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Antimicrobial Efficacy of Three Different Disinfecting Systems against E.Faecalis A comparative- in vitro study

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Abstract

Statement of the Problem: Disinfection is vital part of root canal treatment. Many of the studies have been published over the years on root canal disinfection techniques but the results are contradictory. Hence, this survey provides additional support to detect the most effective root canal disinfection protocol.

Purpose: The purpose of the survey is to determine the antimicrobial efficacy of photo-activated disinfection (PAD), diode laser irradiation, , endo-activator and conventional needle system with 2.5% sodium hypochlorite (NaOCl) on Enterococcus faecalis.

Materials and Method: With Pro Taper next file system, root canals of 60 freshly extracted premolar with single straight canals were prepared and later contaminated with E. faecalis suspension and incubated for 24hrs. All the samples were then randomly distributed into four groups which were irrigated with sodium hypochlorite and disinfected with all four disinfecting devices (Endoactivator, Diode laser, PAD, Conventional needle system).

Results: In the study, a significant decline in the bacterial population after all treatments ($P < 0.001$) were observed. The laser and PAD irrigation were significantly more effective in reduction of bacterial loadi.e1.00 to 1.86x104 CFU/ml than endoactivator and conventional needle systemi.e2.86 to 5.23×104 CFU/ml.

Conclusion: The PAD and laser system were more successful in reducing the bacterial load from the root canal than the endoactivator and NaOCl syringe irrigation alone.

Keywords: Endoactivator, Enterococcus faecalis, Disinfection, Laser, Root canal, Sodium hypochlorite.

INTRODUCTION

The main objective of endodontic treatment is the abolition of bacterial load from the root canal. It is very difficult to get rid of all microbes from the root canal system with mechanical instrumentation alone. Therefore, irrigants and intracanal medicaments are required to eliminate the microbes and debris from intra-radicular space.

The primary etiologic factors in the development of pulpal and periapical lesions is the aggregation of Bacteria. The success of endodontic therapy can be affected by the colonization of biofilm on dentinal walls, along with anatomical complexity of the root canal and the possibility of bacterial invasion in dentinal tubules.[1,2] Although mechanical instrumentation is the primary step in the debridement of root canal. But in cases of

intricate anatomy like fin, narrow isthmus, apical delta ,canal ramification render incomplete debridement by mechanical instrumentation alone.[3]

Moreover, disinfection is the vital part of root canal debridement. With the help of flushing mechanism, it helps in the removal of microorganisms, tissues remnants, and dentinal chips from the root canal. It also dissolves organic and inorganic tissues in the root canal and having antimicrobial properties which actively kills bacteria when introduced in direct contact with the microorganisms. With the help of syringe and needle, irrigants have been traditionally delivered. The drawback with this irrigation technique is the presence of inadequate irrigant throughout the root canal system. The highest streaming velocity present only in the lumen of the needle and around the tip of the needle is the reason of inadequate irrigant.[4] Therefore, disinfection by the needle system is inappropriate and failure rate of root canal treatment increases.

Various mechanical devices have been established to enhance the penetration and effectiveness of irrigation in the root canal space over the last few decades. To vigorously agitate irrigant solution in the root canal, Endoactivator which is a sonic device is used. For deeper penetration of an irrigant to all area of endodontic space and to effectively clean debris from lateral canal, there should be removal of smear layer and dislodging clumps of simulated biofilm may also help in deeper penetration. [5]

New techniques of disinfecting the root canals included use of high-power diode lasers as well as photo-activated disinfection (PAD).

In comparison to chemical disinfectants where depth of penetration is limited to 100 μm only, Diode laser is high power laser which have superior bactericidal effect irradiation and its property is attributed to greater depth of penetration (up to 1000 μm into dentinal tubules). The penetration of irrigants is restricted with progressive reduction in diameter of deep dentinal tubules.[6] Therefore, the factor contributing to its superior antimicrobial efficacy, laser irradiation with its inherent properties of light scattering, local intensity enhancement and attenuation permits penetration of light deep into dentinal tubules.

Destruction to the membrane and DNA of microorganisms is the result of PAD. It is an antimicrobial technique in which low laser energy is used to activate a nontoxic photosensitizer, and the nascent oxygen released from dyes which results in the destruction. [7] It is used as an alternative or a supplement in a root canal treatment for disinfection methods.

Materials and Method

60 freshly extracted human mandibular premolar teeth were collected which were extracted for orthodontic / periodontal reasons. All the teeth were cleaned using ultrasonic scaler and stored in distilled water till further experiment.

Access cavity was prepared using Endo access kit (Dentsply, Maillefer CO., U.S.A) and working length was established by using size 15 K file by radiographic method.

Further all teeth were instrumented using rotary fileProtaper Nextupto X2 and irrigation was done with 2.5%NaOCl and 17% EDTA followed by normal saline.

The apical foramina of all the teeth were sealed off with resin based composite restoration. Samples which were prepared was mounted in small vials and after that bacterial inoculation was done.

Bacterial growth and inoculation:

1) In an incubator at 37 degree C, Enterococcusfaecalis (ATCC-29212) was reactivated in trypton soya broth agar medium by overnight culture. (Figure 1)

Figure1:*E.faecalis(ATCC-29212)*

2) Inoculation with 10 μl of broth containing E.faecalis (2.5×104) using sterilized micropipette (Eppendorf Co., Germany) were done for all the teeth and then incubated for 24hrs at 37ºC.

All the teeth were then divided on basis of disinfecting system into four groups of 15 samples each

1. Group I- The root canal was irrigated using 2.5%NaOClfollowed by agitation with endoactivator for 1minutes

2. Group II- Root canal was irradiated with diode laser for 20sec which was repeated three times at the time period of 10sec.

3. Group III- Root Canal were filled with toulidine blue dye and disinfection was done utilizing PAD system for 1min

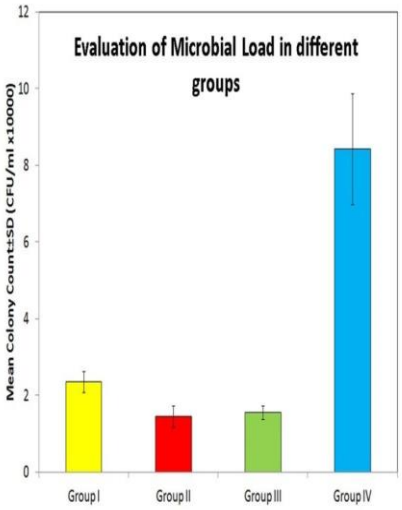
4. Group IV- Was kept as Control in which the root canals were irrigated with 2.5%

Sodium Hypo Chlorite for 1minute with the use of 3-mL syringe and 30-G needle

Evaluation of Bacteria

With the use of size 25 endodontic K file upto the working length, Root canal were filled with Phosphate Buffer Saline and gently filed in a circumferential way. Then the materials of root canal were aspirated using sterile syringe (Hindustan syringe and medical devices ltd co., India) into vials and mean while diluted with Phosphate buffer saline.

In culture plates with TSB agar medium, 100µl of each dilution was retained .The plate were incubated at 37ºC for 24hrs under anaerobic condition. Colony forming unit (were counted after 24hrs in each group by digital colony counter . The cell death or percentage was evaluated in different group (Graph 1) also were delibrated from CFU counted in cell culture plates after 24hrs.



Graph 1: *Evaluation of Microbial Load in different groups.*

Results

For intra group analyses (after disinfection protocol), Mann–Whitney U test was used . After the treatment results were presented graphically (Box and Whisker plot). For the intergroup comparative analysis of data, Kruskal– Wallis test was applied. After the treatment protocol there was reduction in the number of CFUs which was highly significant for all groups ($P < 0.001$) shown in table 4.

Experimental techniques applied for the survey were significantly superior over the control ($P \leq 0.001$) table 4. Diode laser and PAD were equally effective in reduction of E. faecalis populations ($P > 0.03$) (figure) and statistically more effective than the endoactivator and conventional NaOCl syringe irrigation ($P < 0.01$). A significant difference between the high-endoactivator and conventional NaOCl syringe irrigation was observed. ($p \leq 0.01$)

A total of 60 specimens were obtained and of them 15 were randomly allocated to Group I which were treated with Endoactivator, another 15 were allocated to Group II and were treated with Laser, another 15 allocated to Group III and were treated with PAD and rest 15 specimens served as Controls and were classified as Group IV (Table 1). Mean colony count was maximum in Group IV (8.42±1.45x104 CFU/ml) and minimum in Group II (1.45±0.28x104 CFU/ml).

Mean colony count was 1.55±0.17x104 CFU/ml in Group III and 2.35±0.28x104 CFU/ml in Group II. Overall mean colony count was 3.44±3.01x104 CFU/ml.(Table 2)

Table 1: *Group wise distribution of Specimens (N=60)*

Group	Description	No. of specimens	Percentage
Group I	Endoactivator	15	25.0
Group II	Laser	15	25.0
Group III	PAD	15	25.0
Group IV	Control	15	25.0

Table 2: *Evaluation of Microbial Load in different groups Colony count in CFU/ml×104*

Group	N	Minimum	Maximum	Mean	SD	Median
Group I	15	1.80	2.86	2.35	0.28	2.27
Group II	15	1.00	1.86	1.45	0.28	1.50
Group III	15	1.32	1.80	1.55	0.17	1.62
Group IV	15	5.50	10.20	8.42	1.45	8.82
Total	60	1.00	10.20	3.44	3.01	1.83

The mean rank for microbial load was maximum for Controls (53) and minimum for Laser (14.20). Evaluation of box plot shows the order of microbial load to be of highest order for controls followed by endoactivator. Laser and PAD groups had overlapping inter quartile range of microbial load values. Statistically, intergroup difference was significant. (Table 3)

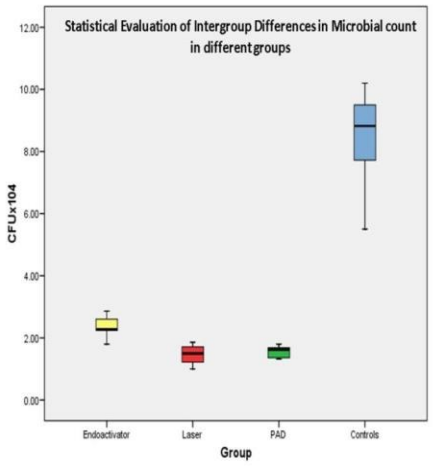
Table 3: *Statistical Evaluation of Intergroup Differences in Microbial count in different groups (Kruskall-Wallis test – Non-parametric ANOVA).*

SN	Group	N	Mean Rank
1.	Group I (Endoactivator)	15	37.90
2.	Group II (Laser)	15	14.20
3.	Group III (PAD)	15	16.90
4.	Group IV (Controls)	15	53.00
	Total	60	

Table 4: *Between Group Comparison of Microbial Load (Mann-Whitney U test)*

SN	Comparison	Mean	S.E.	Z	'p'
1	Group I vs Group II	0.90	0.28	4.628	<0.001
2	Group I vs Group III	0.80	0.28	4.651	<0.001
3	Group I vs Group IV	6.07	0.28	4.670	<0.001
4	Group II vs Group III	0.10	0.28	0.851	0.395
5	Group II vs Group IV	6.96	0.28	4.668	<0.001
6	Group III vs Group IV	6.87	0.28	4.669	<0.001

According to Table 4 , group difference was observed and it was maximum between Group II & Group IV (6.96±0.28) followed by Group III & Group IV (6.87±0.28) while between group difference was found to be minimum between Group II & Group III (0.10±0.28) followed by between Group I &Group III (0.80±0.28) and Group I & Group II (0.90±0.28). All the between group differences except that between Groups II and III were significant statistically .(Graph 2)



Graph 2: *Statistical Evaluation of Intergroup Differences in Microbial count in different groups.*

Discussion

Multiple appointments and inadequate disinfection through out the root canal treatment can result in bacterial colonization of the root canal system causes infection, which may subsequently causes re-infection and hindering the healing of periapical tissues. Therefore, the main objective of root canal treatment is to abolish the bacterial load from the root canal and disinfect effectively. Within the last few decades, more number of studies related to root canal disinfection techniques have been published with doubtful results. However, this survey emphasizes to observe and determine the most effective root canal disinfection protocols.

The antimicrobial effect of four disinfection techniques was determined which is in vitro condition, and is considered as an adjunct to chemo-mechanical canal preparation. E. faecalis was considered as the microbiological marker as it has the property to colonize the root canal in biofilms, which represents the in vivo growth condition. In present study, group IV were irrigated with 2.5% Sodium hypo chlorite with the use of 3mL syringe and 30-G needle and the minimum colony forming count was 5.50×104 CFU/ml while maximum colony was found to be 10.20×104CFU/ml. The high percentage of E.Faecalis in this group is attributed to the fact that very less amount of irrigant has reached to the intricate area to the dentinal tubules .Regarding the conventional syringe system the high percentage of viable bacteria present i.e 8.42±1.45x104 CFU/ml (Figure 5)which can be can be attributed the fact that the above finding are in accordance with study done by previous studies where during conventional syringe irrigation technique delivers irrigating solution not more than 0-1mm beyond the needle tip[8]. The tip of needle is often situated in coronal and middle third of the canal. This is because of the depth of penetration of irrigating solutions and its property to disinfect the dentinal tubules are confined and not reaching to peripheral areas such as anastomoses between canals, fins, and most apical part of the main root canal.

Figure2: *colony forming unit seen in Endoactivator*

In Group I disinfection was done by endoactivator where the root canal was irrigated using 2.5% sodium hypochlorite NaOCl followed by agitation with endoactivator. The result showed that minimum colony count was measured to be 1.80 ×104 CFU/ml while maximum was found to be 2.86×104 CFU/ml(figure 2) The penetration depth of irrigating solution and reduction of bacterial load was greater than conventional syringe technique. This could be due to fact that the endoactivator produce vigorous intra-canal fluid agitation through streaming and cavitations. It is used in our study at a frequency of 1-10KHZ which is capable of cleaning debris from lateral canals, elimination of smear layer and dislodgement of the clumps of simulated biofilms.

According to previous studies where endoactivator and needle irrigation in artificially contaminated root canals against E.Faecalis using 5% NaOCl and showed that endoactivator produced faster and better result in term of tissue dissolution.[9]

In Group II the root canal was irradiated with diode laser for 20sec repeated three times at an interval of 10sec. The minimum colony was

measured to be 1.00 ×104 CFU/ml while maximum colony count was found to be 1.86x104 CFU/ml(Figure 3). So, the elimination of the bacterial load in Diode laser was significantly more. The reason is due to its fibre optic tip which give greater accessibility and availability to formerly unreachable parts of the tubular network resulting in the superior bactericidal effect in the root canal dentin. In the unreachable parts of dentin, the diode laser also causes a thermal photodisruptive action, which results in an enhanced bactericidal effect in the root canal dentin.[10-12]

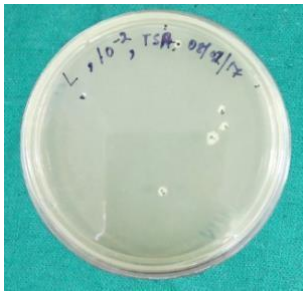


Figure3: *Colony forming unit seen laser*

These survey were congruent with the result of the previous studies where they showed that diode laser results in better antibacterial efficacy and least viable bacteria in comparison to irrigation with conventional needle and endoactivator. [13]

In present study Group III Photoactivated disinfection system was used in which Root Canal were filled with toulidine blue dye and disinfection was done utilizing PAD system for 1min and minimum colony count was measured to be 1.32 ×104 CFU/ml and maximum colony count was found to be 1.80×104 CFU/ml(Figure 4). So, PAD helps in the elimination of E. faecalis from the root canals this could be due its low-energy laser which activate a nontoxic photosensitizer like tolonium chloride, and the singlet (Nascent) oxygen released from dyes damages the membranes and DNA of microorganisms.[14]

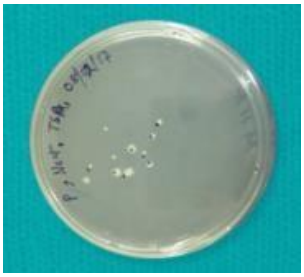


Figure 4: *Colony forming unit seen PAD*

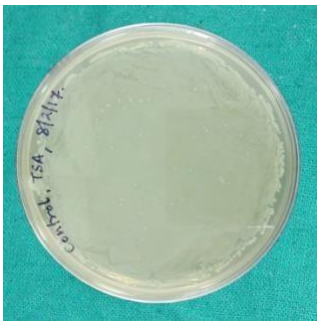


Figure 5: *Colony forming unit seen in control*

In present survey, the PAD and the diode laser were better than endoactivator and NaOCl irrigation syringe system in destruction of intracanal E. faecalis. Therefore, to determine the most effective endodontic disinfection protocol, the efficacy of the techniques must be further observed on multispecies biofilm. After PAD and diode laser with NaOCl, 99.99% reduction is observed in one minute. In vivo studies, it is compulsion to evaluate their real contribution to conventional chemo-mechanical preparation.

Conclusion

The diode laser and PAD had a successful contribution in reducing root canal infection and had the capacity to eradicate E. faecalis. The endoactivator and the conventional syringe irrigation had lower antibacterial effect.

Hence, in the present study amongst all the groups, efficacy of microbial count is as follow

Group II (Laser) ~ Group III (PAD) <Endoactivator (Group I) < Control (Group IV)

However, more long term clinical evaluation of these findings are needed in varied clinical conditions with larger sample size

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