Comparison of clinical parameters, microbiological effects and calprotectin counts in gingival crevicular fluid between Er:YAG laser and conventional periodontal therapies

A split-mouth, single-blinded, randomized controlled trial

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Abstract

Background: The erbium-doped yttrium, aluminum, and garnet (Er:YAG) laser is thought to be the most promising laser for periodontal treatment; however, its application is still under consideration. The aim of this study was to compare Er:YAG laser monotherapy with conventional scaling and root planing (SRP) for chronic periodontitis using clinical parameters, the detection rate of periodontal pathogens, and the calprotectin level in gingival crevicular fluid.

Methods: Twenty-seven participants with moderate-to-advanced chronic periodontitis were included. In a split-mouth design, the 2 half-mouths of each participant were randomly assigned to Er:YAG laser or SRP (combination of ultrasonic and manual instruments) treatment. Clinical parameters were recorded at baseline, 6 weeks, and 3 and 6 months after treatment. At the same time points, gingival crevicular fluid was collected to analyze the detection rate of 6 periodontal pathogens by polymerase chain reaction and the levels of calprotectin by enzyme-linked immunosorbent assay.

Results: Both treatment groups showed significant reductions in probing depth (PD), bleeding index (BI), and clinical attachment level (CAL) from baseline to 6 months. For sites with 4 mm \(\leq\) PD \(\leq\) 6 mm at baseline, SRP resulted in a greater reduction in PD and CAL than Er:YAG laser treatment, and the difference remained at 6 months post-treatment \((P<.01\) and \(P<.01\), respectively). For sites with PD \(\geq\) 7 mm at baseline, the clinical parameters showed similar results between the 2 groups. SRP resulted in a lower detection rate of Porphyromonas gingivalis at 6 months post-treatment. The levels of calprotectin were significantly decreased from baseline to 6 months in both groups, without a significant difference between the groups.

Conclusion: For mild pockets, conventional SRP may still be the preferred choice. For deep pockets, Er:YAG laser treatment could be an effective alternative. Studies are needed to explore more advanced instruments and new application methods for the Er:YAG laser for periodontal treatment in deep pockets.

Abbreviations: BI = bleeding index, CAL = clinical attachment level, Er:YAG = erbium-doped yttrium, aluminum, and garnet, GCF = gingival crevicular fluid, PD = probing depth, Pg = Porphyromonas gingivalis, PLI = plaque index, SRP = scaling and root planing.

Keywords: calcium-binding proteins, chronic periodontitis, lasers, microbiology, nonsurgical periodontal debridement

1. Introduction

Chronic periodontitis is a common inflammatory disease that can result in gingival inflammation, alveolar bone absorption, tooth mobility, and tooth loss. Chronic periodontitis is mainly caused by subgingival microbiota infections combined with the host inflammatory response.\textsuperscript{11} The primary goal of chronic periodontitis treatment is to remove the bacterial biofilm and calculus from the teeth and obtain good root surface biocompatibility.\textsuperscript{12} For many years, scaling and root planing (SRP), using a combination of hand curettes and ultrasonic scalers, has become the standard treatment for periodontal disease, resulting in beneficial outcomes.\textsuperscript{11} However, such conventional treatments have limitations, such as difficulty accessing deep periodontal pockets and other anatomical structures.\textsuperscript{4,3}

Light from an erbium-doped yttrium, aluminum, and garnet (Er:YAG) laser, which has a wavelength of 2940 nm, is highly absorbed by water and hydroxyapatite to form a micro-explosion, performing the clearance of soft and hard tissues
without causing heat damage.\[16\] It has been reported that an Er:YAG laser can effectively remove calculus and diseased cementum without thermal damage\[7,8\] and with good bactericidal effects.\[9\] Several studies and 2 systematic meta-analyses have indicated similar outcomes in terms of clinical parameters between Er:YAG laser treatment and SRP in the short term.\[10\] However, most studies were based on comparisons between Er:YAG laser monotherapy and either manual or ultrasonic SRP alone. The number of comparisons between Er:YAG laser treatment and the combination of ultrasonic and manual instruments, which is the most common nonsurgical treatment, is still limited, and the results are controversial.\[10,14,19\] Thus, additional evidence is needed to comprehensively evaluate the effects of Er:YAG laser treatment in comparison with the commonly used SRP treatment.

Therefore, the objective of this randomized, split-mouth controlled clinical study was to compare the effects of Er:YAG laser monotherapy and common SRP therapy (combination of ultrasonic and manual instruments) for chronic periodontitis over a 6-month follow-up period with regard to 3 aspects: clinical parameters, the detection rate of periodontal pathogens, and the calprotectin level in gingival crevicular fluid (GCF) as an immune-response inflammatory biomarker.

2. Methods

2.1. Study design

This study was a randomized, single-blinded, split-mouth, controlled prospective clinical trial. In a split-mouth design, 2 half-months of each participant were randomly assigned to the Er:YAG laser monotherapy or SRP; thus, 1 half-mouth of each participant was paired with the other half-mouth from the same participant and served as its control.

2.2. Participants

All participants were recruited from the Department of Periodontology, Beijing Stomatological Hospital, Capital Medical University, between September 2013 and October 2016. The Ethics Committee of Beijing Stomatological Hospital evaluated and approved the study protocol (Protocol: 2013–02). Before the study, all participants provided written informed consent.

Inclusion criteria were age ≥18 years; teeth number ≥16; a diagnosis of generalized chronic periodontitis based on the classification of the World Workshop 1999;\[20\] probing depth (PD) ≥4mm and clinical attachment level (CAL) ≥2mm for at least one-third of all mouth sites, and a full-mouth intraoral radiograph was taken to be sure that at least one-third of all approximal sites had bone loss; good general health. Exclusion criteria were periodontal therapy within the previous 6 months; taking antibiotics, steroids, or anti-inflammatory agents within the previous 3 months; current or previous smokers; pregnancy; and systemic diseases, such as diabetes, cardiovascular diseases, or blood diseases.

The sample size was calculated considering 90% power, 5% level of significance, 0.5mm significant difference, and 0.65 mm standard deviation (SD) for PD; using the PASS 11.0 software package for paired means power analysis. At least 20 patients were required for the study. Additional patients were enrolled in the study to compensate for loss during follow-up.

2.3. Oral hygiene program

One week before treatment, all participants were given oral hygiene instructions that reinforced the use of a soft manual toothbrush, dental floss, and interproximal brushes based on individual needs, and also a professional full-mouth supragingival debridement including ultrasonic cleaning and polishing by a single operator, who was blinded to the allocation. The same procedure was performed during recall visits at 6 weeks, and 3 and 6 months after treatment.

2.4. Treatment

2.4.1. Er:YAG laser group. An Er:YAG laser (LAEDL001.1, Doctor Smile, Italy) was applied at a pulse energy of 160mJ and a frequency of 10 Hz with water irrigation. The laser beam was delivered by the hand piece configured with the laser equipment, and the fiber tip was chisel-shaped (1.1 mm × 0.5 mm). During treatment, the fiber tip was held at an angle of 15° to 20° to the root surface and was moved in a coronal to apical direction, with overlapping parallel paths.

2.4.2. Conventional SRP group. The ultrasonic treatment was performed with an ultrasonic hand piece (PS, Satelec, France) and metal tip (H3, H4) at medium or low power, and the manufacturer’s instructions were strictly followed. Gracey curettes (Gracey, SG # 5/6, 7/8, 11/12, 13/14, Hu-Friedy) were then used for root surface planing.

All sites with an initial PD ≥4mm received treatment. The 2 different treatments for each patient were performed at 2 times, and the interval was 1 week. For both groups, the endpoint of the treatment occurred when smooth and thoroughly debrided root surfaces were detected by a pointed probe. Both groups’ treatments were performed by a single experienced operator. To prevent operator bias, another experienced periodontist verified the clinical endpoint. There was no time limit for treatment.

2.5. Clinical measurements

Clinical measurements were performed before and at 6 weeks, 3 months, and 6 months after treatment. The plaque index (PLI, Turesky–Gilmore–Glickman modification of the Quigley–Hein index);\[21\] bleeding index (BI, Mazza index);\[22\] PD, and CAL (distance from the cemento-enamel junction to the bottom of the pocket) were assessed at 6 sites per tooth.

All clinical examinations were performed by the same examiner. Intraxaminer reliability was determined by 2 measurements performed on 10 patients 5 to 7 days apart. The intraclass correlation coefficient value for PD and CAL were 0.97 and 0.91, respectively.

2.6. GCF sampling and processing

Sites with BI ≥2 and PD ≥5 mm at baseline were sampled before and at 6 weeks, and 3 and 6 months after treatment. Before sampling, supragingival plaque was gently removed, and the tooth surface was isolated by cotton rolls and gently dried with an air gun. Absorbent paper points (40#, Meita, South Korea) were carefully inserted into the sampling sites until slight resistance was felt and were held there for 30 seconds. Paper points contaminated by blood or saliva were discarded. The volume of GCF was calculated as the weight difference before and after sampling at a ratio of 1 g/mL. Sampled paper points were then placed separately in sterile tubes and stored at −70°C for further analysis.
Before analysis, the samples were thawed at room temperature, and phosphate buffered solution (pH 7.4, L) was added to achieve a 100-fold dilution. After thorough mixing and centrifugation, the sediment was collected to analyze the microorganism content, and the supernatant was collected to analyze the calprotectin level. All the GCF sampling was performed by the same examiner as performed by the clinical examination, and all the detection was performed by another examiner.

2.7. Detection of microorganisms
DNA was extracted from the GCF using a genomic DNA extraction kit (DP302, Tiangen, China) according to the manufacturer’s instructions. The samples were analyzed to detect Porphyromonas gingivalis (Pg), Tannerella forsythia, Treponema denticola, Prevotella intermedia, Prevotella nigrescens, and Fusobacterium nucleatum using standard PCR methods. The primers for the 6 microorganisms were based on the 16S rRNA gene (see Table, Supplemental Content, http://links.lww.com/MD/C27, which presents the primer sequences for 6 periodontal pathogens).

2.8. Detection of calprotectin
Calprotectin levels were assayed using commercially available ELISA kits (CSB-E12149h; Cusabio, China) and strictly following the manufacturer’s instructions.

2.9. Statistical analysis
The SPSS 19.0 software package was used for the statistical analysis. Qualitative data are reported as frequencies or percentages, and quantitative data are reported as the means and SD. Normality was assessed with the Shapiro-Wilks test. As each participant served as his own control, participants who were present at the recall visits were included in the analysis. A paired t test was used for the intragroup and between-group comparisons. The alpha error was 0.05.

2.10. Randomization, allocation concealment, and blinding
Randomization of half-mouth treatment and treatment order was generated by Microsoft Excel software. Allocation was concealed in opaque envelopes with serial numbers and was stored by a central registrar. The examiner, laboratory personnel, and statistician were blinded to the allocation. The allocation was concealed from the operator until the treatments.

3. Results
3.1. Experimental population and participant characteristics
After 254 periodontal patients were screened, 34 participants received both treatments, and 27 of them attended the first recall visit. The mean age of the 27 participants was 43.6 ± 8.7 years (range 28–56 years), and 13 participants were male. Four participants were absent from the 3-month and 6-month recall visits, and 1 participant did not attend the 6-month recall visit. All absences were for personal reasons (Fig. 1). This study included 612 teeth and 2213 sites (Table 1).

3.2. Clinical parameters
The healing process of both the Er:YAG and SRP groups was uneventful. No abscesses or acute infection symptoms were found during the observation period.
Table 1

Table and site distributions divided according to treatment.

| Treatment | Teeth | Sites 4 mm ≤ PD ≤ 6 mm | Sites PD ≥ 7 mm |
|-----------|-------|-------------------------|----------------|
| ERL       | 304   | 904                     | 183            |
| SRP       | 308   | 948                     | 178            |
| Total     | 612   | 1852                    | 361            |

ERL = Er:YAG laser group, PD = probing depth, SRP = ultrasonic and manual scaling and root planing group.

Table 2 shows the clinical parameters of sites with 4 mm ≤ PD ≤ 6 mm at baseline during the 6-month observation from 2 intervention groups. Both groups showed significant improvements in PD, BI, and CAL from baseline. Compared with Er:YAG treatment, SRP resulted in significantly greater reductions in PD at 6 weeks, 3 months, and 6 months post-treatment (P < .01, P = .04, and P = .01, respectively), in BI at 6 weeks post-treatment (P = .01), and in CAL at 6 weeks, 3 months, and 6 months post-treatment (P = .03, P = .04, and P < .01, respectively).

Table 3 shows the clinical parameters of sites with PD ≥ 7 mm at baseline during the 6-month observation from the 2 intervention groups. Both groups showed significant reductions in PD, BI, and CAL from baseline. There were no significant differences in any clinical parameters between the 2 groups.

3.3. Microorganisms in GCF

A total of 692 samples were collected in this study, which included 348 in the laser group and 344 in the SRP group for 4 visits. Pg, Tf, Td, and Pd were significantly decreased after treatment, whereas Pn and Fn showed no significant differences before and after treatment. There was no significant difference between the 2 groups at any time point, except that the detection...
rate of $Pg$ in the SRP group at 6 months post-treatment was lower than in the Er:YAG group ($P = 0.04$) (Fig. 2).

3.4. Calprotectin levels in GCF

For both groups, the concentration and total amount of calprotectin had decreased significantly at 6 weeks and at 3 and 6 months after treatment compared with baseline. At the same time points, there was no significant difference in the concentration or total amount of calprotectin between the Er:YAG and SRP groups (Fig. 3).

4. Discussion

In our study, both Er:YAG laser and conventional SRP treatments for chronic periodontitis achieved significant improvements in clinical parameters, microbiological effects, and calprotectin levels during the 6-month follow-up. For sites with $4 \text{ mm} \leq \text{PD} \leq 6 \text{ mm}$ at baseline, SRP resulted in a greater reduction in PD and CAL, which remained significant at 6 months post-treatment. For sites with PD $\geq 7 \text{ mm}$ at baseline, the clinical parameters showed similar results between the 2 groups. The $Pg$ detection rate was significantly lower in the SRP group at 6 months after treatment. There was no difference in calprotectin levels between the 2 groups at any time point.

Most of the previous studies were based on comparisons between Er:YAG laser monotherapy and either manual or ultrasonic SRP alone. In the present study, a combination of ultrasonic and hand instruments was used as a control group to simulate common clinical practice as closely as possible, and this combination could provide better results than either instrument alone. To better explore the application of Er:YAG laser, we grouped the sites with $4 \text{ mm} \leq \text{PD} \leq 6 \text{ mm}$ and PD $\geq 7 \text{ mm}$ at baseline as mild pockets and deep pockets, respectively. For mild pockets, we found that SRP resulted in better improvement in PD and CAL compared with Er:YAG laser monotherapy, and the difference lasted until 6 months after treatment. One report by Soo et al. also compared Er:YAG laser monotherapy with a combination of ultrasonic and hand instruments, showed that SRP resulted in significantly greater short-term improvement in clinical parameters. Interestingly, in that study, pockets with PD $< 4 \text{ mm}$ were also included in the treatment and analysis, and the mean value of PD was even lower than our mild pockets. A study by Rotundo et al also compared Er:YAG laser monotherapy with the combination of ultrasonic and hand instruments, in which the mean value of PD in both groups was 5.2 mm, and less PD and CAL gain were also shown in the Er:YAG laser group. For deep pockets, our study showed that Er:YAG laser monotherapy resulted in comparable clinical improvement to SRP. Although we did not show more
between Er:YAG laser and conventional SRP treatments. Calprotectin, which is also called myeloid-related protein or S100A8/A9, is a calcium-binding protein that is mainly produced by polymorphonuclear leukocytes, monocytes, macrophages, and epithelial cells, and is involved in the early immune response.\[33,34]\] It has been reported that a high calprotectin level correlates with deteriorating periodontal status and other biomarkers (interleukin [IL]-1β, prostaglandin E2, collagenase, and aspartate aminotransferase),\[35,36\] and it can be reduced by successful periodontal treatments.\[37–40\] Kaner et al\[37\] observed that sites with a high level of calprotectin in GCF at 3 months post-treatment were more likely to show disease progression from 3 to 6 months post-treatment, indicating that the calprotectin level in GCF could be a useful biomarker for monitoring the effects of periodontal treatment at the site level. A recent study conducted by Eick et al\[41\] evaluated the levels of several biomarkers, including calprotectin, IL-1β, matrix metalloproteinase (MMP)-8, MMP-1, and tissue inhibitor of MMP-1, in the GCF of periodontitis patients before and after SRP. Furthermore, calprotectin in GCF at 3 and 6 months after SRP was the most differentiated biomarker between high and low-treatment response sites at 6 months after SRP, suggesting that calprotectin is a relatively sensitive biomarker to show the effects of periodontal therapy. In our study, the calprotectin level showed similar significant reductions after Er:YAG laser and SRP treatments, and the reduction remained for up to 6 months, indicating comparable effects of these 2 treatments in a more sensitive and predictive way.

There are limitations of the present study. First, only a limited number and type (systemically healthy nonsmokers with chronic periodontitis) of participants were enrolled. Second, microbiological effects were revealed by the detection rate of periodontal pathogens. Third, we detected only the level of calprotectin as a biomarker reflecting the host immune response. Finally, our study investigated only the most common application of the Er:YAG laser. In recent years, more advanced Er:YAG laser instruments have been developed, such as an Er:YAG laser instrument with an automatic calculus detection system,\[42\] a fiberless Er:YAG laser system,\[43\] and modified applications,\[42,43\] and more studies are needed to evaluate their effects.

5. Conclusions

For mild pockets, common SRP may be still the preferred choice, whereas for deep pockets, Er:YAG laser treatment could be an effective alternative. More studies are needed to explore more advanced instruments and new application methods for Er:YAG laser therapy of periodontitis in deep pockets.

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