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Bactericidal effects of using a fiber-less Er:YAG laser system for treatment of moderate chronic periodontitis: Preliminary results

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Objective: The purpose of this study was to evaluate the bactericidal effectiveness of using a fiber-less Er:YAG laser in the first stage of therapy for moderate chronic periodontitis and to compare it with conventional treatment. **Materials and Method:** Two quadrants from 20 patients with moderate chronic periodontitis were treated with Gracey curettes (control), and the contralateral two quadrants in each patient were treated using an Er:YAG laser with total power of 1.5W (test). Subgingival plaque samples from the four deepest pockets in each quadrant were taken immediately before and 1 month after treatment, and the presence of nine marker bacteria were studied using real-time polymerase chain reaction technology. **Results:** A significant reduction of total pathogens and bac-

teria from the red complex was observed 1 month after treatment with both procedures. The results were more significant for the test group ($P = .003$) than for the control group ($P = .005$). Qualitative analysis of sites that had a therapeutically significant number and proportion of marker bacteria also showed significant reduction after treatment. **Conclusion:** The results of the present study suggest that the Er:YAG laser possesses comparable with conventional treatment bactericidal effectiveness against periodontal pathogens in vivo in the initial treatment of moderate chronic periodontitis. More evaluations should be performed to prove these results for a long-term successful clinical outcome. (*Quintessence Int* 201#;##:1-9; doi: ##.###/j.qi.a#####)

Key words: antibacterial, curette, Er:YAG laser, periodontal disease

Chronic periodontitis is one of the most common oral diseases. It is widely accepted that its initiation and progression depends on the presence of marker bac-

teria¹ that have been defined and divided into specific groups, according to their pathogenic potential.² The major goal of periodontal treatment is therefore to eliminate the bacterial biofilm.³

Nonsurgical periodontal treatment, ie scaling and root planing (SRP), eliminates dental plaque, calculus, and bacterial debris from the root surface.⁴ A variety of hand and power-driven instruments are used for this purpose, but they have some limitations, such as difficult access to furcation areas and concavities, as well as some disadvantages: they are time-consuming and require significant operator effort.^{5,6}

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In recent years, lasers have been widely discussed and investigated as tools in periodontal therapy. Among the different systems, Erbium:yttrium-aluminum-garnet (Er:YAG) lasers seem most promising for periodontal treatment because of their ability to ablate both soft and hard tissue. Nd:YAG-, CO₂- and diode lasers are effective only in soft tissues.^{7,8} Investigations have shown that Er:YAG lasers can remove dental calculus and be used for SRP.^{9,10} In the treatment of chronic periodontitis, they have been demonstrated to be as effective as hand instruments and ultrasonic devices.⁹ In-vitro studies have found Er:YAG lasers to be safe for periodontal pocket therapy. They do not cause thermal damage to the root surface or pulp,¹¹ and any damage to the root surface, such as the creation of roughness and grooves, has been found to be minor and dependent on the applied energy and angulation of the laser tip.¹⁰

After Ando et al¹² first reported the bactericidal potential of Er:YAG lasers on periodontal pathogens (*Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*), many investigations evaluated this ability under in-vivo and in-vitro conditions.¹³⁻¹⁷ However, no clear in-vivo evidence has been presented for the bactericidal effect of the Er:YAG laser when used in the first stage of chronic periodontitis therapy.

There are different laser delivery systems like fiber optic, articulated arms, and waveguides. When the laser beam is passing through these kinds of delivery system there can be an energy loss of approximately 30%.¹⁸ The present study used an Er:YAG laser system where the laser beam is generated in the handpiece and with three mirrors is delivered to a sapphire tip, which enables high energy output and prevents loss of energy.

The aim of the present study was to evaluate the antibacterial effectiveness of a fiber-less Er:YAG laser used in the initial treatment of moderate chronic periodontitis. The impact on nine specific periodontal pathogens was tested using real-time polymerase chain reaction (RT-PCR) technology and compared with the bactericidal effect of conventional treatment using hand instruments.

MATERIALS AND METHOD

Patient selection

The study was conducted in the Department of Periodontology and Oral Diseases, Faculty of Dental Medicine, Plovdiv, Bulgaria, and approved by the local Committee of Ethics in Research (number 3/2011).

Twenty subjects (6 men and 14 women, aged from 25 to 62 years, mean 46.49) who came to the Department of Periodontology seeking periodontal therapy were recruited to participate in the study, which was conducted between November 2011 and March 2012. Each patient was diagnosed with moderate chronic periodontitis, as defined by the American Academy of Periodontology.³ The inclusion criteria were:

- a diagnosis of moderate chronic periodontitis¹⁹
- probing pocket depth (PPD) from 3.6 to 6.2 mm
- bleeding on probing
- presence of plaque
- horizontal bone loss
- good general health with no systemic disease that could affect the treatment
- no periodontal treatment within the preceding 6 months
- no antimicrobial treatment within the last 6 months
- no pregnancy.

A signed informed consent form was obtained from every patient.

Study design

A split-mouth design was devised, with the teeth in the first and third quadrants receiving conventional SRP with hand instruments (control group), and those in the second and fourth quadrants (test group) treated using an Er:YAG laser (Lite Touch, Syneron Dental). A total of 80 teeth, one from each quadrant in each of the 20 patients, were included in the study for microbiologic examination.

On the first appointment, clinical examination of the patient was performed, in conjunction with microbiologic sampling, oral hygiene instructions, and supra-gingival cleaning using an ultrasonic unit (Piezon Master 400, Electro Medical Systems), rubber cups, and



Fig 1 Er:YAG laser debridement of the root surface with chisel tip.



Fig 2 Microbiologic sampling method.

abrasive polishing paste. Subgingival treatment was performed within 24 hours after the supragingival cleaning, according to the principles of full-mouth disinfection or debridement.²⁰

Test treatment

For calculus removal, the Er:YAG laser was used with a chisel tip (length 17 mm) and power settings of 100 mJ energy and 15 Hz frequency (average power 1.5 W). For periodontal pocket debridement, a 0.6-mm sapphire tip (length 17 mm) was used at power settings of 50 mJ energy and 30 Hz frequency (average power of 1.5 W). The chisel tip was inserted in the pocket at a 10- to 15-degree angle to the root surface (Fig 1) and kept in constant motion, using coronal to apical movements until the bottom of the pocket was reached. SRP ended when the operator felt a hard and smooth root surface. Pocket debridement was made after SRP in noncontact mode, around the tooth in the pocket.

Control treatment

Periodontal pockets assigned to control treatment were mechanically debrided with a set of seven new Gracey curettes (Hu Friedy). The treatment ended when the operator felt a hard and smooth root surface.

No local anesthesia was used during any of the treatment because patients evaluated the level of pain and discomfort with a visual analog scale (VAS) during and after treatment with both treatment modalities (data not shown). Only 0.9% NaCl solution was used for

irrigation. No other antibacterial solutions were used or prescribed. Patients were instructed in an oral hygiene regimen and included in a clinical assessment program, where they were evaluated in the first, third, sixth, and twelfth month after treatment for PPD, clinical attachment level (CAL), bleeding on probing (BOP), and presence of plaque.

Microbiologic assessment

The deepest pocket from each quadrant, according to the inclusion criteria of PPD from 3.6 to 6.2 mm in each patient, was examined microbiologically (Fig 2). Bacterial samples were taken from the same sites immediately before and 1 month after treatment. Teeth were isolated with paper rolls, and supragingival plaque was removed with a curette and sterile cotton. A sterile paper point (Roeko, ISO 50) was inserted to the bottom of each pocket, kept there for 20 to 25 seconds, and then placed in a transporting box.

Samples were sent to a laboratory (MIP Pharma, Germany) for RT-PCR examination. Nine specific periodontal pathogens (*A actinomycetemcomitans*, *P gingivalis*, *Treponema denticola*, *Tannerella forsythia*, *Prevotella intermedia*, *Peptostreptococcus (Micromonas) micros*, *Fusobacterium nucleatum*, *Eubacterium nodatum*, and *Capnocytophaga gingivalis*) were evaluated for their total numbers and relative proportions within each sample. When this latter parameter exceeded the values presented in Table 1, they were considered to significant for the periodontal disease progression.



| Table 1 Reference values over which the number and relative proportion of the microorganisms in the sample is significant for the disease | | |
|---|--------------------|----------------------------|
| Periodontal pathogens | Therapeutic number | Significant proportion (%) |
| <i>A actinomycetemcomitans</i> | ≥ 10 ³ | ≥ 0.01 |
| <i>P gingivalis</i> | ≥ 10 ⁴ | ≥ 0.25 |
| <i>T denticola</i> | ≥ 10 ⁴ | ≥ 1.0 |
| <i>T forsythia</i> | ≥ 10 ⁴ | ≥ 1.0 |
| <i>P intermedia</i> | ≥ 10 ⁴ | ≥ 2.5 |
| <i>P micros</i> | ≥ 10 ⁵ | ≥ 3.0 |
| <i>F nucleatum</i> | ≥ 10 ⁶ | ≥ 5.0 |
| <i>E nodatum</i> | ≥ 10 ⁵ | ≥ 3.0 |
| <i>C gingivalis</i> | ≥ 10 ⁶ | ≥ 5.0 % |

| Table 2 Quantitative analysis of the indicators for total count of marker bacteria in control group after treatment | | | | | | | |
|---|-----------------|------------|-------------|------------|-------------|-------|------------------|
| Marker bacteria | Treatment group | Parameters | | | | | |
| | | N | Mean | SE | SD | Z | Sig (2-tailed) P |
| Total | H ₀ | 40 | 10711500.00 | 1867294.85 | 11809809.60 | 2.796 | .005* |
| | H ₁ | 40 | 5505500.00 | 1154090.88 | 7299111.62 | | |
| <i>A actinomycetemcomitans</i> | H ₀ | 0 | NA | NA | NA | NA | NA |
| | H ₁ | 0 | NA | NA | NA | | |
| <i>P gingivalis</i> | H ₀ | 22 | 198023.63 | 51995.94 | 243882.59 | 3.312 | .001* |
| | H ₁ | 22 | 49952.72 | 17839.26 | 83673.57 | | |
| <i>T denticola</i> | H ₀ | 23 | 39716.08 | 17208.28 | 82528.02 | 2.677 | .007* |
| | H ₁ | 23 | 15921.30 | 7164.89 | 34361.63 | | |
| <i>T forsythia</i> | H ₀ | 25 | 55052.00 | 14680.12 | 73400.62 | 2.274 | .023* |
| | H ₁ | 25 | 16047.60 | 3021.84 | 15109.22 | | |
| <i>P intermedia</i> | H ₀ | 22 | 67549.54 | 32226.83 | 151157.25 | 1.338 | .181 |
| | H ₁ | 22 | 31867.27 | 14783.27 | 69339.72 | | |
| <i>P micros</i> | H ₀ | 25 | 31366.80 | 22138.79 | 110693.96 | 0.429 | .668 |
| | H ₁ | 25 | 8918.00 | 2788.90 | 13944.52 | | |
| <i>F nucleatum</i> | H ₀ | 14 | 243650.00 | 163107.68 | 610293.06 | 0.471 | .638 |
| | H ₁ | 14 | 88785.00 | 52160.25 | 195165.81 | | |
| <i>E nodatum</i> | H ₀ | 0 | NA | NA | NA | NA | NA |
| | H ₁ | 0 | NA | NA | NA | | |
| <i>C gingivalis</i> | H ₀ | 31 | 13656.12 | 3914.95 | 21797.53 | 0.098 | .922 |
| | H ₁ | 31 | 14180.64 | 4577.35 | 25485.64 | | |

*Statistically significant at P < .05. H₀, control group before treatment; H₁, control group 1 month after treatment; N, number of sites with pathogens; NA, not applicable; SD, standard deviation; SE, standard error; Sig, significance; Z, value of Wilcoxon signed-rank test.

Statistical analysis

Statistical analysis was performed with SPSS for Windows, version 17 (SPSS). The used tests were Wilcoxon signed-rank test, Fisher exact test, and chi-square test. Differences were considered statistically significant when the P value was < .05.

RESULTS

The total number of all microorganisms decreased significantly in both the control (P = .005) and test (P = .003) groups 1 month after treatment, as compared to baseline (Tables 2 and 3). Although the reduction of



Table 3 Quantitative analysis of the indicators for total count of marker bacteria in test group after treatment

| Marker bacteria (count) | Treatment group | Parameters | | | | | |
|--------------------------------|-----------------|------------|-------------|------------|--------------|-------|------------------|
| | | N | Mean | SE | SD | Z | Sig (2-tailed) P |
| Total | L ₀ | 40 | 12107500.00 | 1889075.84 | 11947564.65 | 3.004 | .003* |
| | L ₁ | 40 | 7361250.00 | 2608001.30 | 164944448.51 | | |
| <i>A actinomycetemcomitans</i> | L ₀ | 1 | 13000.00 | NA | NA | NA | NA |
| | L ₁ | 1 | 83.00 | NA | NA | | |
| <i>P gingivalis</i> | L ₀ | 21 | 399759.52 | 201740.99 | 924493.39 | 2.381 | .017* |
| | L ₁ | 21 | 50535.71 | 19419.89 | 88993.15 | | |
| <i>T denticola</i> | L ₀ | 23 | 30500.43 | 10092.12 | 48400.12 | 2.190 | .029* |
| | L ₁ | 23 | 12189.13 | 4908.45 | 23540.12 | | |
| <i>T forsythia</i> | L ₀ | 26 | 50226.92 | 17000.77 | 86687.28 | 3.340 | .001* |
| | L ₁ | 26 | 6663.84 | 2107.00 | 10743.66 | | |
| <i>P intermedia</i> | L ₀ | 17 | 100164.11 | 59372.87 | 244800.64 | 0.970 | .332 |
| | L ₁ | 17 | 69017.64 | 34062.66 | 140443.95 | | |
| <i>P micros</i> | L ₀ | 29 | 7787.24 | 1788.30 | 9630.32 | 0.833 | .405 |
| | L ₁ | 29 | 6014.13 | 1125.97 | 6063.55 | | |
| <i>F nucleatum</i> | L ₀ | 14 | 41887.14 | 74917.85 | 20022.63 | 0.785 | .433 |
| | L ₁ | 14 | 89346.42 | 203206.68 | 54309.27 | | |
| <i>E nodatum</i> | L ₀ | 0 | NA | NA | NA | NA | NA |
| | L ₁ | 0 | NA | NA | NA | | |
| <i>C gingivalis</i> | L ₀ | 30 | 14263.00 | 3651.84 | 20001.95 | 0.339 | .734 |
| | L ₁ | 30 | 23305.33 | 8763.15 | 47977.80 | | |

*Statistically significant at $P < .05$. L₀, test group before treatment, L₁, test group 1 month after treatment; N, number of sites with pathogens; NA, not applicable; Z, value of Wilcoxon signed-rank test.

microorganisms in both groups was statistically significant, the significance was more pronounced in the test group (in the group treated with cures, $Z = 2.796$, and in the group treated with lasers, $Z = 3.004$; Wilcoxon signed-rank test).

When results for each marker bacteria were broken out, significant reductions in *P gingivalis*, *T denticola*, and *T forsythia* were found in both the test and control group samples 1 month after periodontal treatment (Tables 2 and 3). Neither before nor after the treatment were *A actinomycetemcomitans* or *E nodatum* found.

The number and distribution of sites with therapeutically significant numbers of periodontal pathogens for both the test and control groups at baseline are shown in Table 4. The results 1 month after treatment in both groups are presented in the same table. A significant reduction in the marker bacteria in all sites is evident.

Significant difference is observed between both groups for the reduction of *T forsythia* ($P = .007$, Fisher test). This marker bacteria decreased by less than half in the control group, whereas in the test group it was reduced by more than five times.

Table 5 depicts the number and distribution of both control and test sites for which therapeutically significant proportions of periodontal pathogens were found at baseline and after treatment. While the number of significantly infected sites was reduced after treatment with both the control and test methods, the extent of the reduction of sites in the test group with a significant proportion of *T denticola* and *T forsythia* (by four and five times, respectively) is notable. In comparison, the number of positive sites in the control group with a significant proportion of *T denticola* (two) was unchanged; the number of sites with *T forsythia* decreased by less than half (from seven to four).



Table 4 Distribution of sites with therapeutic significant count of periodontal pathogens in both groups before and 1 month after treatment

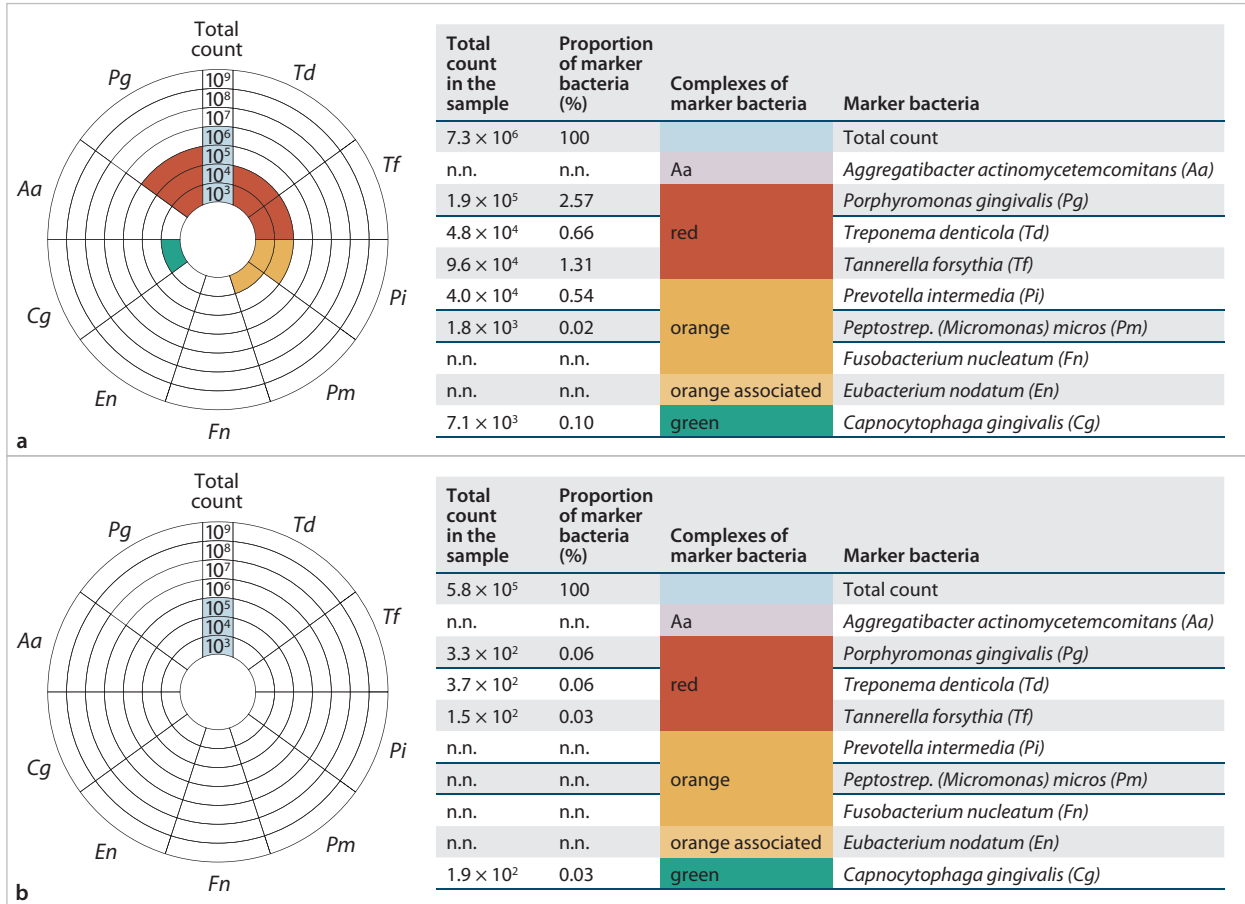
| Marker bacteria | Group | Before | χ^2 | P | After | χ^2 | P |
|--------------------------------|-------|----------|----------|-------|----------|----------|-------|
| <i>A actinomycetemcomitans</i> | H | 1 33.3% | Fisher | 1.000 | NA NA | NA NA | NA NA |
| | L | 1 50.0% | | | NA NA | | |
| <i>P gingivalis</i> | H | 24 80.0% | 2.062 | .151 | 13 56.5% | 0.551 | .458 |
| | L | 21 63.6% | | | 10 45.5% | | |
| <i>T denticola</i> | H | 17 50.0% | 0.875 | .350 | 7 28.0% | 0.104 | .747 |
| | L | 14 44.3% | | | 6 24.0% | | |
| <i>T forsythia</i> | H | 24 68.6% | 0.891 | .345 | 13 52.0% | Fisher | .007* |
| | L | 22 57.9% | | | 4 14.8% | | |
| <i>P intermedia</i> | H | 16 57.1% | 0.155 | .694 | 9 39.1% | 0.023 | .879 |
| | L | 14 51.9% | | | 7 36.8% | | |
| <i>P micros</i> | H | 1 3.0% | Fisher | .485 | NA NA | NA NA | NA NA |
| | L | 0 0% | | | NA NA | | |
| <i>F nucleatum</i> | H | 1 3.8% | Fisher | 1.000 | NA NA | NA NA | NA NA |
| | L | 0 0% | | | NA NA | | |
| <i>E nodatum</i> | H | NA NA | NA | NA | NA NA | NA NA | NA NA |
| | L | 1 100.0% | | | NA NA | | |
| <i>C gingivalis</i> | H | NA NA | NA | NA | NA NA | NA NA | NA NA |
| | L | NA NA | | | NA NA | | |

*Statistically significant at P < .05. Fisher, Fisher exact test; H, control group; L, test group; NA, not applicable; χ^2 , chi-square test.

Table 5 Distribution of sites with therapeutic significant proportion of periodontal pathogens in both groups before and 1 month after treatment

| Marker bacteria | Group | Before | χ^2 | P | After | χ^2 | P |
|--------------------------------|-------|----------|----------|-------|----------|----------|-------|
| <i>A actinomycetemcomitans</i> | H | 1 33.3% | Fisher | 1.000 | 1 100% | NA NA | NA NA |
| | L | 1 50.0% | | | 1 100% | | |
| <i>P gingivalis</i> | H | 21 70.0% | 1.592 | .207 | 15 65.2% | 0.012 | .912 |
| | L | 18 54.5% | | | 14 63.6% | | |
| <i>T denticola</i> | H | 2 5.9% | Fisher | .674 | 2 8.0% | Fisher | 1.000 |
| | L | 4 11.1% | | | 1 4.0% | | |
| <i>T forsythia</i> | H | 7 20.0% | 0.621 | .431 | 4 16.0% | Fisher | .183 |
| | L | 5 13.2% | | | 1 3.7% | | |
| <i>P intermedia</i> | H | 2 7.1% | Fisher | 1.000 | 2 10.5% | Fisher | 1.000 |
| | L | 2 7.4% | | | 2 13.3% | | |
| <i>P micros</i> | H | NA NA | NA | NA | NA NA | NA NA | NA NA |
| | L | NA NA | | | NA NA | | |
| <i>F nucleatum</i> | H | 2 7.7% | Fisher | .491 | 0 0% | Fisher | 1.000 |
| | L | 0 0% | | | 1 5.0% | | |
| <i>E nodatum</i> | H | NA NA | NA | NA | NA NA | NA NA | NA NA |
| | L | NA NA | | | NA NA | | |
| <i>C gingivalis</i> | H | NA NA | NA | NA | 0 0% | Fisher | .492 |
| | L | NA NA | | | 1 3.2% | | |

Fisher, Fisher exact test; H, control group; L, test group; NA, not applicable; χ^2 , chi-square test.



Figs 3a and 3b Laboratory report for one sample about total count and proportion of marker bacteria in a periodontal pocket (a) before and (b) after hand instrumentation (according to Socransky et al²). nn, not provided in the sample.

Figures 3 and 4 present randomly selected laboratory reports about the count and proportion of marker bacteria at baseline and 1 month after the treatment in both groups.

DISCUSSION

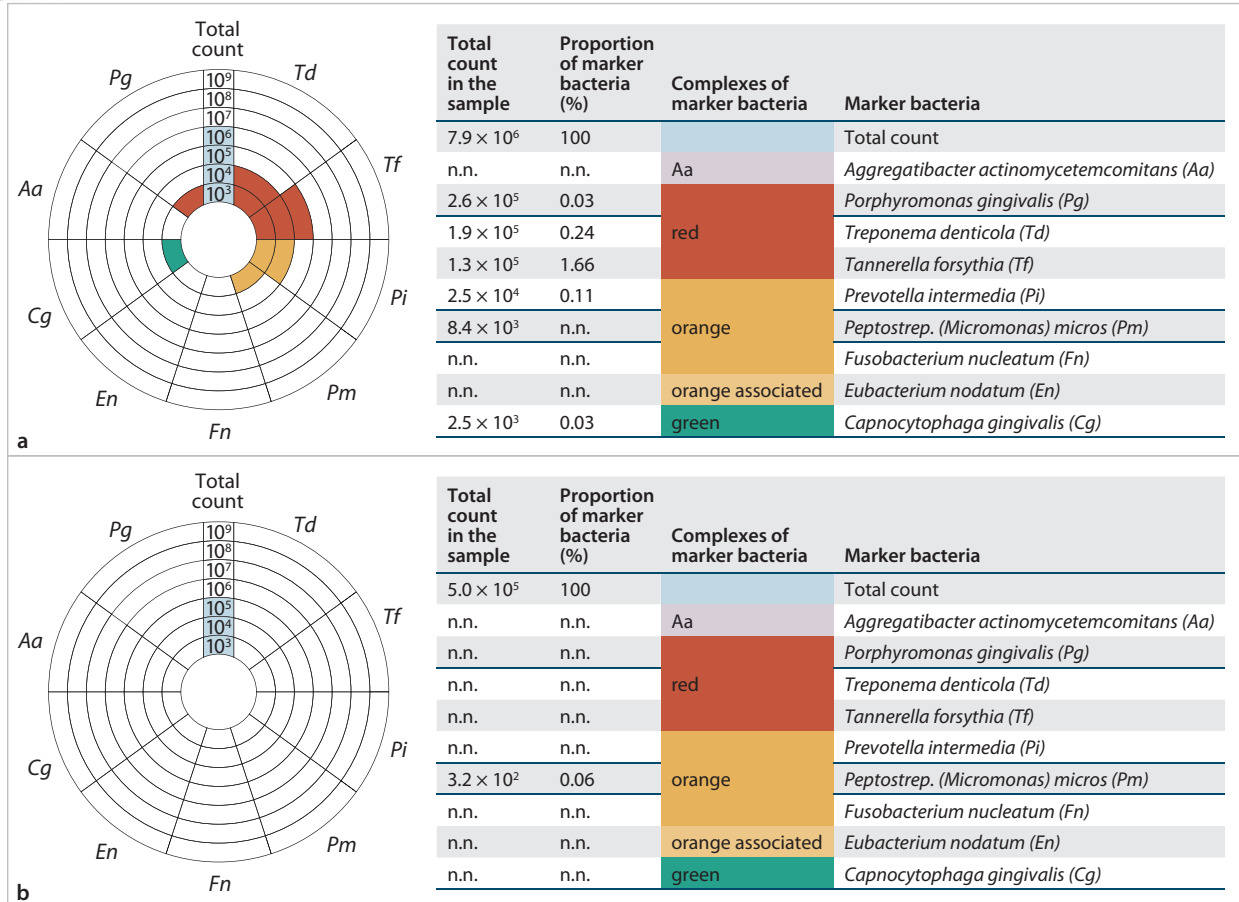
The Er:YAG laser was proved to be as effective as hand and ultrasonic instruments both in nonsurgical or in surgical periodontal therapy.^{21,22} Many studies have shown that Er:YAG lasers possess bactericidal potential. Their antibacterial effectiveness has been demonstrated in connection with caries lesions,²³ root canals,²⁴ and periodontal pockets.^{12,14,15} Thermomechanical ablation is the likely mechanism behind this effect. Water molecules inside the bacterial cells absorb the Er:YAG

laser energy, which leads to their evaporation and the cells destruction.¹³ Effectiveness may also depend on the energy frequency²⁵ and the delivery system.

For this reason, the present study investigated the bactericidal effect of a fiber-less Er:YAG laser system, in which the energy is generated in the handpiece and delivered directly to the working tip. The power and frequency settings were 1.5 W and 50 Hz, respectively, with SRP and pocket decontamination performed in the first stage of periodontal therapy.

Real-time PCR examination was used for marker-bacteria detection because this approach generated both qualitative and quantitative data about the microorganisms.²

The results of the present study demonstrate that *A actinomycetemcomitans* is almost not detected in base-



Figs 3a and 3b Laboratory report for one sample about total count and proportion of marker bacteria in a periodontal pocket (a) before and (b) after hand instrumentation (according to Socransky et al²). nn, not provided in the sample.

line examination, which confirm the diagnosis of chronic periodontitis.²⁶ In contrast to *A actinomycetemcomitans*, pathogens from the red (*P gingivalis*, *T denticola*, *T forsythia*) and orange (*P intermedia*, *F nucleatum*) complexes, as defined by Socransky et al,² were widely distributed before treatment.

One month after periodontal therapy, a significant ($P < .05$) reduction in the total count of all periodontal pathogens in both groups was observed; reductions of *P gingivalis*, *T denticola*, and *T forsythia* in both the test and control groups also were significant. These results confirm the results of other studies that have shown Er:YAG lasers to possess antimicrobial effectiveness in clinical conditions. However, unlike the results of other studies, the present results indicate that the laser system has a higher bactericidal potential than the treat-

ment with hand instruments. Laser treatment had a greater impact on the total number of microorganisms, the proportions in which marker bacteria were presented, and the number of significantly infected sites. The decrease in *T forsythia* in the wake of laser treatment was particularly significant. The total count of *T forsythia* after laser treatment decreased by more than seven times, compared with the three-fold reduction observed after treatment with hand instruments. Furthermore, the number of laser-treated sites where 10^4 or more *T forsythia* were counted and the proportion of this pathogen constituted 1% or more of the total decreased from 5 to 1, dropping only from seven to three in the conventionally treated group. This result is in agreement with the findings of Tomasi et al.¹⁴ Similar results were found for *T denticola*, a bacteria which is



not often evaluated. When Tomasi et al¹⁴ evaluated changes in *T denticola* levels after laser treatment, they found the results to be nonsignificant. In contrast, the present study found significant reduction of *T denticola* 1 month after laser treatment versus conventional SRP.

The Er:YAG laser seems to be a promising tool in antibacterial periodontal therapy because of some specific characteristics. Its wavelength is well absorbed by water molecules, which allows the laser beam to destroy bacteria, as the water molecules inside them are thermally evaporated. The frequency used during root surface debridement and pocket decontamination was higher than that employed in other studies.^{16,17} This may have amplified the Er:YAG laser's beneficial antibacterial effects. Furthermore, for the first time the bactericidal potential of a fiber-less laser system was investigated, where there is no loss of energy through the delivery system.

CONCLUSION

At a time when new technologies are continually available, the general practitioner must know the proper treatment protocol and its benefits for the practice. The present study demonstrated that Er:YAG lasers possess bactericidal potential when used in the initial stage of therapy for moderate chronic periodontitis. Moreover, this laser-assisted treatment compares well with conventional treatment. SRP with the Er:YAG laser can be used as an alternative treatment in periodontitis, because of its proven effectiveness of reduction of periodontal pathogens. Further follow-up bacterial examination should be performed to investigate the long-term bactericidal effectiveness of this laser wavelength.

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