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ANTIBACTERIAL AND REPARATIVE ACTIVITY OF DIODE LASER IN ENDODONTIC TREATMENT OF TEETH WITH PERIAPICAL LESION ANALYZED WITH OPTICAL DENSITY AND RT-PCR

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ABSTRACT **Objectives:** *Modern methods of endodontic treatment of periodontitis include a set of measures to achieve sterilization of the root canal system. Current chemomechanical cleaning methods do not always achieve these goals, and insufficient root canal disinfection is the main reason for endodontic failure. Relatively new approaches for disinfecting and cleaning the root canals include lasers. However, the acceptance of laser technology by clinicians still remains limited. The aim of the study was to evaluate the antibacterial and reparative activity of a diode laser in the endodontic treatment of teeth with a periapical lesion.* **Material And Methods:** *Endodontic treatment in the experimental group included the use of calcium hydroxide three times with an interval of 7-14 days, followed by a long-term temporary obturation with calcium hydroxide with iodoform, ultrasonic activation of sodium hypochlorite, hydrodynamic irrigation, and a diode laser with the following permanent root canal obturation. In the control group the same treatment was applied without the use of a diode laser. The study of the content of microorganisms in the root canal was carried out by Real-Time PCR. Optical density (OD) in the periapical area was performed using the Measure Density Profile program (Sidexis, Sirona).* **Results:** *Under our supervision, there were 62 human permanent teeth with a periapical lesion from 1.8 mm to 5.9 mm in size, divided into two groups (experimental and control), 31 in each. Endodontic treatment performed in both groups ascertained different antimicrobial activity. Complete inhibition of microorganisms was determined in patients of the experimental group. The treatment performed in the control group, which did not include the use of a diode laser, was less active antimicrobiologically. According to optical density in the experimental group, a change in bone density in the periapical area from 35.7 ± 1.76% to 50.6 ± 1.51% ($p < 0.001$) was determined. The improvement in bone density during the treatment period was 14.9 ± 1.38%. In the control group, where antibacterial activity was less pronounced, a change in bone density occurred from 36.0 ± 1.87% to 48.7 ± 1.62% ($p<0.001$). Indicators of improvement in bone density were significantly lower - 12.7 ± 1.28%. ($p<0.05$).* **Conclusion:** *Both treatment regimens tested in groups determined different antimicrobial activity. Complete inhibition of microorganisms was determined in patients of the experimental group. The treatment regimen tested in the control group, which did not include the use of a diode laser, was less active antimicrobiologically. The use of a diode laser in endodontic treatment of the teeth with a periapical lesion enhances the antibacterial activity and promotes more active repair in the bone.*

KEYWORDS : Chronic apical periodontitis, Periapical lesion, RT-PCR, Optical density, Diode laser, Calcium hydroxide with iodoform, Ultrasonic activation of sodium hypochlorite, Hydrodynamic irrigation.

INTRODUCTION

The reason for the development of the inflammatory process in periodontal tissues is bacteria, its metabolic products - endotoxins, which are the main reason for the development of periodontal tissue lesion (Croitoru et al.,2016, Narita et al.,2016,Oliveira et al., 2012).

Modern methods of endodontic treatment of periodontitis include a set of measures to achieve sterilization of the root canal system (Al Khasawnah et al.,2018, Thomas et al., 2012, Azim et al.,2016, Aksel et al., 2017). To eliminate microorganisms from the infected root canal system, a widespread introduction of a modern endodontic treatment strategy is necessary, including thorough mechanical processing and disinfection of the root canal system (Croitoru et al.,2016 , Aksel et al., 2017). Current chemomechanical cleaning methods do not always achieve these goals, and insufficient root canal disinfection is the main reason for endodontic failure (Pereira et al., 2017). Relatively new approaches to disinfecting and cleaning the root canals include lasers (Ashraf et al., 2014, Husejnagic et al., 2019). However, the acceptance of laser technology by clinicians still remains limited (Jurič et al.,2014). PCR diagnostics is a modern and high-precision molecular genetic method for the diagnosis of complex cultivated root canal microflora (Thimmegowada et al.,2019, Shahi et al.,2018). In many instances, to assess the state of periapical tissues, the visual monitoring using x-ray data is used (Estrela et al., 2014). However, optical density (OD) allows a more objective assessment of the ef fectiveness of endodontic treatment (Rózyło-Kalinowska , 2007).

The aim of the study was to evaluate the antibacterial and reparative activity of a diode laser in endodontic treatment of teeth with periapical lesion.

MATERIALS AND METHODS

Under our supervision, there were 62 human permanent teeth treated with a diagnosis of "chronic periodontitis" with a periapical lesion from 1.8 mm to 5.9 mm in size. The teeth were divided into two groups (experimental and control), 31 in each. The treatment time in the experimental group was 138.6 ± 5.10 days, in the control group - 139.4 ± 4.71 days.

Treatment Procedures

Endodontic treatment in the experimental group included the use of calcium hydroxide three times with an interval of 7-14 days, followed by a long-term temporary obturation with calcium hydroxide with iodoform, ultrasonic activation of sodium hypochlorite, hydrodynamic irrigation with *RinsEndo (Dürr)* and a diode laser (*Picasso Lite Dental Laser)* with the following permanent root canal obturation. In the control group the same treatment was applied without the use of a diode laser.

Sample Collection (PCR)

The material was taken before treatment and immediately before the permanent root canal obturation in 20 teeth (10 teeth in each group). All patients did not take antibiotics 3 months before and during the present study.

A comparative quantitative and qualitative analysis was carried out of the 13 most frequently cultivated microorganism strains most commonly found in infected root canals: Porphyromonas endotondalis (P.endotondalis), Porphyromonas gingivalis (P.gingivalis), Aggregatibacter actinomycetemcomitans (A.actinomytomacetoma denticola), Tannerella forsythia (T.forsythia), Prevotella intermedia (P.intermedia), Fusobacterium nucleatum (F.nucleatum), Steptococcus spp (S.spp.)., Enterococcus fecalis (E.fecalis), Enterococcus faecium (E. faecium), Candida albicans (C. albicans), Candida crusei (C. crusei), Candida glabrata (C. glabrata) (Brenda et al., 2008, Siquera Jr et al., 2002) [5, 24].

The technique for the sample collection was as follows: following isolation of the tooth involved with a rubber dam, the field was disinfected with 30% H_2O_2 and followed by 5% tincture of iodine. Caries and/or existing restorations, if present, were removed, and then the cavity was wiped with a sterile cotton pellet slightly wet with 1% buffered NaOCl, with the utmost care that it did not seep into the canal. The halogen disinfectants were inactivated with 5% sodium

thiosulfate. The pulp chamber was then accessed with a new sterile bur. If purulence or serous fluid was present in the canal, this was directly sampled with three size fine paper points. Otherwise, sterile saline was deposited in the canal, making sure that it did not overflow. A size 15 to 30 file (depending on the canal size) was used to negotiate the canal to the estimated length. If the canal was very calcified, Gates Glidden burs sizes 2 and 3 were used so that the paper point could penetrate to a depth close to the estimated canal length. Three fine paper points were then used to obtain the sample. The last paper point was left in the canal for 30 sec. In multicanaled teeth, one paper point sample was obtained from each canal unless the canals were very calcified, in which case sampling of the canal in the root with the largest periapical lesion and the largest canal was done (Fig. 1). The paper points were placed in sterile, DNA- and RNA-free vials containing 1 ml of filter-sterilized 10 mM Tris-HCl, 1 mM EDTA (pH 8), and 0.5 g of sterile glass beads (diameter, 0.71 to 1.18 mm). The vials were frozen at −70°C until used (Ashraf et al., 2002) [19].

Fig. 1. Stages Of Sampling For PCR Analysis

Optical Density

The optical density of the bone tissue destruction site in the periapical area of 62 teeth was carried out using the Measure Density Profile program (Sidexis, Sirona). Densitometry was performed in the periapical area, drawing a straight line along the center of the lesion, and the program provided a diagram for visualizing the state and the average digital value of the Optical density of the bone in percent (Fig. 2). If the tooth was multi-rooted, studies were carried out in the root area, which corresponded to the objectives of the study. Assessment of the state of periapical tissues according to optical density was performed before treatment and immediately before permanent obturation of the root canals based on digital intraoral x-ray examination.

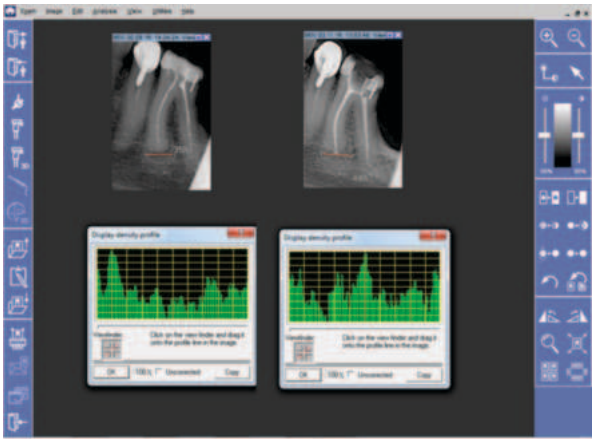

Fig. 2 The Optical Density Technique.

RESULTS

Bacterial contamination

In the experimental group of patients, a quantitative analysis of the root canal microflora, carried out before the endodontic treatment, revealed P.endotondalis in four out of ten examined patients (40%), respectively, with a titer of 2.0 × 10/2 GE/mL, 3.7 × 10/2 GE/mL, 1.8 × 10/3 GE/mL and 3.5 × 10/3 GE/mL; P.gingivalis in two patients (20%) with a titer of 1.7 × 10/3 GE/mL and 1.5 × 10/4 GE/mL; T.forsythia in six patients (60%), respectively, with a titer of 2.6 × 10/3 GE/mL, 6.2 × 10/2 GE/mL, 2.6 × 10/2 GE/mL, 2.3 × 10/3 GE/mL, 5.3 × 10/4 GE/mL and 3.5 × 10/3 GE/mL; P.intermedia in two patients (20%) with a titer of 3.1 × 10/3 GE/mL and 2.9 × 10/3 GE/mL; F.nucleatum in ten patients (100%), respectively, with a titer of 48 GE/mL, 2.8 × 10/3 GE/mL, 1.8 × 10/2 GE/mL, 1.8 × 10/2 GE/mL, 2.1 × 10/2 GE/mL, 50 GE/mL, 3.0 × 10/4 GE/mL, 1.6 × 10/2 GE/mL, 1.7 × 10/4 GE/mL and 2.2 × 10/2 GE/mL. A qualitative analysis of the microflora of the root canal, also carried out before the start of endodontic treatment, revealed S.spp. in ten patients (100%), E.fecalis / E.faecium in two patients (20%), C.albicans in two patients (20%). A quantitative analysis of the root canal microflora in this group of patients did not reveal the presence of the microorganism A.actinomycetemcomitans, T. denticola, and a qualitative one did not reveal the microorganisms C. crusei and C.glabrata (Fig.2).

In the control group of patients, a quantitative analysis of the root canal microflora, carried out before the endodontic treatment, revealed P.endotondalis in four out of ten (40%) of the examined patients, respectively, with a titer of 2.0 × 10/3 GE/mL, 3.1 × 10/10 GE/mL, 2.0 × 10/2 GE/mL and 3.0 × 10/3 GE/mL; P.gingivalis in two patients (20%), respectively, with a titer of 1.2 × 10/2 GE/mL and 4.7 × 10/4 GE/mL; T. denticola in four patients (40%) with a titer of 4.6 × 10/5 GE/mL, 2.8 × 10/3 GE/mL, 4.5 × 10/4 GE/mL and 3.6 × 10/3 GE/mL; T.forsythia in six patients (60%) with a titer of 1.9 × 10/4 GE/mL, 12 GE/mL, 4.7 × 10/5 GE/mL, 4,5 × 10/4 GE/mL, 15 GE/mL and 2.0 × 10/6 GE/mL; P.intermedia in two patients (20%) with a titer of 1.0 × 10/4 GE/mL and 1.2 × 10/4 GE/mL; F.nucleatum in ten patients (100%), respectively, with a titer of 5 × 10/3 GE/mL, 5.4 × 10/5 GE/mL, 3.6 × 10/3 GE/mL, 1.2 × 10/2 GE/mL, 3.9 × 10/4 GE/mL, 5.8 × 10/3 GE/mL, 5.2 × 10/5 GE/mL, 4 × 10/3 GE/mL, 3.5 × 10/3 GE/mL and 1.3 × 10/4 GE/mL. A qualitative analysis of the microflora of the root canal, also carried out before the endodontic treatment, revealed S.spp. in ten patients (100%), C. albicans and C. crusei in two patients (20% each). A quantitative analysis of the root canal microflora in this group of patients did not reveal the presence of a qualitative A.actinomycetemcomitans, E.fecalis, E.faecium and C.glabrata.

Repeated quantitative and qualitative PCR analysis performed immediately before completion of endodontic treatment in the experimental group of patients determined the complete inhibition of all types of microorganisms identified before treatment. PCR analysis performed in the control group of patients showed a significant change in the composition of microflora identified before treatment. Quantitative analysis determined the complete inhibition of the microorganisms T. denticola and P. intermedia. P.endotondalis were completely suppressed in three patients (30%), and one showed significant inhibition (10%) from a titer of 2.0 × 10/2 to 85 GE/mL; P.gingivalis were completely suppressed in one patient (10%), and significant inhibition was determined in one patient (10%) from a titer of 4.7 × 10/4 to 7.4 × 10/2 GE/mL; T.forsythia were completely suppressed in five patients (50%), and in one (10%) significant inhibition was determined from a titer of 4.5 × 10/4 to 56 GE/mL; F.nucleatum were completely suppressed in six patients (60%), and in four (40%) significant inhibition was detected, respectively, from a titer of 5.4 × 10/5 to 1.8 × 10/3 GE/mL, from a titer of 1.2 × 10/2 to 75 GE/mL, 5.8 × 10/3 to 3.8 × 10/3 GE/mL and 5.2 × 10/5 to 1.6 × 10/3 GE/mL. Qualitative analysis determined complete inhibition (100%) in the root canals of the microorganisms C. albicans, C. crusei and S. sp., In six out of ten patients (Fig.2).

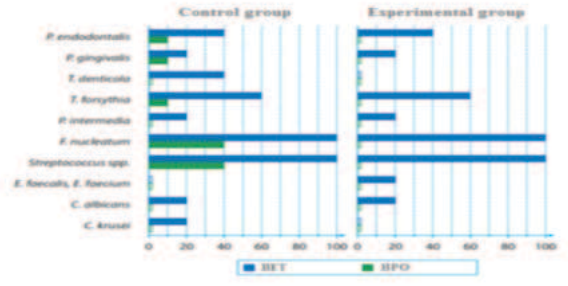

Fig.3 Bacterial Load In Root Canals Before Endodontic Treatment (BET) And Before Permanent Obturation (BPO).

Densitometric Analysis

Against the background of high antibacterial activity of the approved endodontic treatment in the experimental group, the study of reparative processes in the bone according to optical density determined the change in bone density in the periapical area from 35.7 ± 1.76% to 50.6 ± 1.51% (p< 0.001). As a result, the improvement in bone density during the treatment period was 14.9 ± 1.38%. In the control group, where antibacterial activity was less pronounced, a change in bone density after endodontic treatment occurred from 36.0 ± 1.87% to 48.7 ± 1.62% (p<0.001). Indicators of improved bone density in control group were significantly lower - 12.7 ± 1.28%. (p>0.05). (Table 1).

Table 1. The Mean And Standard Deviation Values Of The Optic Density Of The Groups, %.

Optic Density, %					
	Experimental group		Control group		P - value
	Mean	SD	Mean	SD	
Before treatment	35.7	1.76	36.0	1.87	<0.05*
After treatment	50.6	1.51	48.7	1.62	
Improvement	14.9	1.38	12.7	1.28	
P - value	<0.001**		<0.001**		

All statistical tests were performed using Stata 14.2 software (Stata Crop, College Station, TX). Non-parametric methods were used to assess the statistical accuracy of the difference between the indicators - Mann-Whitney's U-test for independent groups and Wilcoxon's double test for dependent groups. The statistical difference between the groups is considered significant when p <0.05.

SD: Standard Deviation; *Significant (p<0.05); **Significant (p<0.001)

CONCLUSION

Both treatment regimens tested in groups determined different antimicrobial activity. Complete inhibition of microorganisms was determined in patients of the experimental group. The treatment regimen tested in the control group, which did not include the use of a diode laser, was less active antimicrobiologically.

The use of a diode laser in endodontic treatment of the teeth with periapical lesion enhances the antibacterial activity and promotes more active repair in the bone.

Highlights

Optical density allows a more objective assessment of the ef fectiveness of endodontic treatment of the teeth with periapical lesion.
The bacterial load in the root canal is highly recommended to be done by PCR.

The use of a diode laser in endodontic treatment of the teeth with a periapical lesion enhances the antibacterial activity and promotes more active repair in the bone.

Source of Support: Nil

Conflict of Interest: Nil

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