=1.00$, $P<0.001$) and group 3 ($\rho=1.00$, $P<0.001$) (Table X and Figs. 2-4).

Table IX. Comparison of the GCF NRG4 and ErbB4 levels (in ng/ml) between baseline and 3 months in each group.

Parameter	Groups	Time	No. of patients	Median	IQR	Median Diff	P-value
GCF NRG4	Group 1	Baseline	15	6.49	1.39	0.24	<0.001
		3 Months	15	6.23	1.40		
	Group 2	Baseline	15	8.51	1.25	0.74	<0.001
		3 Months	15	7.89	1.10		
	Group 3	Baseline	15	12.01	3.18	1.37	<0.001
		3 Months	15	10.94	4.39		
GCF ErbB4	Group 1	Baseline	15	0.77	0.16	0.22	<0.001
		3 Months	15	0.57	0.17		
	Group 2	Baseline	15	1.80	0.61	0.22	<0.001
		3 Months	15	1.57	0.60		
	Group 3	Baseline	15	3.04	0.68	0.15	<0.001
		3 Months	15	2.82	0.60		

Data were analyzed using the Wilcoxon signed rank test. Values in bold font indicate statically significant differences. GCF, gingival crevicular fluid; NRG4, neuregulin-4.

Table X. Spearman's rank correlation analysis to assess the correlation between clinical parameters, and GCF NRG4 and ErbB4 levels at baseline in each group.

Groups	Markers	Values	ErbB4	PI	GI	BOP	PD	CAL
Group 1	GCF NRG4	Rho	-0.29	0.09	0.09	-0.17	0.25	.
		P-value	0.30	0.76	0.76	0.54	0.36	.
	GCF ErbB4	Rho	-	-0.30	-0.31	0.06	-0.30	.
		P-value	-	0.27	0.26	0.82	0.28	.
Group 2	GCF NRG4	Rho	1.00	0.63	0.63	0.63	0.68	0.68
		P-value	<0.001	0.01	0.01	0.01	0.005	0.005
	GCF ErbB4	Rho	-	0.64	0.64	0.64	0.69	0.70
		P-value	-	0.01	0.01	0.01	0.004	0.004
Group 3	GCF NRG4	Rho	1.00	0.72	0.72	0.72	0.77	0.77
		P-value	<0.001	0.002	0.002	0.002	<0.001	<0.001
	GCF ErbB4	Rho	-	0.74	0.74	0.74	0.78	0.79
		P-value	-	0.002	0.002	0.002	<0.001	<0.001

Values in bold font indicate statically significant differences. GCF, gingival crevicular fluid; NRG4, neuregulin-4; PI, plaque index; GI, gingival index; BOP, bleeding on probing; PD, probing depth; CAL, clinical attachment level.

At the time point of 3 months, no significant correlations were observed between the GCF NRG4 or ErbB4 levels and the periodontal clinical parameters in group 1. In group 2, GCF NRG4 demonstrated significant positive correlations with PI ($\rho=0.65$, $P=0.009$), GI ($\rho=0.62$, $P=0.013$), BOP ($\rho=0.65$, $P=0.009$), PD ($\rho=0.68$, $P=0.006$) and CAL ($\rho=0.70$, $P=0.004$). GCF ErbB4 also exhibited significant positive correlations with PI ($\rho=0.62$, $P=0.013$), GI ($\rho=0.59$, $P=0.019$), BOP ($\rho=0.62$, $P=0.014$), PD ($\rho=0.65$, $P=0.008$) and CAL ($\rho=0.67$, $P=0.006$). In group 3, GCF NRG4 exhibited significant positive correlations with PI ($\rho=0.71$, $P=0.003$), GI ($\rho=0.74$, $P=0.002$), BOP ($\rho=0.74$, $P=0.002$), PD ($\rho=0.79$, $P<0.001$) and CAL ($\rho=0.79$, $P<0.001$). Similarly, GCF ErbB4 demonstrated significant positive correlations

with PI ($\rho=0.72$, $P=0.002$), GI ($\rho=0.75$, $P=0.001$), BOP ($\rho=0.74$, $P=0.002$), PD ($\rho=0.81$, $P<0.001$) and CAL ($\rho=0.81$, $P<0.001$). A very strong positive correlation was also observed between the GCF NRG4 and ErbB4 levels in group 2 ($\rho=1.00$, $P<0.001$) and group 3 ($\rho=1.00$, $P<0.001$) (Table XI and Figs. 5-7).

Discussion

Periodontitis is driven by the response of the host to a dysbiotic microbial biofilm, with inflammatory mediators, such as IL-1, IL-6, TNF- α and prostaglandin E2, contributing to connective tissue degradation and alveolar bone resorption (1). T2DM further increases periodontal susceptibility

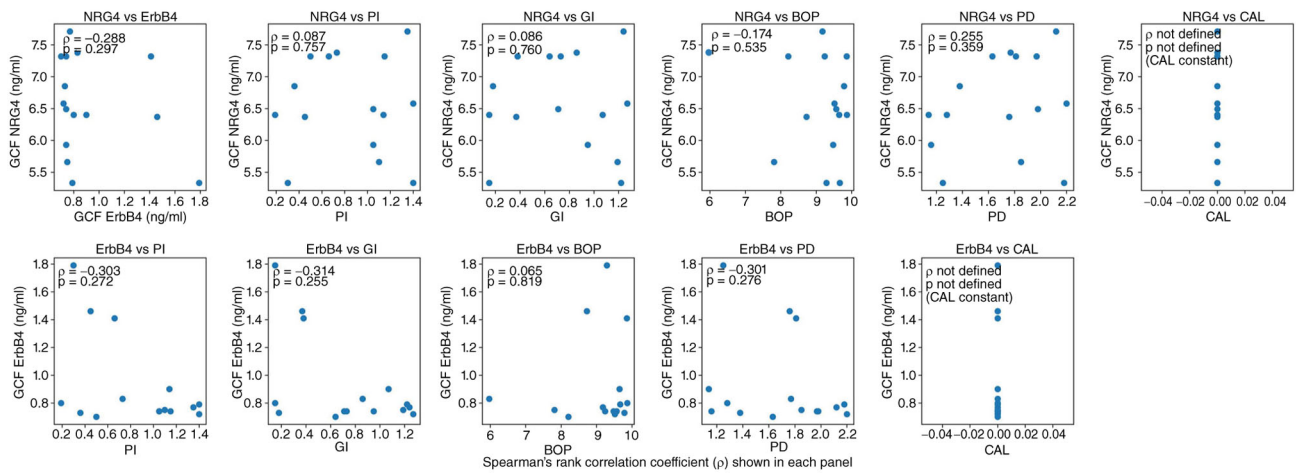


Figure 2. Spearman's correlation plots at baseline for group 1 (n=15). NRG4, neuregulin-4; GI, gingival index; PI, plaque index; BOP, bleeding on probing; PD, probing depth; CAL, clinical attachment level.

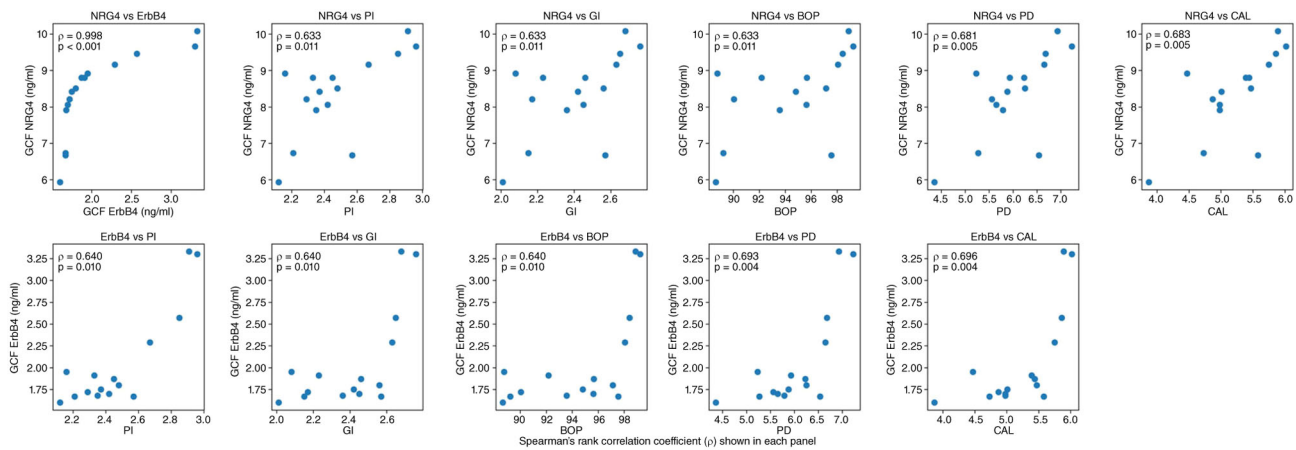


Figure 3. Spearman's correlation plots at baseline for group 2 (n=15). NRG4, neuregulin-4; GI, gingival index; PI, plaque index; BOP, bleeding on probing; PD, probing depth; CAL, clinical attachment level.

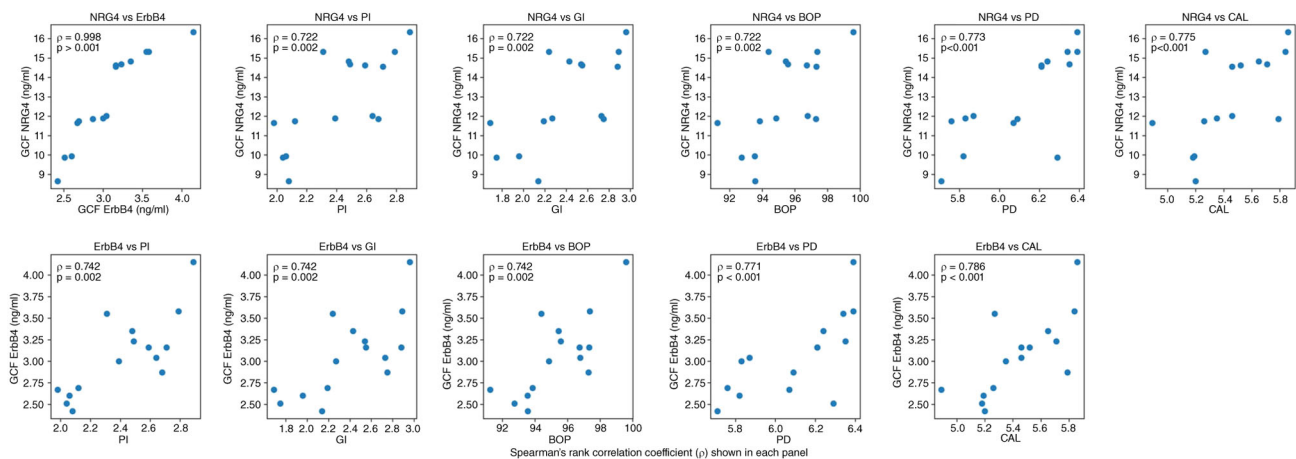


Figure 4. Spearman's correlation plots at baseline for group 3 (n=15). NRG4, neuregulin-4; GI, gingival index; PI, plaque index; BOP, bleeding on probing; PD, probing depth; CAL, clinical attachment level.

through advanced glycation end products, oxidative stress and impaired immune responses, while periodontal inflammation may adversely affect glycemic control (2,3).

Previous epidemiological studies have demonstrated greater periodontal destruction among diabetic individuals; Shlossman *et al* (13), along with Taylor *et al* (14), Emrich *et al* (15)

Table XI. Spearman's rank correlation analysis to assess the correlation between clinical parameters, and GCF NRG4 and ErbB4 levels at 3 months in each group.

Groups	Markers	Values	ErbB4	PI	GI	BOP	PD	CAL
Group 1	GCF NRG4	Rho	-0.23	0.00	0.02	-0.11	0.05	.
		P-value	0.41	0.99	0.95	0.69	0.86	.
	GCF ErbB4	Rho	-	-0.33	-0.32	0.12	-0.44	.
		P-value	-	0.22	0.25	0.68	0.10	.
Group 2	GCF NRG4	Rho	1.00	0.65	0.62	0.65	0.68	0.70
		P-value	<0.001	0.009	0.013	0.009	0.006	0.004
	GCF ErbB4	Rho	-	0.62	0.59	0.62	0.65	0.67
		P-value	-	0.013	0.019	0.014	0.008	0.006
Group 3	GCF NRG4	Rho	1.00	0.71	0.74	0.74	0.79	0.79
		P-value	<0.001	0.003	0.002	0.002	<0.001	<0.001
	GCF ErbB4	Rho	-	0.72	0.75	0.74	0.79	0.81
		P-value	-	0.002	0.001	0.002	<0.001	<0.001

Values in bold font indicate statically significant differences. GCF, gingival crevicular fluid; NRG4, neuregulin-4; PI, plaque index; GI, gingival index; BOP, bleeding on probing; PD, probing depth; CAL, clinical attachment level.

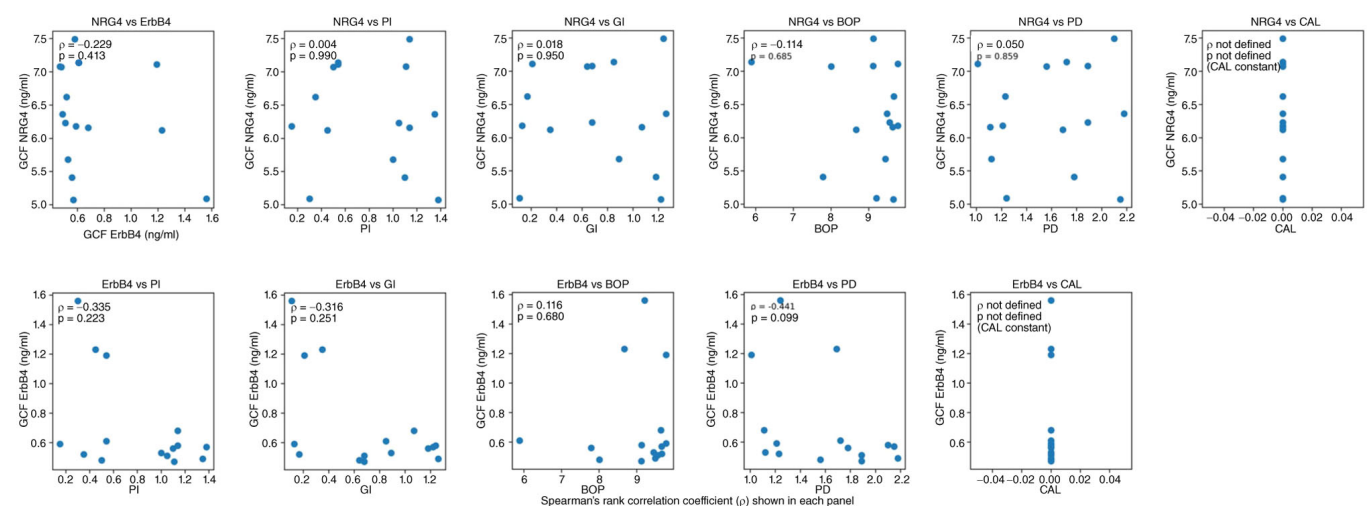


Figure 5. Spearman's correlation plots at 3 months for group 1 (n=15). NRG4, neuregulin-4; GI, gingival index; PI, plaque index; BOP, bleeding on probing; PD, probing depth; CAL, clinical attachment level.

and Nelson *et al* (16) reported increased prevalence and severity of periodontal disease among patients with diabetes.

NRG4 is a novel adipokine that functions primarily through ErbB4, and is involved in metabolic homeostasis, inflammation and insulin sensitivity (17). Kang *et al* (18) demonstrated an association between NRG4 and diabetes mellitus, while Attique *et al* (19) reported that reduced NRG4 levels were associated with impaired insulin sensitivity. Mechanistic studies by Díaz-Sáez *et al* (20) suggested that NRG4 downregulation may contribute to insulin resistance, reduced glucose transporter type 4 expression, increased pro-inflammatory cytokine production and mitochondrial dysfunction (21). Experimental models with impaired ErbB4 signaling exhibit obesity and insulin resistance, supporting the metabolic importance of Nrg4-ErbB4 signaling (22). The activation of this pathway may also suppress inflammatory signaling, including NF- κ B (23).

Although NRG4 has been extensively studied in metabolic disorders, its periodontal role remains less established. GCF provides a useful site-specific fluid for the evaluation of periodontal inflammatory changes; however, limited evidence is available regarding NRG4 and ErbB4 in GCF, particularly in periodontitis with T2DM (1,4,24,25).

In the present study, baseline NRG4 and ErbB4 levels were significantly higher in patients with periodontitis than in healthy individuals, with the highest concentrations observed in patients with both periodontitis and T2DM. This pattern suggests that periodontal inflammation and systemic metabolic dysregulation may influence the NRG4-ErbB4 axis.

Following NSPT, significant improvements were observed in PI, GI, BOP, PPD and CAL, accompanied by significant reductions in GCF NRG4 and ErbB4 levels. The decrease in biomarker levels may reflect the reduced inflammatory burden

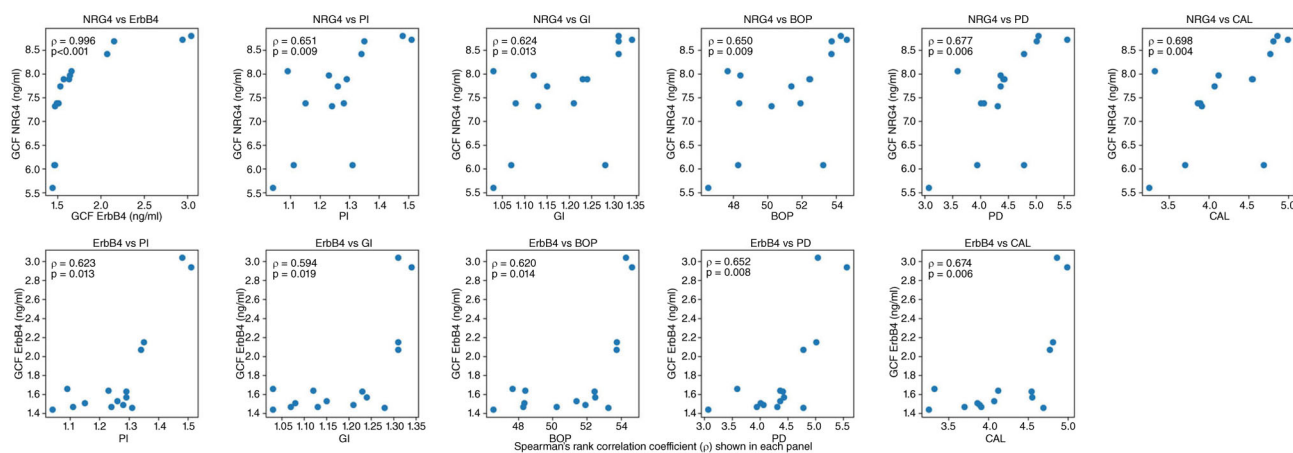


Figure 6. Spearman's correlation plots at 3 months for group 2 (n=15). NRG4, neuregulin-4; GI, gingival index; PI, plaque index; BOP, bleeding on probing; PD, probing depth; CAL, clinical attachment level.

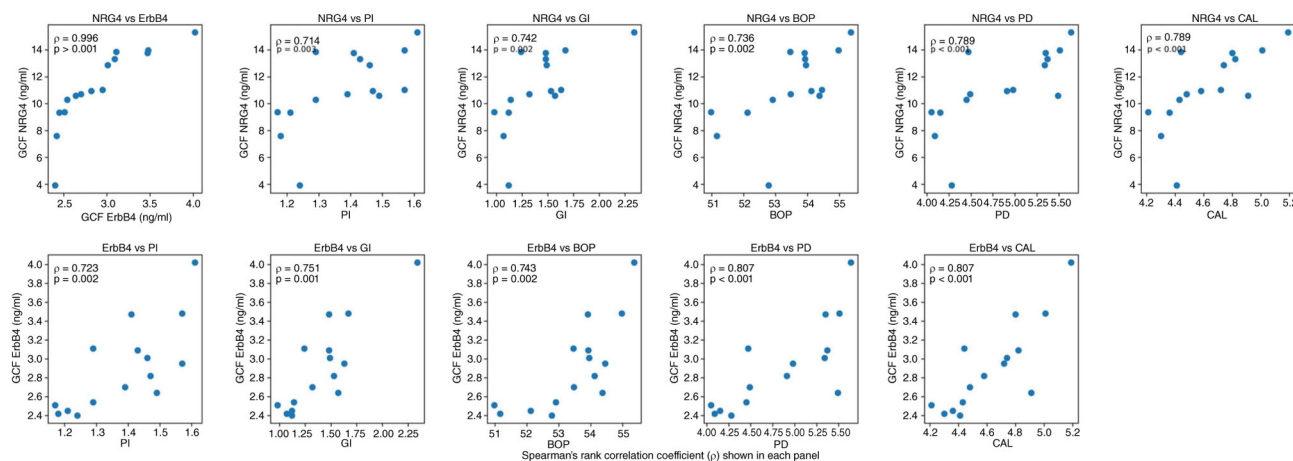


Figure 7. Spearman's correlation plots at 3 months for group 3 (n=15). NRG4, neuregulin-4; GI, gingival index; PI, plaque index; BOP, bleeding on probing; PD, probing depth; CAL, clinical attachment level.

following removal of subgingival microbial deposits and subsequent periodontal healing.

A positive association between the NRG4 and ErbB4 levels and clinical periodontal parameters was observed at baseline, suggesting that higher biomarker concentrations were associated with greater disease burden. Following treatment, these associations were reduced, possibly reflecting improvements in periodontal health and reduced inflammation.

The present findings are consistent with previous reports. Meraci *et al* (4) reported elevated GCF NRG4 and ErbB4 levels in periodontal disease, and these levels were decreased following NSPT. Abdel Fattah *et al* (1) similarly reported increased levels of these biomarkers in advanced periodontitis, particularly among patients with T2DM. Zhang and Ma (26) reported that an increased expression of ErbB4 was associated with periodontal inflammation and bone loss. The observed reduction following NSPT is also consistent with evidence demonstrating decreased inflammatory mediators after periodontal therapy (27).

Collectively, NRG4 and ErbB4 levels were associated with clinical periodontal parameters and changes following NSPT in the present study cohort. However, the present findings do

not establish their independent utility as biomarkers of disease activity or treatment response. Their presence in GCF suggests potential relevance as site-specific indicators of periodontal inflammation; however, their usefulness for monitoring disease progression or treatment outcomes requires validation in larger longitudinal studies. The findings also do not establish the NRG4-ErbB4 axis as a therapeutic target.

The present study has several limitations, including the relatively small sample size, single-center design, short 3-month follow-up and absence of an independent validation cohort. Furthermore, only patients with well-controlled T2DM were included; therefore, the findings cannot be extrapolated to patients with poorly controlled diabetes. Larger, longitudinal, multicenter and mechanistic studies are required to clarify the clinical significance and potential utility of NRG4 and ErbB4.

In conclusion, GCF NRG4 and ErbB4 levels were elevated in patients with stage III periodontitis, particularly among patients with well-controlled T2DM, and significantly decreased following NSPT. Their association with clinical periodontal parameters and changes following therapy suggest that NRG4 and ErbB4 may be involved in the inflammatory and metabolic interplay between periodontitis and T2DM.

However, their independent value as biomarkers of disease activity or treatment response cannot be established from the present study. Further larger, longitudinal, multicenter and mechanistic studies are required to validate these findings and determine their clinical significance.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

KSP was involved in the conception and design of the study, literature search, clinical procedures, data acquisition, statistical analysis and the drafting of the manuscript. SS contributed to the study design, study supervision, data interpretation, and critical revision of the manuscript. NKJ contributed to the conception and design of the study, overall supervision of the study, institutional support, data interpretation, and critical revision of the manuscript. KSP and SS confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board and Ethics Committee of Vydehi Institute of Dental Sciences and Research Centre, Bangalore, India (Approval no. VIDSIEC/UG/AAP/2024/136). Written informed consent was obtained from all participants prior to their inclusion in the study, in accordance with the principles of the Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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