ORIGINAL ARTICLE

Comparative evaluation of root canal disinfection by conventional method and laser: An *in vivo* study

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Abstract

Objective: The aim of this study was to comparatively evaluate *in vivo* the disinfecting ability of conventional method and lasers in root canals.

Materials and Methods: Study criteria included 60 single rooted teeth, which were indicated for root canal therapy followed to dental caries and trauma with intact crowns. Such selected patients were randomly divided into 2 groups, namely, Group A (30 teeth) and Group B (30 teeth). All clinical procedures were carried out under strict aseptic precautions. The teeth in Group A were subjected to biomechanical preparation followed by the treatment with the help of diode laser containing the gallium aluminum and arsenic, which emitted 980 nm wavelengths. The teeth in Group B were treated with routine method of biomechanical preparation along with irrigation using sodium hypochlorite and hydrogen peroxide. The microbiological samples were taken immediately after the access preparation and after the completion of the root canal disinfection and were sent for microbiological analysis.

Results: The teeth in Group A showed presence of common strains of bacteria ranging from *Streptococci*, *Staphylococci*, *Klebsiella,* and *Pseudomonas*. Reduction in the growth of microorganisms was found for all types of microorganisms. Only 8 samples exhibited the growth after treatment with laser. Results of Group B also showed the presence of common strains of anaerobic and aerobic bacteria as shown in earlier studies, predominantly *Staphylococcus*, *Streptococci,* and *Pseudomonas*. Statistical analysis showed non-significant *P* values for the microorganisms; however, only 3 samples showed the growth after treatment with conventional technique using sodium hypochlorite and hydrogen peroxide.

Conclusion: Conventional method by using sodium hypochlorite and hydrogen peroxide as irrigating solutions is highly effective in disinfecting the root canal. Lasers when used can also reduce the bacterial load of the infected root canal.

Key words: Diode laser, endodontics, root canal disinfection

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Introduction

Root canal disinfection is paramount because successful elimination of the microbial flora is the only guarantee for the ultimate success of the endodontic treatment. Disinfection of the root canal system has always remained a challenge for the clinician in the field of endodontics, prior to the obliteration of the root canal system. Routinely the root canal disinfection is established through biomechanical preparation along with the use of irrigants.

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As the popular endodontics axiom goes what is taken out of the canal is more important than what is put inside the canal, the role of chemomechanical debridement of the root canal cannot be overemphasized. In addition to mechanical alterations in the root canal system using biomechanical preparation and irrigation for the elimination of the root canal flora which has its own limitations.[1]

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Lasers have been claimed to disinfect the root canal system with considerable consistency and efficiency; however, very few *in vivo* studies exist, which show a comparison with the traditionally used techniques to eliminate the microbial flora from the root canal.[2]

Materials and Methods

For this *in vivo* study a total of 60 anterior single rooted teeth were selected as a sample.

Criteria kept in mind while selecting the patients.

- Any single rooted tooth requiring root canal therapy due to irreversible pulpitis, apical periodontitis, or trauma
- Preference given while selecting more than one sample in the patient with same criteria of selection
- Care taken to evaluate the health of periodontium and possibility of fracture.

Following patients are eliminated:

- Teeth with an incompletely formed apex or presence of large periapical lesion, internal or external resorption medically compromised patients and teeth where rubber dam could not be applied
- Every patient was well-informed about the objective, methodology, purpose, and any other related aspect of the study and a known consent was taken from the patient.

The samples were randomly divided into 2 groups.

- Group A: In this group, laser was used to disinfect the canal followed to conventional biomechanical preparation using normal saline as irrigant (30 teeth)
- Group B: These teeth were disinfected with the conventional technique using NaOCl and H_2O_2 as irrigating solutions (30 teeth).

All clinical procedures were carried out under strict aseptic precautions and rubber dam isolation (Hygiene, Italy). For the purpose of standardization, we have carried out all the clinical procedures ourselves.

Methodology

Strict antiseptic precautions were observed by cleaning the teeth with 15% iodine. Isolation of the teeth was carried out using rubber dam [Figure 1]. Access cavity preparation was carried out using Mani diamond points, BR – 46 bur for entry and TR – 13 bur for extension of cavity [Figure 2]. No. 15 k file was used to check the patency of the canal (Mani, Japan).

At this stage paper points of the corresponding sizes were used to take the sample from the root canal [Figure 3] and transferred to the tubes containing normal saline (for Aerobic culture) and Robertson cooked meat broth (anaerobic) [Figure 4].

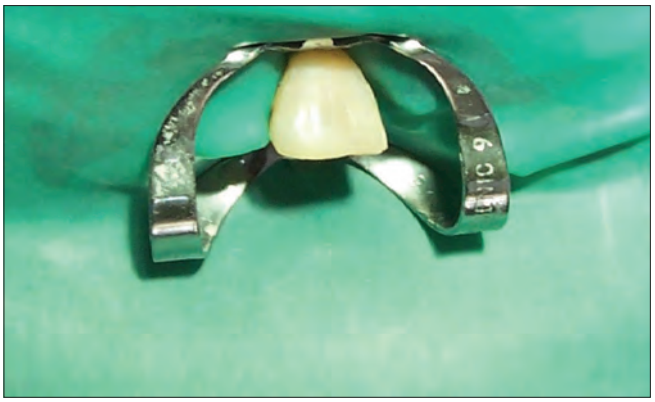


Figure 1: Isolation under rubber dam

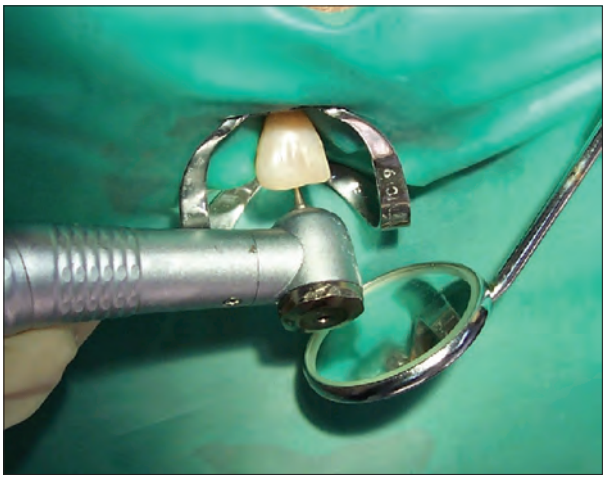


Figure 2: Access preparation with round bur

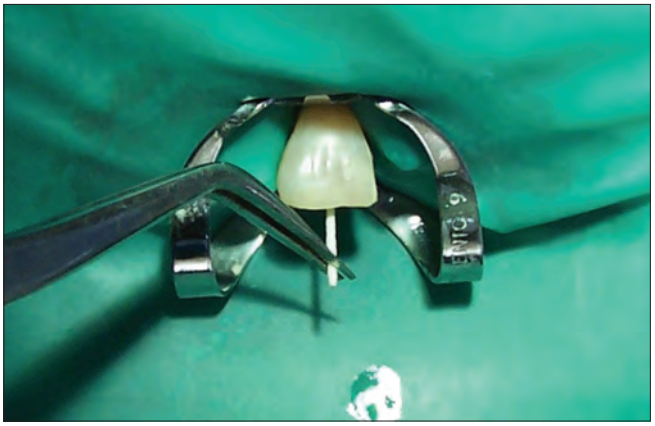


Figure 3: Collection of samples with the help of paper points

Following to the obtaining of specimen in Group A, standard norms and methods in conventional root canal therapy were strictly followed while measuring the root canal length and debridement of the canal.

Biomechanical preparation in Group A was carried out using the H files (Mani, Japan) and following circumferential filing to ensure entire length of the canal is included and

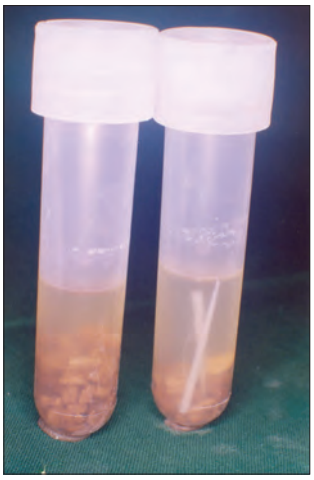


Figure 4: Robertson cooked meat broth as transport medium for anaerobic culture

normal saline (Parentral drugs India. Pvt. Ltd) was used as an irrigating solution to avoid any disinfecting action. Biomechanical preparation was carried out until 3 file sizes more than the initial binding file. After the completion of biomechanical preparation, they were irradiated with laser.

The Laser used in this study was a diode Laser (DIODENT) containing the gallium aluminum and arsenic which emitted 980 nm wavelengths. On the basis of *in vitro* study by Gutknecht, *et al*. a power setting of 2.8 W with 5 s irradiation pulse with 5 s of rest of total 4 cycles was selected and roots were exposed accordingly.[3] After this laser treatment the microbiological samples were taken using the sterile paper points.

In-Group B, Access cavity preparation was done using Mani diamond points, BR – 46 bur for entry and TR – 13 bur for extension of cavity. No. 15 k file was used to check the patency of the canal. At this stage, the microbiological samples were taken using sterile paper points as above.

Biomechanical preparation was done using H files and following circumferential filing to ensure entire length of the canal is included. The canals were irrigated with NaOCl (Prime dental products) and H_2O_2 (Parentral drugs India. Pvt. Ltd.) alternatively. Biomechanical preparation was carried out until 3 file sizes more than the initial binding file. Paper points of the corresponding sizes were used to take the sample as explained above.

The samples were submitted for microbiological culture.

Microbiological analysis

The samples obtained were incubated at 37°C until they were processed further. Samples were inoculated on blood agar plates and Mconkys agar plates to evaluate the growth of aerobic bacteria, growth found on these plates were identified by biochemical methods, for evaluating anaerobic growth the samples inoculated on Neomycin agar with Trypticase soy agar (TSA) as the basal media and menadione and hemin as supplement for enhancing growth.

Plates were incubated in a dyanox Jar (principle Rosenthal 1937, Marshall 1960) at 37°C for 48 h-1 week. A chemical indicator was used (Fildes and Mcintosh indicator).

It consists of three stock solutions as follows

1. 6% glucose in distilled water.
2. 6 ml, 0.1 M sodium hydroxide diluted to 100 ml with distilled water
3. 3 ml, 0.5% aqueous methylene blue distilled to 100 ml with distilled water.

Growth of microorganisms were noted and recorded for the two groups. The data collected was analyzed for significance of association between the time intervals (pre-treatment and after pre-treatment) and occurrence of growth by means of Pearson's Chi-square test.

Chi-square test was used for assessing statistical significance as the data to be analyzed was of nominal (descript) type and had a non-parametric distribution.

The results were as follows

The teeth in Group A showed presence of common strains of bacteria ranging from *Streptococci*, *Staphylococci*, *Klebsiella*, and *Pseudomonas* as recorded in the earlier studies. A statistical analysis using the Chi-square test was done. Significant *P* value (<0.05) was noted for organisms *Staphylococcus aureus* and *Streptococci*. Reduction in the growth of microorganisms was found for all types of microorganisms. Only 8 samples exhibited the growth after treatment with laser.

Results of Group B also showed the presence of common strains of anaerobic and aerobic bacteria as shown in earlier studies, predominantly staphylococcus, *Streptococci* and *Pseudomonas*. Statistical analysis showed non-significant *P* values for the microorganisms; however, only 3 samples showed the growth after treatment with conventional technique using the sodium hypochlorite and hydrogen peroxide.

When Group A and Group B were compared statistically for their effectiveness, they did not show any statistical difference [Tables 3].

In order to find the root canal flora of the infected teeth the pre-Biomechanical (BMP) findings of both groups were combined which showed [Tables 1 and 2].

Streptococcal species constituted 48% of the flora and *Pseudomonas* constituted 21% of the flora. The remaining 31% of the flora was constituted by the anaerobic species ranging from coagulase negative *Staphyllococci*, anerobic *Streptococci, Klebsiella*, and anaerobic gram-negative bacilli.

Table 1: Root canal flora before biomechanical preparation , group A and B

Pre-BMP	Group	
	A	B
Anaerobic gram negative bacilli		
No.	0	1
%	0.00	3.30
Anerobic cocci		
No.	0	1
%	0.00	3.30
Coagulase negative staphyllococci		
No.	1	1
%	3.30	3.30
Klebsiella, Anerobic cocci		
No.	1	1
%	3.30	3.30
Streptococcus (anaerobic)		
No.	5	2
%	16.70	6.70
Pseudomonas aeroginosa		
No.	7	6
%	23.30	20.00
Streptococcal Species		
No.	6	10
%	20.00	33.30
Streptococcal species, Coagulase negative staphyllococci		
No.	2	1
%	6.70	3.30
Streptococcus +++		
No.	2	1
%	6.70	3.30
Streptococcus viridans		
No.	6	6
%	20.00	20.00
Total		
No.	30	30
%	100.00	100.00

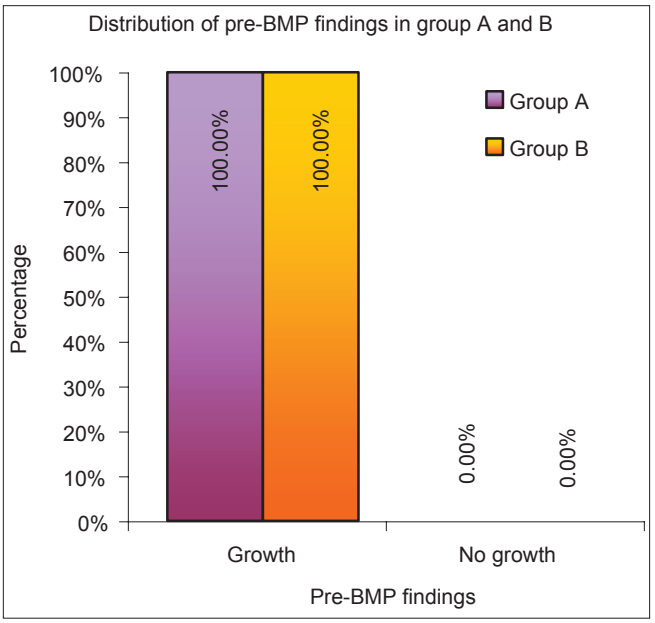
BMP=Biomechanical preparationws

Table 2: Root canal flora before BMP, group A and B

Distribution of pre-BMP findings in group A and B

Pre-BMP	Group		Total
	A	B	
Growth			
No.	30	30	60
%	100.00	100.00	100.00
No growth			
No.	0	0	0
%	0.00	0.00	0.00
Total			
No.	30	30	60
%	100.00	100.00	100.00

BMP=Biomechanical preparationws



After treatment in both the groups it was found that *Pseudomonas* species were not eradicated totally in both the groups; however, the heavy growth of the streptococcal species were reduced wherever found but not totally eradicated.

Discussion

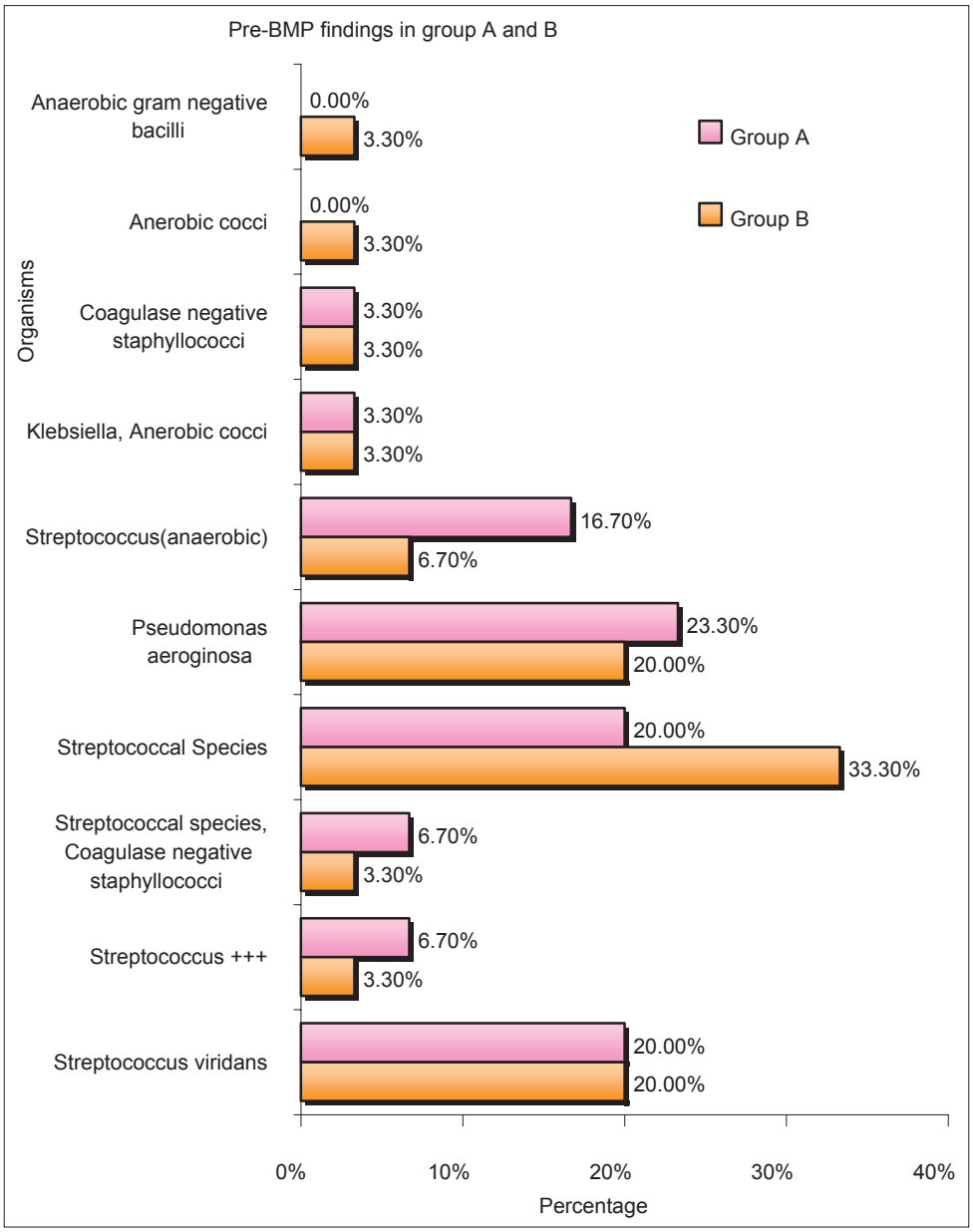
Endodontic infections are polymicrobial in nature. Microorganisms such as *Streptococci*, *staphyllococi*, *eubacterium*, *peptococcus*, *peptostreptococcus*, *pervotella*, *porphyromonas*, and *fusobacterium* predominate the infected root canal.[4]

Witgow and Sabiston investigated the root canal of intact teeth with pulp necrosis and found 80% of the cultures yielded positive for obligate anaerobic bacteria. Similar studies by sundqvist on traumatized but intact teeth with necrotic pulps have shown the presence of aerobic and anaerobic bacteria.[3,5,6]

The microbial flora of the canal after failure of root canals are predominated by gram positive species, facultative anaerobes especially E. faecalis are the most commonly isolated species. However poly microbial infection and obligate anaerobes were frequently found in the canal of symptomatic root filled teeth.[7]

Other studies have proven the polymicrobial nature of the root canal with pulp necrosis mainly involving obligate anaerobes and also showed infection may persists after treatment. Studies investigating the presence of microorganisms in closed periapical lesions associated with both refractory endodontic therapy and pulpal calcification found the presence of predominantly obligate and facultative anaerobes.[8,9]

Conditions may exists in the root canal that permit the growth of anaerobic bacteria capable of fermenting amino



acids, peptides, whereas bacteria that mainly obtain energy by fermenting carbohydrates by lack of available nutrients.[8]

Common endodontic cultures are thioglycollate, trypticase soybroth (with 1% agar), dextrose broth, brain heart infusion broth, and serum dextrose broth. The importance of culturing medium is noted in several studies. In this study, the samples were collected in Robertson's cooked meat media (RCM).

RCM was introduced by Robertson and is widely used for cultures of anaerobes. The cooked sterile muscle tissue contains reducing substances particularly glutathione, which permit the growth of many strict anaerobes without the application of other anaerobic methods. The meat in addition to its reducing activity provides a variety of nutritional substances for bacterial growth.

A number of preparation techniques have been advocated for use in preparation of the root canals ranging from the stepback, crowndown, modified stepdown, hybrid technique or the balance force concept. While each technique has its own advantages and disadvantages, the operator must choose the technique suitable for the particular case.[4]

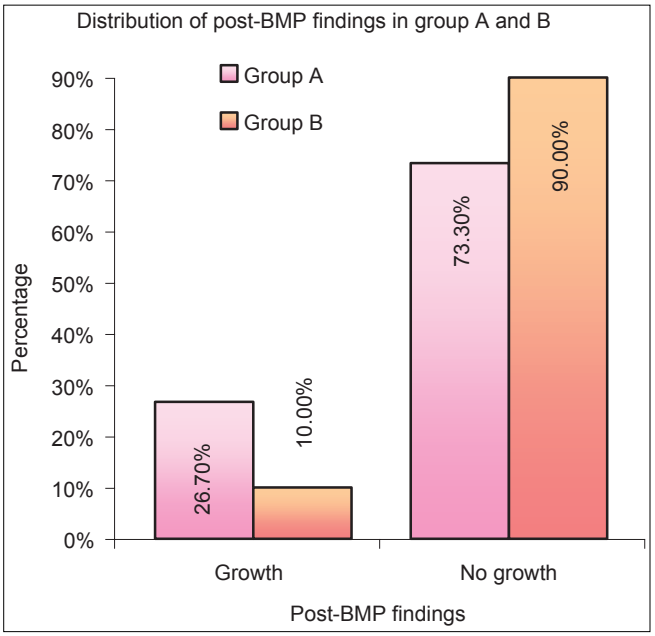
In this study, the conventional preparation was used to enlarge the canals because the teeth samples used belonged to the anterior teeth which can be correctly, convincingly and effectively prepared by this technique.[10]

Irrigating solutions have been used from the beginning of the endodontics till date in order to control the microbial growth inside the canal. A partial list of irrigants used in modern day include sodium hypochlorite, saline, water, anesthetic

Table 3: Root canal flora after BMP in group A and B

Distribution of post-BMP findings in group A and B			
Post-BMP	**Group**		**Total**
	A	**B**	
Growth			
No.	8	3	11
%	26.70	10.00	18.30
No growth			
No.	22	27	49
%	73.30	90.00	81.70
Total			
No.	30	30	60
%	100.00	100.00	100.00

BMP=Biomechanical preparationws



solutions, hydrogen peroxide, ethylenediaminetetraacetic acid (EDTA), chlorhexidine etc., Irrigants like sodium hypochlorite not only provide antibacterial property against the microorganisms, but also serve as a lubricants and dissolves vital and non-vital tissues.[4]

Siqueira, *et al*. in an *in vitro* study (1974), a 4% sodium hypochlorite solution provided the largest average zone of bacterial inhibition and was significantly superior when compared with the other solutions, except 2.5% sodium hypochlorite ($P < .05$).[11]

Raphael, *et al*. tested 5.25% sodium hypochlorite on *Streptococcus faecalis, S. aureus,* and *Pseudomonas aeruginosa* at 21°C and 37°C and found that increasing the temperature made no difference on antimicrobial efficacy and may even have decreased it.[12]

Buttler and Crawford, using *Escherichia coli* and *Salmonella typhosa*, studied 0.58%, 2.7%, and 5.20% sodium hypochlorite for its ability to detoxify endotoxin. All 3 concentrations were equally effective; however, large amounts of *E. coli* endotoxin could not be detoxified by 1 ml of 0.58% or 2.7% sodium hypochlorite.[13]

A Loyola University *in vitro* study reported that full strength clorox (sodium hypochlorite) and gly-oxide (urea peroxide), used alternately, were 100% effective against bacteroides melaninogenicus, which has been implicated as an endodontic pathogen.[14]

With this background sodium hypochlorite and hydrogen peroxide were selected as irrigating solutions in the conventional group.

For the laser group use of sterile saline solution was made as an irrigant. Saline will not enhance or synergistically help the antibacterial effect and will only provide a means of a lubricating agent and to help the flushing out of debris during biomechanical preparation. Lasers have been claimed to disinfect the root canal system with considerable consistency and efficiency; however, very few *in vivo* studies exist which show a comparison with the traditionally used techniques to eliminate the microbial flora from the root canal.[4]

Various types of lasers have been introduced to the endodontics such as diode laser, carbon dioxide laser, erbium family of laser, and argon laser.

Diode lasers are mainly used as soft-tissue lasers, but are proved to be particularly effective in disinfection of the root canal and do not alter the shape of the canal as hard tissue lasers such as carbon di oxide lasers and erbium lasers do.[15-17]

The Laser used in this study was a diode Laser (DIODENT) containing the gallium aluminum and arsenic, which emitted 980 nm wavelengths. On the basis of *in vitro* study by Gutknecht, *et al*. a power setting of 2.8W with 5s irradiation pulse with 5s of rest of total 4 cycles was selected and roots were exposed accordingly.[16]

Gutknecht and Nuebler-Moritz studied bactericidal effect of a holmium: Yttrium-aluminum-garnet laser on root canals *in vitro*. The efficiency of different laser settings was compared. The results showed that on an average 99.98% of the bacteria injected in the root canal could be eliminated.[17]

Klimm, *et al.* studied the bactericidal effects of neodymium: Yttrium- aluminum garnet laser irradiation in the depth of the root canal dentin. The results revealed highly significant elimination of bacteria for all thicknesses following laser irradiation. Although the intensity of the laser irradiation decreased after penetration of a 1000-micron dentin slice, the bactericidal mode of action was still effective.[18]

Moritz *et al.* (1997) examined the suitability of the diode laser with a wavelength of 810 nm for reducing bacteria in root canals. Infrared spectroscopic examinations revealed that irradiation at 4W under even circling movements resulted in a maximum rise in temperature of 6°C on the root surface. Stain penetration tests and scanning electron microscopic examinations revealed complete closure of the dentinal tubules on the irradiated root canal walls.[19]

Kreisler *et al.* investigate the bactericidal effect of an 809 nm semiconductor laser alone, and in combination with $NaOCl/H_2O_2$ irrigation in root canals *in vitro*. The results indicated that application of the diode laser might be an adjunct to conventional endodontic treatment when used in combination with a $NaOCl/H_2O_2$ solution.[20]

The teeth in Group A showed presence of common strains of bacteria ranging from *Streptococci*, *Staphylococci*, *Klebsiella,* and *Pseudomonas* as recorded in the earlier studies. A statistical analysis using the Chi-square test was done. Significant *P* value (<0.05) was noted for organisms *S. aureus* and *Streptococci*. Reduction in the growth of microorganisms was found for all types of microorganisms. Only 8 samples exhibited the growth after treatment with Laser.

When laser energy is absorbed by the target, a reaction may occur as a result of which it may prove to be fatal to the bacteria. Supraphysiological heating seems to contribute to the different bacterial killing during laser irradiation.

Results of Group B also showed the presence of common strains of anaerobic and aerobic bacteria as shown in earlier studies, predominantly *Staphylococcus*, *Streptococci* and *Pseudomonas*. Statistical analysis showed non-significant *P* values for the microorganisms; however, only 3 samples showed the growth after treatment with conventional technique using sodium hypochlorite and hydrogen peroxide.

This may be attributed to the bactericidal action of the sodium hypochlorite which takes place in 2 phases: (1) Penetration into the bacterial cell. (2) Chemical combination with the bacterial cell protoplasm that destroys it.[6]

When both the solutions are combined they have advantages as:

1. Effervescent action by the hydrogen peroxide pushes the debris out
2. Solvent action of the sodium hypochlorite on the organic tissue debris
3. Release of the free radicals like nascent oxygen.

When Group A and group B were compared statistically for their effectiveness, they did not show any statistical difference.

In order to find the root canal flora of the infected teeth the pre-BMP findings of both groups were combined which showed:

- Streptococcal species constituted 48% of the flora
- *Pseudomonas* constituted 21% of the flora.

The remaining 31% of the flora was constituted by the anerobic species ranging from coagulase negative *Staphyllococci*, anerobic *Streptococci*, *Klebsiella* and anaerobic gram-negative bacilli which is in line with the earlier microbiological studies.

After treatment in both groups, it was found that *Pseudomonas* species were not eradicated totally in both groups; however, the heavy growth of the streptococcal species were reduced wherever found, but not totally eradicated. It may be attributed to the ability of the *Pseudomonas* species to invade deep into the dentin as shown in the studies by Mehl *et al.*[21]

It cannot be just said or concluded that the laser was effective or not effective as a tool to disinfect the root canal in comparison with routinely used method of biomechanical preparation along with copious use of irrigation. It can be said from this study that one cannot ignore the importance of biomechanical preparation and use of irrigation, may be intracanal medicaments on one hand and also cannot underestimate newer technology introduced in the field of endodontics. Further studies in this regard are recommended.

Conclusions

Conventional method by using the sodium hypochlorite and hydrogen peroxide as irrigating solutions is highly effective in disinfecting the root canal.

Lasers when used can also reduce the bacterial load of the infected root canal.

Organisms such as *Pseudomonas* and anaerobic *Streptococci* are difficult to eradicate from the root canal.

Clinical significance

It can be said from this study that one cannot ignore the importance of conventional biomechanical preparation and use of irrigation.

Lasers when used can also reduce microbial load. Further studies are indicated in the field to ascertain the efficiency of different lasers systems like carbon dioxide laser and neodymium: Yttrium-aluminum garnet Nd YAG laser that are marketed today. The efficiency of laser systems in retreatment cases should also be evaluated.

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