Research Protocol

Project Name：Characteristics of Subgingival Microbiota in Patients with Stage III-IV Periodontitis in Response to Nd:YAG Laser-Assisted Therapy

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1.Research Background and Rationale

Periodontitis is a microbially associated, host-mediated chronic inflammatory disease. Stage III-IV periodontitis, representing the advanced phase of disease progression, is characterized by severe alveolar bone loss and irreversible clinical attachment loss (CAL). According to the latest Global Burden of Disease Study, periodontitis ranks as the sixth most prevalent condition globally, with Stage III-IV cases constituting a significant proportion of affected individuals[1].

The primary therapeutic objectives in periodontitis management are plaque control and inflammation resolution. Conventional scaling and root planing (SRP), while foundational, exhibits inherent limitations including incomplete biofilm removal and iatrogenic tissue trauma[2]. The neodymium-doped yttrium-aluminium-garnet (Nd:YAG) laser (wavelength: 1064 nm), a near-infrared laser modality, demonstrates selective absorption for melanin and hemoglobin while maintaining low water absorption coefficients (0.61 cm^(-1)). This unique photophysical property enables deep tissue penetration and localized photothermal effects[3]. Recognized for its potent bactericidal, hemostatic, and soft tissue ablation capabilities, Nd:YAG laser has emerged as a minimally invasive adjunct to periodontal therapy. Nevertheless, the response patterns of subgingival microbiomes to Nd:YAG laser-assisted treatment remain insufficiently characterized in the current literature.

2.Study Objectives

This study aims to comparatively evaluate the clinical efficacy of Nd:YAG laser-assisted scaling and root planing (SRP) versus conventional SRP alone in the treatment of stage III-IV periodontitis. Furthermore, we seek to investigate the effects of Nd:YAG laser adjunctive therapy on the compositional structure and diversity of subgingival microbiota. By elucidating the potential

role of Nd:YAG laser in modulating subgingival microbial equilibrium, this research will provide novel theoretical insights and evidence-based guidance for the clinical management of periodontitis.

3. Study Design

3.1 Study Setting and Participants

From August 2024 to March 2025, 120 patients with stage III-IV periodontitis were recruited from the Department of Stomatology, The First Affiliated Hospital of Sun Yat-sen University, according to predefined eligibility criteria. All participants provided written informed consent. The study subjects were randomly allocated to either the conventional subgingival scaling and root planing (SRP) group (n=62) or the Nd:YAG laser-assisted SRP group (n=63).

3.1.1 Inclusion Criteria （1）Diagnosis of stage III-IV periodontitis based on the 2018 classification system jointly proposed by the European Federation of Periodontology (EFP) and the American Academy of Periodontology (AAP)[4, 5] , with: Maximum interdental clinical attachment loss (CAL) ≥5 mm, and Radiographic bone loss extending to the mid-third of the root or beyond; （2）Age 18–80 years;（3）Minimum of 14 natural teeth remaining in the dentition;（4）Willingness to provide informed consent and comply with study protocols.

3.1.2 Exclusion Criteria（1）Systemic conditions affecting periodontal treatment outcomes; （2）Periodontal therapy within the past 6 months; （3）Recent use of antibiotics or medications associated with gingival enlargement; （4）Pregnancy or lactation;（5）Acute periodontal lesions;（6）Inability to cooperate.

3.2 Sample Size Calculation

Based on preliminary data, the mean difference in post-treatment probing depth (PD) between the SRP group and Nd:YAG-assisted SRP group was estimated at 0.45 mm, with a standard deviation (SD) of 0.58 mm. Using PASS 25.0 software, we calculated the required sample size with the following parameters: Significance level (α): 0.05 (two-tailed) and Power (1-β): 90%. The initial computation indicated a minimum of 18 participants per group. To account for a potential ≤20% dropout rate, the final sample size was adjusted to at least 23 subjects per group (46 total).

3.3 Study Procedures

3.3.1 Periodontal Examination

One week after supragingival scaling, a single-blinded periodontist performed full-mouth examinations at baseline and 3-month follow-up, following the WHO Oral Health Surveys Basic Methods guidelines[6] . For each tooth, six sites (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, distolingual) were assessed for probing depth (PD)， clinical attachment loss (CAL)， gingival index (GI)， plaque index (PI) and bleeding on probing (BOP). The mean value of each parameter per patient was recorded as the final measurement.

3.3.2 Periodontal Treatment Protocol

Periodontal treatment was performed by a trained periodontist using a standardized quadrant approach, with half-mouth treatments completed per session separated by one-week intervals to ensure patient comfort and procedural consistency. Both treatment groups received full-mouth scaling and root planing using an EMS AIR-FLOW MASTER PIEZON® ultrasonic scaler and Gracey curettes, followed by comprehensive oral hygiene instructions and scheduling for 3-month follow-up visits.

The experimental group additionally received immediate Nd:YAG laser therapy post-SRP using a Wiser Waterlase MD system (Vista Dental) with manufacturer-recommended settings (150mJ/pulse, 20Hz frequency, water level 1 and air level 3 coolant). The laser was applied in a continuous zig-zag pattern across root surfaces and pocket linings, maintaining a 30° angle and delivering 2-second irradiation per treatment site to ensure thorough antimicrobial coverage while minimizing thermal damage to surrounding tissues.

All procedures were conducted under local anesthesia when clinically indicated, with strict adherence to infection control protocols and standardized patient positioning. Treatment fidelity was maintained through operator calibration, use of timer-controlled laser application, and periodic quality assurance checks to ensure consistent intervention delivery across all study participants throughout the trial duration.。

3.3.3 Subgingival Plaque Sampling and Microbiome Analysis

Subgingival plaque sampling procedure: Subgingival plaque samples were collected from six designated teeth (#16, #21, #24, #36, #41, #44) at baseline and 3-month follow-up, with adjacent

teeth substituted if target teeth were missing. After isolation with sterile cotton rolls and removal of supragingival plaque, subgingival plaque was obtained using sterile Gracey curettes and immediately placed in 1.5 mL sterile microtubes containing phosphate-buffered saline (PBS). All samples were stored at -80°C until analysis. The collected samples were subsequently sent to MajorBio Corporation for 16S rDNA high-throughput sequencing (V3-V4 hypervariable regions, Illumina platform). Bioinformatic analysis was performed using QIIME2 pipeline with SILVA 138 database for taxonomic classification. The sequencing results were then integrated with clinical parameters for comprehensive cross-sectional and longitudinal analyses to evaluate microbial shifts following treatment.

3.4 Data Management and Statistical Analysis Plan

Data collection and entry were performed according to standardized operating procedures (SOPs), with researchers manually entering all study data into a unified Excel spreadsheet.

Statistical analyses were conducted using SPSS 25.0 software. For continuous variables, the Shapiro-Wilk test was employed to assess normality of distribution. Normally distributed measurement data were expressed as mean ± standard deviation (SD), with between-group comparisons analyzed using independent samples t-tests and within-group pre-post treatment comparisons evaluated with paired t-tests. Categorical data were presented as percentages (%) and compared using chi-square tests. A two-sided P-value < 0.05 was considered statistically significant for all analyses.

3.5 Ethical Approval and Informed Consent

The study protocol was reviewed and approved by the Institutional Review Board/Ethics Committee in accordance with standard regulatory procedures. All participating volunteers provided written informed consent prior to enrollment.

4. References

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