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

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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 (2x10⁷ 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. However, adjunct treatments such as probiotic therapy (PT) have been reported 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. Probiotics are used for the management of a variety of disorders, including ulcerative colitis, mood disorders (such as depression), pancreatitis and cancer. The role of PT in the management of clinical and experimentally induced periodontal inflammatory conditions (PIC) has also been evaluated. In an experimental
SRP, probiotics, cortisol, and periodontal inflammation

study, Messora et al\textsuperscript{13} assessed the influence of orally administered PT on the treatment of ligation-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\textsuperscript{13}. The study concluded that PT reduces ABL in rats with LIP\textsuperscript{13}. Similarly, in another study\textsuperscript{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\textsuperscript{14}. Clinically, results from a randomized placebo-controlled clinical trial\textsuperscript{15} 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\textsuperscript{18} compared the efficacy of PT and chlorhexidine in reducing PIC. The results showed that PT is a suitable alternative to chlorhexidine for managing PIC\textsuperscript{18}. Matsubara et al\textsuperscript{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)\textsuperscript{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\textsuperscript{16}. These results\textsuperscript{14-16} 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\textsuperscript{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\textsuperscript{22}. Significantly high cortisol levels (CL) have been reported in patients with PIC\textsuperscript{23,24}; and one study\textsuperscript{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\textsuperscript{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) \(\geq 3\) mm in at least 30% sites; and (d) bleeding on gentle probing in at least 30% sites\textsuperscript{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”; individuals who had attained an additional two years’ education after graduation from school were classified as having attained “college-level education”; and individuals who reported to have attended a university were categorized as having attained “University-level education”.

**Periodontal Parameters**

Clinical and radiographic periodontal parameters were assessed at baseline and at 6-week follow-up. In all patients, PD, plaque index (PI), gingival index (GI) and clinical attachment loss (clinical AL) 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. All clinical and radiographic assessments were performed by a trained 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. 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. In summary, participants in the NSMD + PT and PT + OHI groups received *Lactobacillus rhamnosus* SP1 [2×10⁷ colony-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...
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

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**Table I.** General characteristics of included patients.

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

NSMD: Nonsurgical mechanical debridement, PT: Probiotic therapy, OHI: Oral hygiene instructions.
Table II. Periodontal status at baseline and 6 weeks’ follow-up.

<table>
<thead>
<tr>
<th>Periodontal Parameters</th>
<th>Baseline</th>
<th>6 weeks’ follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NSMD alone</td>
<td>NSMD+PT</td>
</tr>
<tr>
<td>Plaque index</td>
<td>0.81±0.07*</td>
<td>0.84±0.05†</td>
</tr>
<tr>
<td>Gingival index</td>
<td>0.88±0.006*</td>
<td>0.8±0.004†</td>
</tr>
<tr>
<td>Probing depth</td>
<td>5.1±0.3 mm*</td>
<td>5.3±0.2 mm†</td>
</tr>
<tr>
<td>Clinical attachment loss</td>
<td>0.6±0.07</td>
<td>0.8±0.04</td>
</tr>
<tr>
<td>Marginal bone loss (mesial)</td>
<td>0.50±0.2 mm</td>
<td>0.5±0.05</td>
</tr>
<tr>
<td>Marginal bone loss (Distal)</td>
<td>0.7±0.3 mm</td>
<td>0.6±0.07</td>
</tr>
<tr>
<td>Number of missing teeth (n)</td>
<td>1.4±0.4 teeth</td>
<td>1.5±0.6 teeth</td>
</tr>
</tbody>
</table>

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.
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;

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

<table>
<thead>
<tr>
<th>Periodontal Parameters</th>
<th>Baseline</th>
<th></th>
<th></th>
<th>6 weeks' follow-up</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NSMD alone</td>
<td>NSMD + PT</td>
<td>PT + OHI</td>
<td>PT alone</td>
<td>NSMD alone</td>
<td>NSMD + PT</td>
</tr>
<tr>
<td>Salivary flow rate (ml/min)</td>
<td>0.11±0.005</td>
<td>0.12±0.007</td>
<td>0.1±0.002</td>
<td>0.11±0.005</td>
<td>0.1±0.004</td>
<td>0.11±0.002</td>
</tr>
<tr>
<td>Cortisol levels (pg/ml)</td>
<td>602.4±115.3</td>
<td>589.4±89.2</td>
<td>571.7±98.6</td>
<td>612.8±114.2</td>
<td>489.4±57.6</td>
<td>446.1±74.5</td>
</tr>
</tbody>
</table>

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.
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 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), which in turn is a major source of psychological stress in vulnerable populations. 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. Advancing age is a risk factor for periodontitis. According to Javed et al, 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., 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. Although questionnaires are reliable tools for assessing oral health, 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.
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Conflict of Interest
The authors declare that they have no conflict of interest.

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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
SRP, probiotics, cortisol, and periodontal inflammation


