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Original article

# INVESTIGATION OF ANTIBACTERIAL ACTIVITY OF ND: YAG - LASER AND STANDARD ENDODONTIC TREATMENT

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**ABSTRACT**

**Introduction:** The microbial infection is one of the main causes of the dental pulp and periodontal diseases. Previously used methods for its elimination are not fully effective, and often some microorganisms in root canals (RC) remain unaffected after treatment.

Another modern method for disinfection of root canal system is laser disinfection. Different types of lasers are used - Nd: YAG, Er: YAG, Diode laser.

**Purpose:** The purpose of our study is to compare the antibacterial activity of ND: YAG laser and conventional endodontic therapy in the treatment of infected root canals.

**Materials and methods** The study involved 36 teeth of patients diagnosed with pulp gangrene or chronic periapical periodontitis, requiring endodontic treatment. They were divided into two groups of 18 teeth each one. The teeth in both groups are prepared by Protaper Universal rotary instruments (Maillefer Instruments SA, Ballaigues, Switzerland). In group 1the root canals disinfection is performed with a Nd: YAG laser (source of Nd: YAG laser (1064 nm) is the AT Fidelis - Fotona d.d., Ljubljana laser system). In group 2 was used the following protocol of root canal disinfection: 2.5% sodium hypochlorite solution and 17% EDTA divided by irrigation with distilled water. Then a sterile paper point is placed in the root canals, and a microbiological sample is taken again.

**Results:** In all compared pairs, there was no significantly different effect regarding the number of microorganisms.

**Conclusions:** The disinfection rinsing method with NaOCl has the strongest antimicrobial effect in clinical studies (90% against all microbial isolates). The use of Nd: YAG laser independently is not always sufficient for root canal disinfection - the effect is about 66%.

**Keywords:** Endodontic treatment, root canal disinfection, lasers in endodontics,

## INTRODUCTION

The microbial infection is one of the main causes of the dental pulp and periodontal diseases. The most common methods (irrigation with 2.5% sodium hypochlorite solution and 17% EDTA ) used for its elimination are not fully effective, and often some microorganisms in root canals (RC) remain unaffected after treatment. [1, 2].

Another modern method for disinfection of root canal system is laser disinfection. Different types of lasers are used - Nd: YAG, Er: YAG, Diode laser. They also have antibacterial action [3, 4, 5, 6, 7]. In endodontic treatment, photothermic and photomechanical effects of lasers of different wavelengths interact with the dental tissues (dentin, residual pulp), the polluting layer, and microorganisms. Using various capacities, all types of lasers can destroy the cell walls of microorganisms due to the photothermal effect [8, 1, 4].

**The aim** of our study was to compare the antibacterial activity of ND: YAG laser and conventional endodontic therapy in the treatment of infected root canals.

## MATERIALS AND METHODS

The study involved 36 teeth of patients diagnosed with pulp gangrene or chronic periapical periodontitis, requiring endodontic treatment.

**Case selection**

Patients' complete medical history was taken. Those who have systemic diseases or have taken antibiotics for the last three months are excluded from the study. Each patient signs an informed consent. In each case (tooth), a preliminary X-ray is taken to detect the presence of periapical changes and get an idea of the morphology, length and number of root canals.

The teeth are isolated with a rubber dam. Then they are quenched with 2% chlorhexidine solution. Sterile burs are used for the preparation of the endodontic cavity and access. Old obturations and carious lesions are carefully removed. Microbiological samples are taken by placing a sterile paper point in the root canal for 60 seconds. After removing the paper point from the root, it is immediately placed in a sterile transport environment and then transported to a microbiological laboratory. **This is the initial microbiological sample.**

The treated teeth are divided into **two groups** depending on the method used for root canal disinfection:

**I group** - 18 cases – root canals are prepared by Protaper

Universal rotary instruments (Maillefer Instruments SA, Ballaigues, Switzerland). Root canal rinsing is performed with sterile saline solution, after working with each instrument. Once the root canals are prepared, they are dried with a sterile paper point. **In this group, the root canals disinfection is performed with a Nd: YAG laser** (source of Nd: YAG laser (1064 nm) is the AT Fidelis - Fotona d.d., Ljubljana laser system). The following parameters of laser radiation are used - pulse mode of operation with frequency 15 Hz, without water or air cooling. The laser power is 1.5 W. Root canal irradiation is performed by means of a handpiece and a fiber-optic fiber with a diameter of 200 ìm for 1 minute. The fiber moves continuously in the root canal with circular movements in the apical-coronary direction, reaching up to 1 mm of the working length. A sterile paper point is then placed in the root canals, and a microbiological sample is retaken.

**II group** - 18 cases - root canals are prepared by Protaper Universal rotary instruments (Maillefer Instruments SA, Ballaigues, Switzerland). After working with each canal instrument, the root canals are rinsed with 2.5% sodium hypochlorite solution and 17% EDTA. Root canals are dried with a sterile point, and a second microbiological sample is taken.

## RESULTS

In all compared pairs, there was no significantly different effect regarding the number of microorganisms (Table 1, 2, 3, 4).

**Table 1.** Antibacterial activity of the Nd: YAG laser in infected root canals

| Sample number | Isolated microorganisms prior to treatment | Amount of micro-organisms /cfu/ml | Isolated micro-organisms after disinfection with Nd: YAG laser | Amount of micro-organisms /cfu/ml |
|---|---|---|---|---|
| 1 | *Streptococcus mitis* | 1000 000 | No | No |
| | *Neisseria perflava* | 100 000 | No | No |
| 2 | *Str. mitis* | 1000 000 | No | No |
| | *A.actinomycetemcomitans* | 100 000 | No | No |
| | *Corynebacterium xerosis* | 10 000 | No | No |
| 3 | *Streptococcus mitis* | 1000 000 | *S. mitis* | 1 000 |
| | *E.faecalis* | 100 000 | No | No |
| | *Bacillus brevis* | 100 000 | *Bacillus brevis* | 1 000 |
| 4 | *Actinomyces neuii* | 100 000 | No | No |
| | *K. oxytoca* | 100 000 | *K.oxytoca* | 1 000 |
| | *Candida albicans* | 1000 | No | No |
| 5 | *Enterobacter cloaceae* | 100 000 | *Enterobacter cloaceae* | 1 000 |
| 6 | *Staphylococcus aureus* | 1000 000 | Staph.aureus | 1 000 |
| | *Streptococcus mutans* | 1000 000 | | |
| | *Actinomyces viscosus* | 10 000 | Str. mutans | 100 |
| 7 | *E. faecalis* | 10 000 000 | *E.faecalis* | 1000 |
| | *Streptococcus parasanguis* | 100 000 | No | No |
| | *Corynebacterium striatum* | 10 000 | *C. striatum* | 10 |
| 8 | *Staphylococcus aureus* | 10 000 | No | No |
| | *Streptococcus mitis* | 100 000 | No | No |
| 9 | *Enterococcus faecalis* | 100 000 | No | No |
| | *Bacillus circulans* | 100 000 | *B.circulans* | 100 |
| 10 | *Streptococcus mutans* | 1000 000 | No | No |
| 11 | *Streptococcus sanguis* | 100 000 | | |
| | *Neisseria flavescens* | 100 000 | No | No |
| 12 | *Staphylococcus aureus* | 1000 000 | *S.aureus* | 1000 |
| 13 | *Streptococcus gordonii* | 100 000 | No | No |
| 14 | *Enterococcus faecalis* | 10 000 | No | No |
| | *Corynebacterium propiniqum* | 10 000 | No | No |
| 15 | *Streptococcus mitis* | 100 000 | No | No |
| 16 | *Staphylococcus aureus* | 1000 000 | No | No |
| | *Enterobacter cloaceae* | 100 000 | *E.cloaceae* | 1 000 |
| 17 | *Staphylococcus aureus* | 10 000 | No | No |
| 18 | *Enterococcus faecalis* | 10 000 | No | No |

**Table 2.** Antibacterial activity of 2,5% NaOCl and 17% EDTA in infected root canals

| Sample number | Isolated microorganisms prior to treatment | Amount of micro-organisms /cfu/ml | Isolated microorganisms after disinfection with 2,5% NaOCl and 17% EDTA | Amount of micro-organisms /cfu/ml |
|---|---|---|---|---|
| 1 | *E.faecalis* | 5x100 000 | No | No |
| | *A.viscosus* | 3x10 000 | No | No |
| 2 | *S.parasanguis* | 4x100 000 | No | No |
| | *K.denitrificans* | 7x10 000 | No | No |
| 3 | *K.pneumoniae* | 9x100 000 | *K.pneumoniae* | 1x100 |
| 4 | *C.albicans* | 3x 10 000 | No | No |
| 5 | *S.mitis* | 2x 10 000 | No | No |
| 6 | *E.faecalis* | 3x100 000 | No | No |
| | *E. cloaceae* | 2x10 000 | No | No |
| 7 | *A.haemolyticum* | 8x 100 000 | No | No |
| | *E. faecalis* | 5x10 000 | No | No |
| | *Bacillus brevis* | 6x10 000 | No | No |
| 8 | *S.sanguis* | 4x1000 000 | Str.sanguis | 2x1000 |
| | *Coryneacterium ulcerans* | 1x10 000 | No | No |
| 9 | *E. faecalis* | 8x100 000 | No | No |
| | *E.corrodens* | 9x100 000 | No | No |
| 10 | *S.pyogenes* | 6x 100 000 | No | No |
| | *Lactobacillus fermentum* | 6x10 000 | No | No |
| 11 | *S.gordonnii* | 6x 100 000 | No | No |
| | *Kingella kingae* | 5x100 000 | No | No |
| | *N.polysaccharea* | 2x10 000 | No | No |
| 12 | *S.aureus* | 6x10 000 | No | No |
| | *E.faecalis* | 8x10 000 | No | No |
| | *S.constelatus* | 3x 100 000 | Str.constelatus | 9x100 |
| 13 | *G.morbillorum* | 8x100 000 | No | No |
| | *Staph.aureus* | 2x10 000 | No | No |
| 14 | *E.faecalis* | 7x100 000 | No | No |
| | *C. matruchotii* | 5x10 000 | No | No |
| 15 | *E.faecalis* | 6x10 000 | No | No |
| | *A.neuii* | 7x100 000 | No | No |
| 16 | *M.morganii* | 8x100 000 | M.morganii | 3x10 |
| | *S.aureus* | 9x10 000 | No | No |
| 17 | *E.faecalis* | 4x1 000 000 | No | No |
| | *S.anginosus* | 6x10 000 | No | No |
| | *Neisseria mucosa* | 5x 10 000 | No | No |
| 18 | *E.coli* | 2x100 000 | No | No |
| | *Candida albicans* | 3x 1 000 | No | No |

**Table 3.** Numbers of microorganisms before and after treatment

| Method | Before and after treatment | N | Amount of microorganisms /cfu/ml | | | | p |
|---|---|---|---|---|---|---|---|
| | | | Mean | Median | Min | Max | |
| NaOCl/EDTA | Before treatment | 18 | 1 035 722,2 | 755 000,0 | 20 000,0 | 4 110 000,0 | <0.001 |
| | After treatment | 18 | 168,3 | 0,0 | 0,0 | 2 000,0 | |
| Nd: YAG | Before treatment | 18 | 1 093 388,9 | 200 500,0 | 10 000,0 | 10 110 000,0 | <0.001 |
| | After treatment | 18 | 456,1 | 0,0 | 0,0 | 2 000,0 | |

In all two methods, significant differences in the amounts of microorganisms before and after treatment ($p < 0.001$) have been observed (Table 3).

**Table 4.** Numbers of microorganisms before and after treatment

| Before and after treatment | Method | Number of samples | Amount of microorganisms | | | | p |
|---|---|---|---|---|---|---|---|
| | | | Mean | Median | Min | Max | |
| Before treatment | NaOCl/EDTA | 18 | 1 035 722,2 | 755 000,0 | 20 000,0 | 4 110 000,0 | 0,366 |
| | Nd:YAG | 18 | 1 093 388,9 | 200 500,0 | 10 000,0 | 10 110 000,0 | |
| After treatment | NaOCl/EDTA | 18 | 168,3 | 0,0 | 0,0 | 2 000,0 | 0,105 |
| | Nd:YAG | 18 | 456,1 | 0,0 | 0,0 | 2 000,0 | |

NaOCl has the most pronounced antimicrobial activity in vivo. The remaining causative agents, after the impact of this disinfection method, are in a small amount – only 10%.

After in vivo therapy with Nd: YAG laser 34% of untreated etiological agents remain. Reduction of these microbial species again from KES, *Enterobacter spp., Klebsiella spp*. is about 2 log, but they remain at a microbial number of approximately 1000 cfu / ml. Unlike the other method, there is a lack of complete eradication in some other more sensitive bacterial species, as well. (Table 4)

## DISCUSSION

From the two tasted groups are isolated predominantly polymicrobial associations and rarely mono-infection with a predominance of Gram-positive species

It is noteworthy that the most pronounced antimicrobial activity in vivo has NaOCl (only 10% of the initially isolated microorganisms remain after the action of this disinfection method - the effect is 90%). Microorganisms remaining after treatment are Gram-positive cocci - oral streptococci that are likely to reinfect the dental canals despite the successful removal of the other causative agents from the original association from which they were isolated. Other microorganisms that remain after treatment are Enterobacteriaceae of the KES group, *Enterobacter spp., Klebsiella spp*. These species form extremely rigid biofilms due to the overproduction of substances in capsule form and many other adhesion molecules on their cell wall surface as outer membrane proteins, lipopolysaccharide, and adhesive piles. They are also polyresistant to many antimicrobial agents and are the cause of problematic in-hospital infections.

After Nd: YAG laser therapy, 34% of microorganisms stay unaffected - the effect is 66%. Microorganisms that are observed after treatment are again of KES group, *Enterobacter spp., Klebsiella spp*. Unlike the other two methods, there is a lack of complete eradication in some other more sensitive bacterial species - *S. aureus* and *E. faecalis*, several species of bacilli and associated with them corynebacteria. Similar results were also established by other authors who did not get a good antibacterial effect while using the Nd: YAG laser - Blum et al. (1997) [1], Jukic et al. (2004) [9]. The photothermal effect of the lasers for microorganism destruction is used in the endodontic treatment [10]. The lower response rate of E. faecalis can be due to the greater durability of this heat microorganism [2, 11]. In contrast, Gutknecht et al. (1996) [12] and Hardee et al. (1994) [4] received 99% bacterial reduction in their studies. This can be due to various parameters of the laser radiation or different exposure duration. The conducted clinical study allows for the reduction of microorganisms in RC after treatment applying the two methods. The used microbiological method permits reading the remaining microorganisms only in RC lumen, i.e., those microorganisms adhered to the paper pin while taking the second microbiological sample.

It does not give an idea of the microorganisms that have stuck to the canal walls and that have entered the dentinal tubules and micro-canals of the apical delta. They are the cause of root canal system re-infection after filling the canal and the appearance of periodontitis after the treatment of infected RC or the failure to treat existing periodontitis. However, the microbiological method used makes it possible to gain a comparative assessment of the two methods' effectiveness.

Nd: YAG laser disinfection at this stage can be used as a selection method, although it has the lowest antimicrobial effect. However, the power of the laser and the duration of procedures should be very carefully selected so that the limitations of heating the root canals and surrounding tissues are not exceeded.

## CONCLUSION

The disinfection rinsing method with NaOCl has the strongest antimicrobial effect in clinical studies (90% against all microbial isolates).

The use of Nd: YAG laser independently is not always sufficient for root canal disinfection - the effect is about 66%.

We believe that the established antimicrobial effect of Nd: YAG laser makes the method appropriate both as complementary to routine one and as a method of choice in situations severely impeding the conventional method of rinsing with antiseptic solutions.

The microbiological studies were conducted at Bulgarian Academy of Sciences, Microbiology Institute "Stefan Angelov" (associated with the Institute "Pasteur" in Paris and the Department of Microbiology at the Medical Faculty of Medical University - Sofia). They were carried out by Assoc. Prof. R. Gergova (Department of Medical Microbiology, Medical Faculty, Medical University of Sofia).

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