European Review for Medical and Pharmacological Sciences 2023; 27: 8360-8370

Influence of mechanical debridement with adjunct probiotic therapy on clinical status and salivary cortisol levels in patients with periodontal inflammation

N. ALHAMOUDI[1], T. ABDULJABBAR[2], F. VOHRA[2], F. JAVED[3,4]

[1]Department of Periodontics and Community Dentistry, College of Dentistry, King Saud University, Riyadh, Saudi Arabia
[2]Department of Prosthetic Dental Sciences, College of Dentistry, King Saud University, Riyadh, Saudi Arabia
[3]General Dentistry, Brighton Dental Care, Brighton, NY, USA
[4]Department of Orthodontics and Dentofacial Orthopedics, Eastman Institute for Oral Health, University of Rochester, NY, USA

Abstract. – OBJECTIVE: The null hypothesis is that there is no difference in periodontal parameters and salivary cortisol levels (CL) among patients with periodontal inflammatory conditions (PIC) who receive non-surgical mechanical debridement (NSMD) with or without adjunct probiotic therapy (PT). The aim of this study was to assess the influence of NSMD with and without adjunct PT on clinical status and whole salivary CL in patients with PIC.

PATIENTS AND METHODS: Participants were randomly divided into four groups: Group-1: NSMD alone (n=19); Group-2: NSMD + PT (n=18); Group-3: PT + oral hygiene instructions (OHI) (n=17); and Group-4: PT alone (n=18). *Lactobacillus rhamnosus* SP1 (2×10^7 colony-forming units/day) was used for PT. Plaque and gingival indices (PI and GI), probing depth (PD) and clinical attachment loss (AL) were assessed, and whole salivary CL was assessed at baseline and at 6-week follow-up. Marginal bone levels were assessed at baseline and demographic data was collected using a questionnaire. Sample-size estimation was performed, and statistical significance was determined at $p<0.05$.

RESULTS: At follow-up, PI ($p<0.01$), GI ($p<0.01$), and PD ($p<0.01$) were significantly higher in PT + OHI and PT alone groups compared with individuals who underwent NSMD + PT and NSMD alone. At baseline and follow-up, there was no significant difference in CL in all groups. There was no statistically significant correlation between age, gender, PI, PD, GI, clinical AL, salivary flow rate, education status and salivary CL in all groups at baseline and follow-up.

CONCLUSIONS: NSMD continues to be the "gold standard" and most reliable treatment strategy for managing PIC. It is imperative to reach a consensus regarding the duration, dosage, and frequency of PT that would yield optimal results for managing PIC.

Key Words:
Alveolar bone loss, Clinical attachment loss, Cortisol, Probing depth, Probiotic therapy, Non-surgical mechanical debridement, Saliva.

Abbreviations

ABL, Alveolar bone loss; AL, Attachment loss; CL, Cortisol levels; DM, Diabetes mellitus; GI, Gingival index; LIP, Ligature-induced periodontitis; MBL, Marginal bone loss; NSMD, Non-surgical mechanical debridement; OHI, Oral hygiene instructions; PD, Probing depth; PI, Plaque index; PIC, Periodontal inflammatory conditions; PT, Probiotic therapy; RCT, Randomized clinical trial; RS, Restraint stress.

Introduction

Traditionally, non-surgical mechanical debridement (NSMD) is performed using hand instruments such as curettes and ultrasonic scalers for the treatment of periodontal diseases[1,2]. However, adjunct treatments such as probiotic therapy (PT) have been reported[3-5] to enhance the overall anti-inflammatory effect of NSMD compared to NSMD alone.

Probiotics are live bacteria and yeasts that offer health when either ingested or applied topically[6-8]. Probiotics are used for the management of a variety of disorders, including ulcerative colitis, mood disorders (such as depression), pancreatitis and cancer[9-12]. The role of PT in the management of clinical and experimentally induced periodontal inflammatory conditions (PIC) has also been evaluated[13-16]. In an experimental

Corresponding Author: Nawwaf Al-Hamoudi, MD; e-mail: nalhamoudi@ksu.edu.sa

study, Messora et al[13] assessed the influence of orally administered PT on the treatment of ligature-induced periodontitis (LIP) in rats. Rats in the PT (test group) and control (no PT) groups were euthanized after 44 days (approximately 6 weeks) and the jaws were histomorphometrically assessed. The results showed that alveolar bone loss (ABL) was significantly higher in the control than in the test group[13]. The study concluded that PT reduces ABL in rats with LIP[13]. Similarly, in another study[14], the efficacy of PT in reducing ABL in rats exposed to LIP and restraint stress (RS). The results showed that PT reduces periodontal tissue damage in unstressed rats but not in animals exposed to RS[14]. Clinically, results from a randomized placebo-controlled clinical trial[17] with an 8-week follow-up showed that NSMD with adjunct PT offers additional clinical and immunological benefits for the management of PIC compared with NSMD alone. In a recent systematic review and meta-analysis, Henrique Soares et al[18] compared the efficacy of PT and chlorhexidine in reducing PIC. The results showed that PT is a suitable alternative to chlorhexidine for managing PIC18. Matsubara et al[19] also reported that PT helps reduce PIC and help develop a healthy plaque microbiome via immunological and microbiological pathways. However, results from a recent randomized clinical trial (RCT)[15] with a 30-day follow-up showed no additional benefits when PT was used as an adjunct to NSMD for the management of PIC. Similar results were reported in another double-blind, paralleled-arm, placebo-controlled RCT[16]. These results[13-16] suggest that there is still a controversy over the effectiveness of PT for the clinical management of PIC.

Cortisol is a glucocorticoid, which is regulated by the hypothalamic pituitary adrenal axis (HPAA) and controls the release of stress hormones, including cortisol[20,21]. The HPAA is activated in response to inflammatory and emotional insults, thereby increasing the release of cortisol. Cortisol exhibits anti-inflammatory properties and protects the host in challenging environments such as psychological stress[22]. Significantly high cortisol levels (CL) have been reported in patients with PIC[23,24]; and one study[24] showed that NSMD helps reduce inflammation as well as CL in non-smokers with PIC. This suggests that NSMD, when performed with adjunct PT, is more effective in reducing periodontal inflammation and salivary CL than NSMD alone. However, results from an experimental study[14] showed that PT after NSMD offers no additional perks in terms of reducing ABL in rats with LIP. To our knowledge, there are no studies that have assessed whole salivary CL in relation to NSMD with and without adjunct PT in patients with PIC.

The aim of the present study was to assess the influence of NSMD with and without adjunct PT on clinical status and whole salivary CL in patients with periodontal inflammation. The present investigation is based on the null hypothesis that there is no difference in periodontal parameters and CL among patients with periodontal inflammation who receive NSMD with or without adjunct PT.

Patients and Methods

Ethical Statement

The study was designed, conducted and reported following the Consolidation Standards of Reporting Trials (CONSORT) Statement. The present study was performed following guidelines recognized by the Declaration of Helsinki as revised in 2013 for experimentation involving human patients (OR/SCC/1019-D). All participants were requested to read and sign a written informed consent form. The participants were invited to ask questions and were also informed that they could withdraw their participation at any stage of the investigation without consequences. Oral hygiene instructions (OHI) were given to all individuals irrespective of their decision to participate and withdraw from the present study.

Study Design, Location, and Timing

The present case-control study was performed at the Department of Periodontology and Implant Dentistry, Specialist Care Center, Riyadh, Saudi Arabia. The study was conducted between July and November 2022.

Inclusion and Exclusion Criteria

Adult individuals with any of the following characteristics were invited to participate in the present study (a) visible supragingival plaque on clinical examination; (b) patients with self-reported "bleeding gums"; (c) probing depth (PD) ≥3 mm in at least 30% sites; and (d) bleeding on gentle probing in at least 30% sites[25,26]. Third molars, supernumerary teeth, and grossly carious teeth, and remaining root remnants were not assessed. Self-reported combustible and smokeless tobacco product users, individuals using electronic nicotine delivery systems, and individuals with self-reported systemic diseases such as diabetes mellitus

(DM), prediabetes, acquired immune deficiency syndrome, hepatic and renal diseases and cardiovascular disorders were not included. In addition, edentulous patients and individuals who had undergone NSMD within the past three months were not sought. Individuals undergoing steroid, non-steroidal anti-inflammatory drugs, antibiotics or/and cancer therapy were also excluded.

Allocation Concealment, Randomization, Blinding and Grouping

Individuals who volunteered to participate were therapeutically randomly divided into four groups as follows: Group-1: Individuals who received NSMD alone; Group-2: Individuals who underwent NSMD with adjunct PT; Group-3: Individuals who received PT with stringent OHI; and Group-4: Individuals who received PT alone. The principal investigator performed allocation concealment by allotting code numbers to participants, which were used in a randomization software (www.randomization.com) to allocate patients into the respective study groups. All investigators involved in the clinical, radiographic, laboratory and statistical analyses were blinded to the study groups.

Questionnaire

Information regarding age, gender, daily toothbrushing frequency (once or twice), daily flossing (yes or no), and the most recent visit to a dentist and/or dental hygienist (within 6 months, within 6 to 12 months and over a year) was collected. This questionnaire also gathered information regarding the education status (ES) of participants. Individuals who reported to have attended a school up to Grade 10 were categorized as having "school-level education"[27]; individuals who had attained an additional two years' education after graduation from school were classified as having attained "college-level education"[27]; and individuals who reported to have attended a university were categorized as having attained "University-level education"[27].

Periodontal Parameters

Clinical and radiographic periodontal parameters were assessed at baseline and at 6-week follow-up. In all patients, PD[28], plaque index (PI)[29], gingival index (GI)[30] and clinical attachment loss (clinical AL)[31] were assessed on four surfaces per tooth. The number of missing teeth was also recorded. Clinical AL and PD were assessed using a graded probe (UNC-15, Hu-Friedy, Chicago, IL, USA). Full-mouth digital intraoral radiographs were taken using the long-cone paralleling technique. Marginal bone loss (MBL) was gauged as the perpendicular distance (in millimeters) from two millimeters below the cementoenamel junction to the alveolar crest[32]. All clinical and radiographic assessments were performed by a trailed and calibrated investigator (Kappa score 0.9). Clinical parameters were assessed at baseline and at 6-week follow-up.

Assessment of Whole Salivary Cortisol Levels

Unstimulated whole saliva samples (UWSS) were collected between 7:30 am and 8:30 am with all participants being in a fasting state. The protocol for UWSS collection is described in other studies[33,34]. After determining the salivary flow rate (SFR), UWSS were centrifuged at 1,500 rpm at 4°C and the supernatant was collected and stored in plastic tubes with lid (Fisherbrand™ Premium Microcentrifuge-Tubes, Waltham, MA, USA). Supernatants were immediately assessed in duplicates for CL using commercial ELISA kits (RayBio®, RayBiotech Life, Inc., Atlanta, GA, USA), which were used according to manufacturers' instructions. According to this kit (RayBio®, RayBiotech Life, Inc., Atlanta, GA, USA), the detection of CL ranged between 100-1,000,000 picograms per milliliter (pg/mL). The ELISA plates were read at 450 nanometers using a microplate reader (StatFax 2100, Awareness Tech. Inc., Palm City, FL, USA). All UWSS were analyzed by one trained and calibrated examiner (Kappa score 0.89).

Non-surgical Mechanical Debridement and Probiotic Therapy

Sterile hand instruments (Hu-Friedy, Chicago, IL, USA) and ultrasonic scalers (DTE Ultrasonic Scaler D1 Unit, CA, USA) were used to perform NSMD. In all patients, NSMD was performed by one experienced and calibrated investigator (Kappa score 0.89). In the present study, PT was performed using the protocol described by Morales et al[35]. In summary, participants in the NSMD + PT and PT + OHI groups received *Lactobacillus rhamnosus* SP1 [2×10^7colony-forming units (CFU)/day] (Macrofood S.A., Santiago de Chile, Chile) for six weeks. In the NSMD alone and PT alone groups, participants were given placebo sachets that were identical to the probiotic sachets. The dosage of placebo and probiotic sachets was one sachet taken orally daily. Individuals were instructed to dilute one sachet in water (150 mL) and ingest it once daily every morning after tooth brushing.

Sample Size Estimation

Sample-size estimation was based on data from a pilot investigation using the change in PD as the primary outcome variable. It was estimated that at least 15 individuals must be included in each group in order to detect a 2 mm difference in PD with an alpha of 5%. With these assumptions, the study was estimated to have a power of 89.8%.

Statistical Analysis

Data normality was assessed using the Shapiro-Wilk test. Data were presented as means ± standard deviations and group comparisons were done using one-way analysis of variance. For multiple comparisons, the Bonferroni post-hoc adjustment was performed. Correlation between CL and age, gender, ES, and clinical periodontal parameters (PD and clinical AL) was assessed using logistic regression models. Probability values that were lower than 5% were selected as indicators of statistical significance. Statistical significance was set at $p<0.05$.

Results

Study Population

Ninety-four individuals were invited to participate in the present study. Seventeen (5 males and 12 females) individuals refused to sign the consent form and five females declined participation without providing any reason. In total, seventy-two individuals (53 males and 19 females) agreed to participate in the present study and signed the consent form (Figure 1). Nineteen (mean

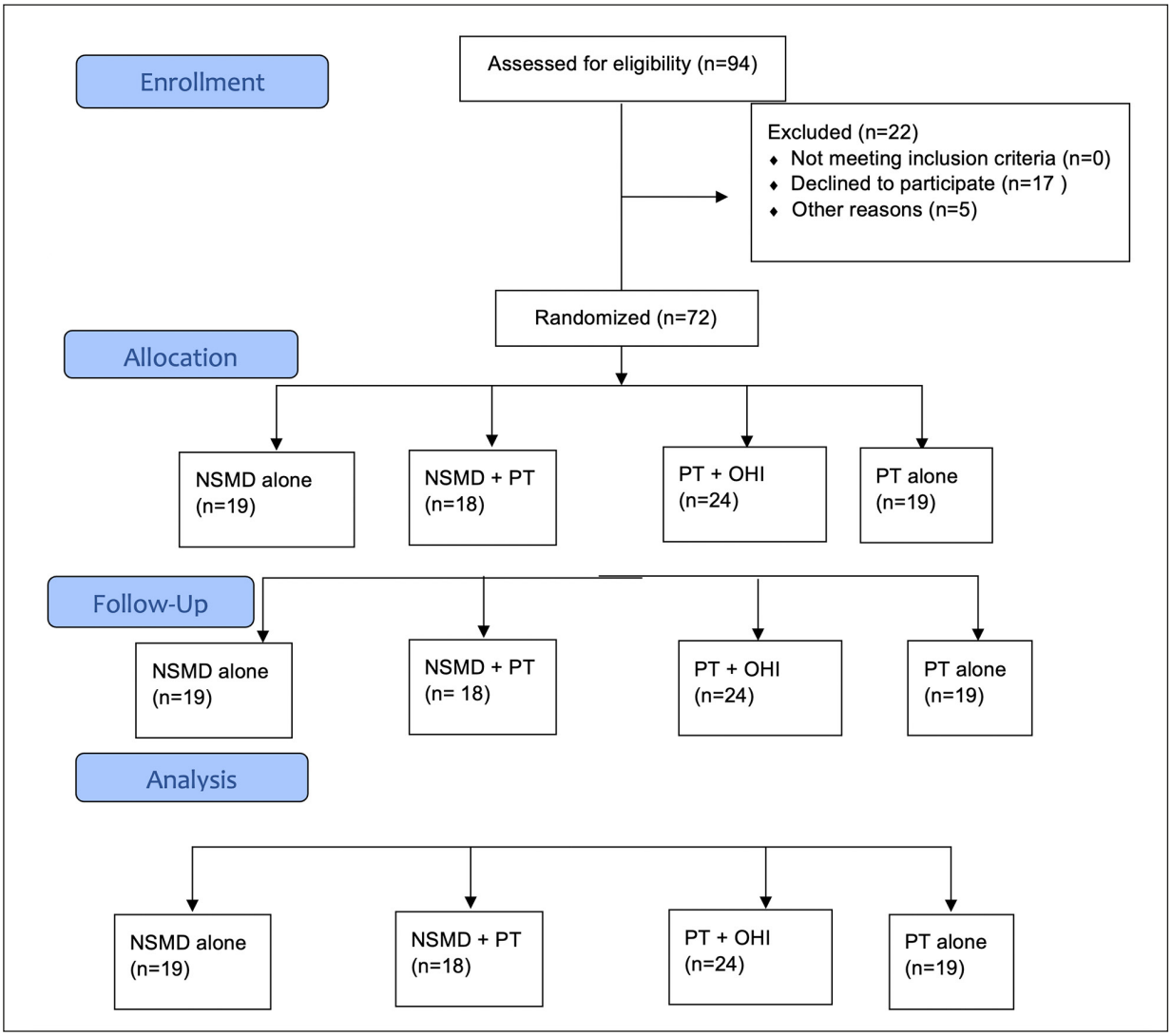

Figure 1. Protocol for recruitment of study participants.

age 37.6±2.5 years) and 18 (mean age 35.3±2.1 years) individuals were randomly assigned to the NSMD alone and NSMD + PT groups, respectively. In the PT + OHI and PT alone groups, 17 (mean age 36.5±2.7 years) and 18 individuals (mean age 37.7±0.8 years) were randomly assigned. University-level education was attained by 5.3% of individuals in the NSMD alone group and none in the remaining groups. In all groups, at least 72% of individuals reported that they were performing toothbrushing once daily. Daily flossing was reported by none of the individuals, and all participants reported to have visited an oral healthcare provider over a year ago (Table I).

Periodontal Status

At baseline, there was no significant difference in PI, GI, PD, clinical AL and MBL in all groups. At follow-up, there was a statistically significant reduction in PI ($p<0.01$), GI ($p<0.01$) and PD ($p<0.01$) in patients who underwent NSMD alone and NSMD + PT compared with their respective baseline scores. At follow-up, PI ($p<0.01$), GI ($p<0.01$) and PD ($p<0.01$) were significantly higher in PT + OHI and PT alone groups compared with individuals who underwent NSMD + PT and NSMD alone. At follow-up, there was no statistically significant difference in PI, GI and PD among patients who underwent NSMD + PT and NSMD alone. Throughout the study duration, there was no difference in clinical AL in all groups (Table II).

Salivary Flow Rate and Cortisol Levels

The whole SFR was similar in all groups at baseline and follow-up. Whole salivary CL at baseline and follow-up are shown in Table III. At baseline and follow-up, there was no significant difference in CL in all groups. Comparison of baseline and follow-up CL showed no statistical significance.

Logistic Regression Analysis

There was no statistically significant correlation between PD and whole salivary CL at baseline and follow-up in all groups (Figures 2 and 3). There was no statistically significant correlation between age, gender, PI, GI, clinical AL, SFR, ES, and salivary CL in all groups at baseline and follow-up (data not shown).

Discussion

The results of the present investigation are in accordance with the null hypothesis, according to which, there is no difference in periodontal parameters and whole salivary CL among patients with periodontal inflammation who receive MD with or without adjunct PT. In the present study, participants were mainly divided into four groups (a) individuals who underwent MD alone; (b) individuals who underwent MD with adjunct PT; (c) individuals who received PT and OHI and (d) individuals who received PT alone. The authors would like to clarify that there were initially two additional groups included in the initial study protocol submitted for ethical approval. These subgroups comprised individuals who either received no treatment (Group A) or received OHI alone (Group B) for the treatment of periodontal inflammation. The Ethics Council that reviewed

Table I. General characteristics of included patients.

Parameters	NSMD alone	NSMD + PT	PT + OHI	PT alone
Patients (n)	19	18	17	18
Male : Female	14 : 5	12 : 6	13 : 4	14: 4
Mean age in years	37.6±2.5 years	35.3±2.1 years	36.5±2.7 years	37.7±0.8 years
Education status				
School level	10 (52.6%)	12 (66.7%)	14 (82.4%)	14 (77.8%)
College level	8 (42.1%)	6 (33.3%)	3 (17.6%)	4 (22.2%)
University level	1 (5.3%)	None	None	None
Toothbrushing				
Once daily	14 (73.7%)	15 (83.3%)	13 (72.2%)	14 (77.8%)
Twice daily	4 (26.3%)	3 (16.7%)	4 (27.8%)	4 (22.2%)
Flossing (once daily)	None	None	None	None
Last visit to dentist/hygienist	None	None	None	None
Within 6 months	None	None	None	None
Within 6-12 months	None	None	None	None
Over a year ago	19 (100%)	18 (100%)	17 (100%)	18 (100%)

NSMD: Nonsurgical mechanical debridement, PT: Probiotic therapy, OHI: Oral hygiene instructions.

Table II. Periodontal status at baseline and 6 weeks' follow-up.

	Baseline				6 weeks' follow-up			
Periodontal Parameters	**NSMD alone**	**NSMD+PT**	**PT+OHI**	**PT alone**	**NSMD alone**	**NSMD+PT**	**PT+OHI**	**PT alone**
Plaque index	0.81±0.07*	0.84±0.05†	0.79 ±0.02	0.81±0.03	0.28±0.04‡	0.26±0.05	0.66±0.04	0.75±0.08
Gingival index	0.88±0.006*	0.8±0.004†	0.85± 0.07	0.88±0.004	0.16±0.005‡	0.2±0.007	0.79±0.03	0.81±0.05
Probing depth	5.1±0.3 mm*	5.30.2 mm†	5.3±0.2 mm	5.2±0.3 mm	1.2±0.1 mm	1.3±0.2 mm	4.8±0.2 mm	5.02±0.1 mm
Clinical attachment loss	0.6±0.07 mm	0.8±0.04 mm	0.7±0.2 mm	1.05±0.08 mm	0.5±0.05 mm	0.7±0.08 mm	0.7±0.1 mm	1.1±0.07 mm
Marginal bone loss (mesial)	0.50±0.2 mm	0.5±0.05 mm	0.8±0.1 mm	0.5±0.07 mm	NA	NA	NA	NA
Marginal bone loss (Distal)	0.7±0.3 mm	0.6±0.07 mm	0.6±0.05 mm	0.4±0.08 mm	NA	NA	NA	NA
Number of missing teeth (n)	1.4±0.4 teeth	1.5±0.6 teeth	2.07±0.3 teeth	2.2±0.4 teeth	1.4±0.4 teeth	1.5±0.6 teeth	2.07±0.3 teeth	2.2±0.4 teeth

NSMD: Nonsurgical mechanical debridement, PT: Probiotic therapy, OHI: Oral hygiene instructions. *Compared with NSMD alone at 6-weeks' follow-up (p<0.01), †Compared with NSMD + PT at 6-weeks' follow-up (p<0.01), ‡Compared with PT + OHI (p<0.01) and PT alone groups (p<0.01), NA: Not applicable.

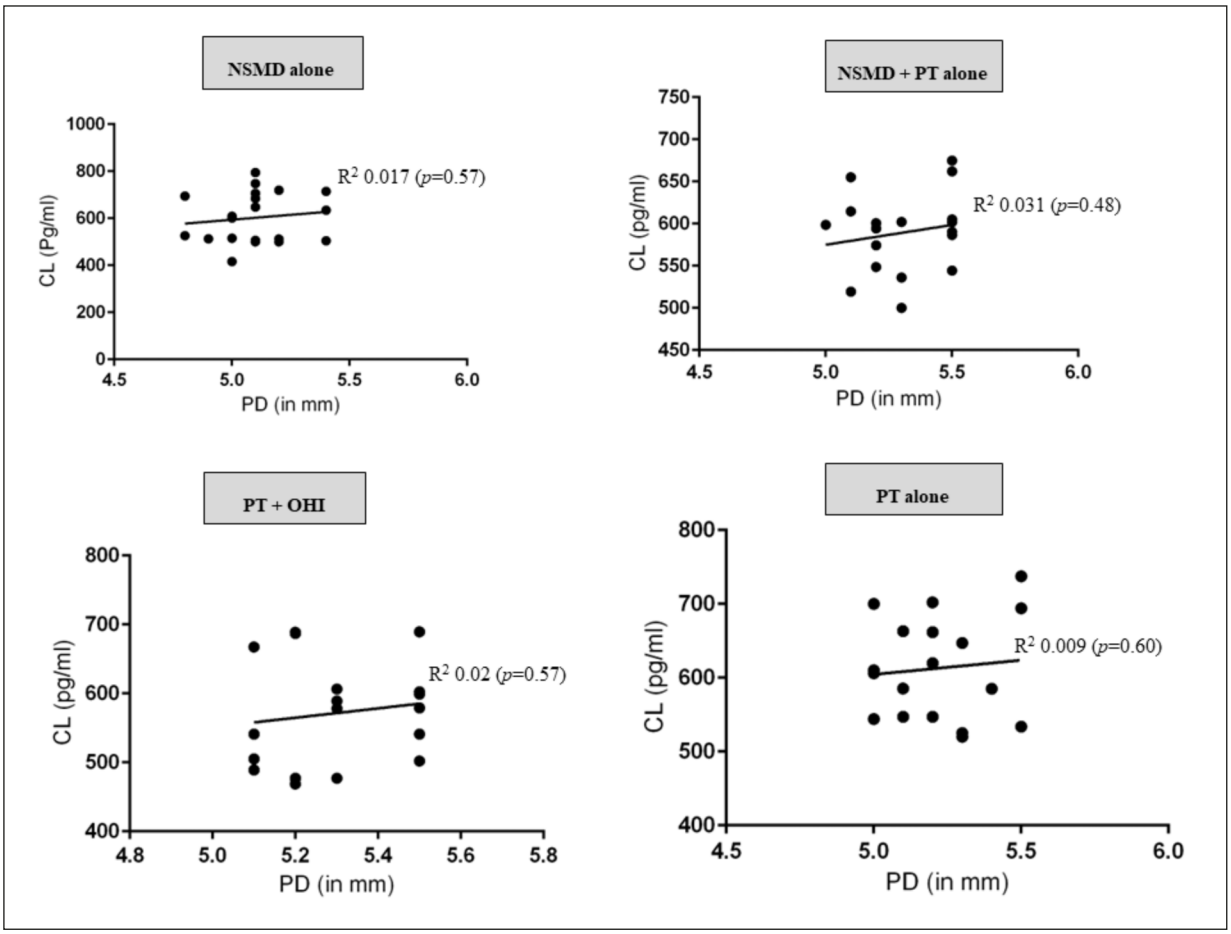

Figure 2. Correlation between probing depth and cortisol levels in the study groups at baseline.

Table III. Salivary flow rate and cortisol levels at baseline and 6 weeks' follow-up.

	Baseline				6 weeks' follow-up			
Periodontal Parameters	NSMD alone	NSMD+PT	PT+OHI	PT alone	NSMD alone	NSMD+PT	PT+OHI	PT alone
Salivary flow rate (ml/min)	0.11±0.005 ml/min	0.12±0.007 ml/min	0.1±0.002 ml/min	0.11±0.005 ml/min	0.1±0.004 ml/min	0.11±0.002 ml/min	0.11±0.003 ml/min	0.12±0.002 ml/min
Cortisol levels (pg/ml)	602.4±115.3 (pg/ml)	589.4±89.2 (pg/ml)	571.7±98.6 (pg/ml)	612.8±114.2 (pg/ml)	489.4±57.6 (pg/ml)	446.1±74.5 (pg/ml)	498.7±85.6 (pg/ml)	468.4±69.4 (pg/ml)

NSMD: Nonsurgical mechanical debridement, PT: Probiotic therapy, OHI: Oral hygiene instructions. *Compared with NSMD alone at 6-weeks' follow-up (p<0.01), †Compared with NSMD + PT at 6-weeks' follow-up (p<0.01), ‡Compared with PT + OHI (p<0.01) and PT alone groups (p<0.01), NA: Not applicable.

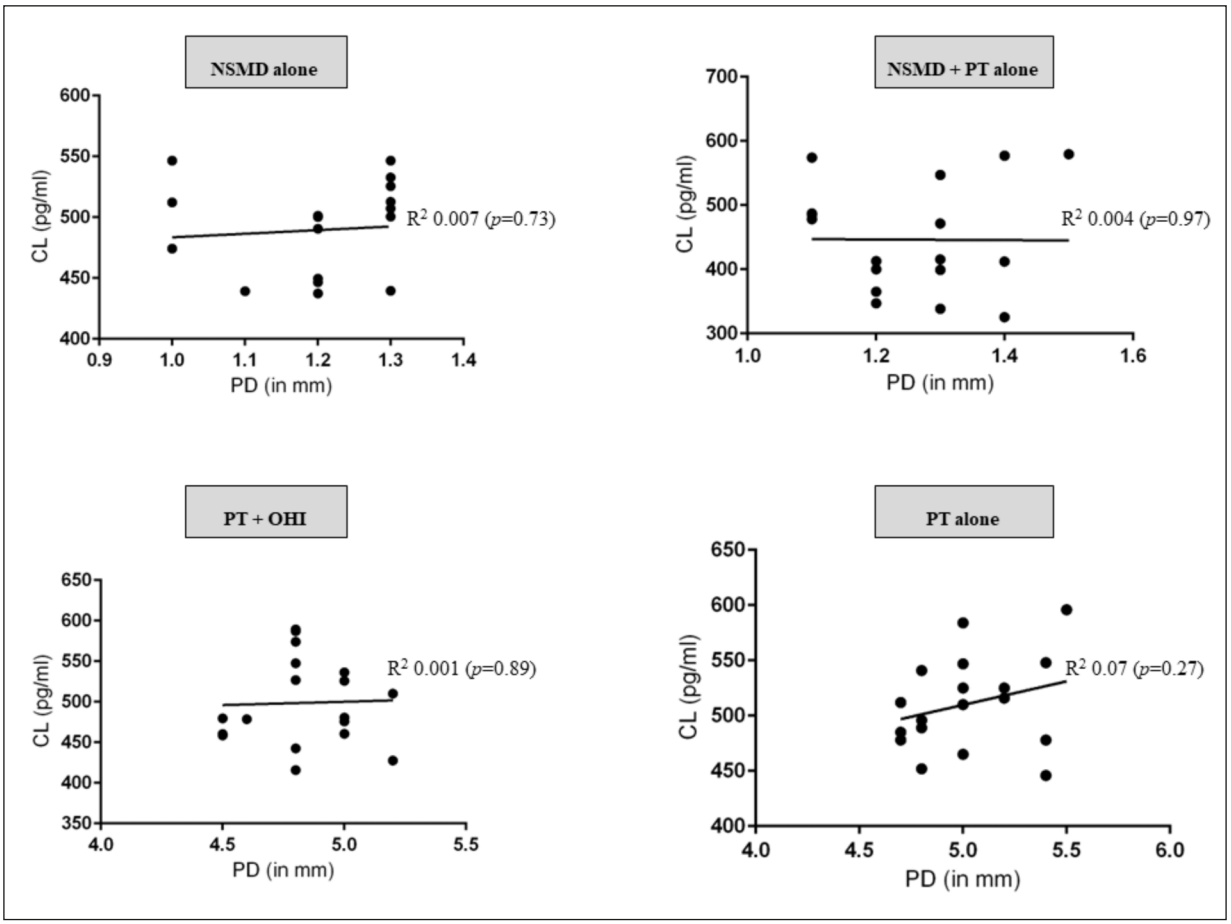

Figure 3. Correlation between probing depth and cortisol levels in the study groups at follow-up.

the research protocol prior to approval raised concerns in this regard and claimed that all patients in the current investigation must receive some form of therapy for the management of periodontal disease. Therefore, the investigators were instructed by the Review Committee to remove subgroups A and B prior to officially approving the research study. An interesting finding in the present study was that at follow-up, there was no difference in clinical periodontal parameters in patients who underwent PT + OHI or PT alone, as shown in Table III. Similarly, as demonstrated in Table III, whole salivary CL showed no statistically significant difference when baseline levels were compared to follow-up. It is demanding to present an absolute explanation for these findings;

however, patients' ES may have contributed to this regard. Based on the demographic results, the highest level of education reported by at least 50% of the individuals in each study group was up to Grade 10 (school-level education) (Table I). It has been reported[32,36] that a deprived ES is a risk factor for periodontal disease and psychological stress among adults. Moreover, an underprivileged ES is usually associated with a deprived socioeconomic status (SES)[36], which in turn is a major source of psychological stress in vulnerable populations[37]. It is, therefore, likely that the patient population assessed in the present study had a deprived SES, possibly due to a poor ES. This may have exposed these individuals to additional risk factors, such as psychological disorders, including anxiety. The authors hypothesize that improvements in socioeconomic and education statuses help improve the overall oral health status and reduce salivary CL in patients with oral inflammatory conditions, including periodontal diseases. However, further studies are needed to test this hypothesis.

It is worth mentioning that none of the participants were diagnosed with periodontitis in the present study. The average mesial and distal MBL assessed at baseline in the study groups was lower than 2 and 2 mm, which reflects health periodontal osseous health[38,39]. Advancing age is a risk factor for periodontitis[32]. According to Javed et al[32] individuals aged 60 years and older are more susceptible to PIC compared with individuals aged 45 years or younger. It is pertinent to note that all participants who agreed to participate in the present study were approximately 35 years old. This factor may have also contributed to masking the potential benefits of PT in these individuals. It is hypothesized that PT, when administered as an adjunct to NSMD, is more effective in treating severe forms of periodontal diseases such as Stage III periodontitis than NSMD alone.

Nevertheless, controversial results have also been reported. Pelekos et al[16], in a double-blinded placebo-controlled RCT with a follow-up of up to 180 days, showed that PT, when performed as an adjunct to NSMD, offers no additional benefits in contrast to NSMD for the management of periodontitis. To the authors' knowledge, there are no standard guidelines for PT for managing periodontitis. In this regard, it is imperative to determine a precise protocol and reach a global scientific consensus based on factors including but not limited to (a) the most effective type of probiotic microbe, (b) the concentration of the probiotic, (c) mode of delivery (local or systemic), duration of treatment and (e) daily dosage. Further power-adjusted and well-designed placebo-controlled RCTs are needed to justify the effectiveness of PT for managing oral diseases.

Limitations

One limitation of the present study is that MBL was assessed only at baseline. The reasoning for this is that since the present investigation had a follow-up duration of merely six weeks, it was challenging to expose patients to a second round of radiation exposure. Moreover, the presence of psychological diseases such as anxiety and/or depression was not asked for in the questionnaire. Moreover, the exclusion of patients using combustible and/or non-combustible nicotinic products and patients with systemic diseases such as DM was based on self-reported information, and this data was not validated via laboratory-based investigations such as assessment of serum cotinine and hemoglobin A1c levels. It is well-known that tobacco usage and persistent hyperglycemia compromise the outcomes of oral interventions such as NSMD[40-42]. Although questionnaires are reliable tools for assessing oral health[43,44], the risk of including patients with undiagnosed medical diseases, such as DM, that may potentially bias results cannot be overlooked.

Conclusions

Within the limits of the present study, the significance of PT as an adjunct to NSMD cannot be completely overlooked. There is a lack of consensus regarding the duration, dosage, and frequency of PT that would yield optimal results in terms of managing PIC. NSMD continues to be the "gold standard" and most reliable treatment strategy for managing periodontal disease.

Informed Consent

All participants were requested to read and sign a written informed consent form.

Authors' Contributions

N. Alhamoudi: formal analysis, methodology, formal analysis, writing the manuscript, and revisions before submission. T. Abduljabbar: supervision; funding acquisition, methodology, writing the manuscript, and revisions before submission. F. Vohra: methodology, formal analysis, methodology, formal analysis, writing the manuscript and revisions before submission. F. Javed: Writing the manuscript and revisions before submission.

Funding
The authors are grateful to the Researchers supporting project at King Saud University for funding through Researchers supporting project (RSP2023R44), Riyadh, Saudi Arabia.

Conflict of Interest
The authors declare that they have no conflict of interest.

ORCID ID
N. Alhamoudi: 0000-0002-5106-9871
T. Abduljabbar: 0000-0001-7266-5886
F. Vohra: 0000-0002-6204-7985
F. Javed: 0000-0002-9253-1989

Ethics Approval
The study was designed, conducted, and reported following the Consolidation Standards of Reporting Trials (CONSORT) Statement. The present study was performed following guidelines recognized by the Declaration of Helsinki as revised in 2013 for experimentation involving human patients. The study protocol was reviewed and approved by the Research Ethics Committee at the Specialist Care Center in Riyadh, Saudi Arabia (OR/SCC/1019-D).

References

1) Christgau M, Männer T, Beuer S, Hiller KA, Schmalz G. Periodontal healing after non-surgical therapy with a new ultrasonic device: a randomized controlled clinical trial. J Clin Periodontol 2007; 34: 137-147.
2) Heitz-Mayfield LJ, Lang NP. Surgical and nonsurgical periodontal therapy. Learned and unlearned concepts. Periodontol 2000 2013; 62: 218-231.
3) Nguyen T, Brody H, Radaic A, Kapila Y. Probiotics for periodontal health-Current molecular findings. Periodontol 2000 2021; 87: 254-267.
4) Stamatova I, Meurman JH. Probiotics and periodontal disease. Periodontol 2000 2009; 51: 141-151.
5) Teughels W, Van Essche M, Sliepen I, Quirynen M. Probiotics and oral healthcare. Periodontol 2000 2008; 48: 111-147.
6) Barbian ME, Patel RM. Probiotics for prevention of necrotizing enterocolitis: Where do we stand? Semin Perinatol 2023; 47: 151689.
7) Bernard NJ. Probiotics boost immunotherapy. Nat Immunol 2023; 24: 732.
8) Gao J, Zhao L, Cheng Y, Lei W, Wang Y, Liu X, Zheng N, Shao L, Chen X, Sun Y, Ling Z, Xu W. Probiotics for the treatment of depression and its comorbidities: A systemic review. Front Cell Infect Microbiol 2023; 13: 1167116.
9) Besselink MG, van Santvoort HC, Buskens E, Boermeester MA, van Goor H, Timmerman HM, Nieuwenhuijs VB, Bollen TL, van Ramshorst B, Witteman BJ, Rosman C, Ploeg RJ, Brink MA, Schaapherder AF, Dejong CH, Wahab PJ, van Laarhoven CJ, van der Harst E, van Eijck CH, Cuesta MA, Akkermans LM, Gooszen HG. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet 2008; 371: 651-659.
10) Palumbo VD, Romeo M, Marino Gammazza A, Carini F, Damiani P, Damiano G, Buscemi S, Lo Monte AI, Gerges-Geagea A, Jurjus A, Tomasello G. The long-term effects of probiotics in the therapy of ulcerative colitis: A clinical study. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2016; 160: 372-377.
11) Schaub AC, Schneider E, Vazquez-Castellanos JF, Schweinfurth N, Kettelhack C, Doll JPK, Yamanbaeva G, Mählmann L, Brand S, Beglinger C, Borgwardt S, Raes J, Schmidt A, Lang UE. Clinical, gut microbial and neural effects of a probiotic add-on therapy in depressed patients: a randomized controlled trial. Transl Psychiatry 2022; 12: 227.
12) Zaharuddin L, Mokhtar NM, Muhammad Nawawi KN, Raja Ali RA. A randomized double-blind placebo-controlled trial of probiotics in post-surgical colorectal cancer. BMC Gastroenterol 2019; 19: 131.
13) Messora MR, Oliveira LF, Foureaux RC, Taba M, Jr., Zangerônimo MG, Furlaneto FA, Pereira LJ. Probiotic therapy reduces periodontal tissue destruction and improves the intestinal morphology in rats with ligature-induced periodontitis. J Periodontol 2013; 84: 1818-1826.
14) Foureaux Rde C, Messora MR, de Oliveira LF, Napimoga MH, Pereira AN, Ferreira MS, Pereira LJ. Effects of probiotic therapy on metabolic and inflammatory parameters of rats with ligature-induced periodontitis associated with restraint stress. J Periodontol 2014; 85: 975-983.
15) de Oliveira AM, Lourenço TGB, Colombo APV. Impact of systemic probiotics as adjuncts to subgingival instrumentation on the oral-gut microbiota associated with periodontitis: A randomized controlled clinical trial. J Periodontol 2022; 93: 31-44.
16) Pelekos G, Ho SN, Acharya A, Leung WK, McGrath C. A double-blind, paralleled-arm, placebo-controlled and randomized clinical trial of the effectiveness of probiotics as an adjunct in periodontal care. J Clin Periodontol 2019; 46: 1217-1227.
17) Invernici MM, Salvador SL, Silva PHF, Soares MSM, Casarin R, Palioto DB, Souza SLS, Taba M, Jr., Novaes AB, Jr., Furlaneto FAC, Messora MR. Effects of Bifidobacterium probiotic on the treatment of chronic periodontitis: A randomized clinical trial. J Clin Periodontol 2018; 45: 1198-1210.
18) Henrique Soares K, Firoozi P, Maria de Souza G, Beatriz Lopes Martins O, Gabriel Moreira Falci S, Rocha Dos Santos CR. Efficacy of Probiotics Compared to Chlorhexidine Mouthwash in Improving Periodontal Status: A Systematic Review and Meta-Analysis. Int J Dent 2023; 2023: 4013004.

19) Matsubara VH, Fakhruddin KS, Ngo H, Samaranayake LP. Probiotic Bifidobacteria in Managing Periodontal Disease: A Systematic Review. Int Dent J 2023; 73: 11-20.

20) Karachaliou CE, Koukouvinos G, Goustouridis D, Raptis I, Kakabakos S, Petrou P, Livaniou E. Cortisol Immunosensors: A Literature Review. Biosensors (Basel) 2023; 13: 285.

21) Vega-Beyhart A, Araujo-Castro M, Hanzu FA, Casals G. Cortisol: Analytical and clinical determinants. Adv Clin Chem 2023; 113: 235-271.

22) Obulareddy VT, Chava VK, Nagarakanti S. Association of Stress, Salivary Cortisol, and Chronic Periodontitis: A Clinico-biochemical Study. Contemp Clin Dent 2018; 9: S299-S304.

23) Botelho J, Machado V, Mascarenhas P, Rua J, Alves R, Cavacas MA, Delgado A, João Mendes J. Stress, salivary cortisol and periodontitis: A systematic review and meta-analysis of observational studies. Arch Oral Biol 2018; 96: 58-65.

24) Alhumaidan AA, Al-Aali KA, Vohra F, Javed F, Abduljabbar T. Comparison of Whole Salivary Cortisol and Interleukin 1-Beta Levels in Light Cigarette-Smokers and Users of Electronic Nicotine Delivery Systems before and after Non-Surgical Periodontal Therapy. Int J Environ Res Public Health 2022; 19: 11290.

25) Armitage GC. Classifying periodontal diseases--a long-standing dilemma. Periodontol 2000 2002; 30: 9-23.

26) Armitage GC. Periodontal diagnoses and classification of periodontal diseases. Periodontol 2000 2004; 34: 9-21.

27) Javed F, Al-Zawawi AS, Allemailem KS, Almatroudi A, Mehmood A, Divakar DD, Al-Kheraif AA. Periodontal Conditions and Whole Salivary IL-17A and -23 Levels among Young Adult Cannabis sativa (Marijuana)-Smokers, Heavy Cigarette-Smokers and Non-Smokers. Int J Environ Res Public Health 2020; 17: 7435.

28) Armitage GC, Svanberg GK, Löe H. Microscopic evaluation of clinical measurements of connective tissue attachment levels. J Clin Periodontol 1977; 4: 173-190.

29) Silness J, Loe H. Periodontal disease in pregnancy. ii. correlation between oral hygiene and periodontal condtion. Acta Odontol Scand 1964; 22: 121-135.

30) Loe H, Silness J. Periodontal disease in pregnancy. i. prevalence and severity. Acta Odontol Scand 1963; 21: 533-551.

31) Armitage GC, Dickinson WR, Jenderseck RS, Levine SM, Chambers DW. Relationship between the percentage of subgingival spirochetes and the severity of periodontal disease. J Periodontol 1982; 53: 550-556.

32) Javed F, Näsström K, Benchimol D, Altamash M, Klinge B, Engström PE. Comparison of periodontal and socioeconomic status between subjects with type 2 diabetes mellitus and non-diabetic controls. J Periodontol 2007; 78: 2112-2119.

33) Ali D, Kuyunov I, Baskaradoss JK, Mikami T. Comparison of periodontal status and salivary IL-15 and -18 levels in cigarette-smokers and individuals using electronic nicotine delivery systems. BMC Oral Health 2022; 22: 655.

34) Ali D, Qasem SS, Baskaradoss JK. Periodontal Clinicoradiographic Status and Whole Saliva Soluble Urokinase Plasminogen Activation Receptor and Tumor Necrosis Factor Alpha Levels in Type-2 Diabetic and Non-diabetic Individuals. Oral Health Prev Dent 2021; 19: 481-488.

35) Morales A, Gandolfo A, Bravo J, Carvajal P, Silva N, Godoy C, Garcia-Sesnich J, Hoare A, Diaz P, Gamonal J. Microbiological and clinical effects of probiotics and antibiotics on nonsurgical treatment of chronic periodontitis: a randomized placebo- controlled trial with 9-month follow-up. J Appl Oral Sci 2018; 26: e20170075.

36) Husain N, Chaudhry N, Jafri F, Tomenson B, Surhand I, Mirza I, Chaudhry IB. Prevalence and risk factors for psychological distress and functional disability in urban Pakistan. WHO South East Asia J Public Health 2014; 3: 144-153.

37) Cundiff JM, Bennett A, Carson AP, Judd SE, Howard VJ. Socioeconomic status and psychological stress: Examining intersection with race, sex and US geographic region in the REasons for Geographic and Racial Differences in Stroke study. Stress Health 2022; 38: 340-349.

38) Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, Flemmig TF, Garcia R, Giannobile WV, Graziani F, Greenwell H, Herrera D, Kao RT, Kebschull M, Kinane DF, Kirkwood KL, Kocher T, Kornman KS, Kumar PS, Loos BG, Machtei E, Meng H, Mombelli A, Needleman I, Offenbacher S, Seymour GJ, Teles R, Tonetti MS. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol 2018; 45 Suppl 20: S162-S170.

39) Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, Flemmig TF, Garcia R, Giannobile WV, Graziani F, Greenwell H, Herrera D, Kao RT, Kebschull M, Kinane DF, Kirkwood KL, Kocher T, Kornman KS, Kumar PS, Loos BG, Machtei E, Meng H, Mombelli A, Needleman I, Offenbacher S, Seymour GJ, Teles R, Tonetti MS. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol 2018; 89 Suppl 1: S173-S182.

40) Apatzidou DA. The role of cigarette smoking in periodontal disease and treatment outcomes of dental implant therapy. Periodontol 2000 2022; 90: 45-61.

41) Javed F, Al-Rasheed A, Almas K, Romanos GE, Al-Hezaimi K. Effect of cigarette smoking on the clinical outcomes of periodontal surgical procedures. Am J Med Sci 2012; 343: 78-84.

42) Ko KI, Sculean A, Graves DT. Diabetic wound healing in soft and hard oral tissues. Transl Res 2021; 236: 72-86.

43) Gurses HN, Saka S, Zeren M, Bayram M. Validity and reliability of the Turkish version of breathlessness beliefs questionnaire. Physiother Theory Pract 2023; 39: 834-839.

44) Pilegaard MS, Nielsen KT, Enemark Larsen A, Wæhrens EE. Reliability and validity of the Danish version of the Self-Assessment of Modes Questionnaire. Scand J Occup Ther 2023; 30: 497-504.