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Comparative Evaluation of Microbial Flora in Primary Teeth Following Disinfection with Diode Laser- An Invivo Study

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Abstract

Background: Diode laser (980 nm) has been in use lately for root canal disinfection. It is seen to aid in effectively disinfecting the root canals in permanent teeth, and make it free of most bacteria, which is important for the success of endodontic therapy. The residual bacteria in the deeper layers of the periluminal dentin is not removed effectively by disinfection and debridement in the course of the root canal treatment. This in vivo study was done on primary teeth to evaluate the effectiveness of Diode laser in disinfecting the primary root canals.

Aims and Objectives: To comparatively evaluate the microbial flora remaining in primary root canals following disinfection with Diode laser.

Materials and Methods: After ethical clearance and parental consent, the study was done on 10 teeth which were indicated for pulpectomy, in children aged 4-7 years. After access cavity preparation, the specimens were collected on paper points, incubated in BHI medium for 24 hours, and the baseline count of bacteria was obtained in all the teeth. Biomechanical preparation was done using Kedo S rotary files. The teeth were then subjected to Diode laser. The base line count of bacteria was assessed. The remaining prominent bacteria were identified.

Results: There was a significant reduction in bacterial count after Laser treatment. The resistant bacteria were found to be mostly gram-positive organisms.

Conclusion: Use of Diode laser for disinfection of root canals of primary teeth can effectively reduce the bacterial count, especially the gram-negative microorganisms.

Keywords: Diode Laser, Disinfection, Enterococcus Faecalis, Brain Heart Infusion Medium (BHI), Blood Agar Plate (BAP).

Introduction

Due to the change in lifestyle and food habits, there is an increased incidence of dental caries and pulpitis in young children especially those under 6 years of age. It affects their nutrition and aesthetics as well as speech. The main objective of pulp therapy is the elimination of bacteria and necrotic pulp tissue from the primary root canals so as to keep the children free of pain and infection.1 The microorganisms are comprised of a complex mixture of bacteria, both aerobic and anerobic organisms, gram positive and gram negative. Studies have shown that they are capable of penetrating the periluminal dentin to more than 1100micrometers and thus, eliminating these bacteria becomes achallenge.2 The conventional chemical irrigants penetrate not more than 130 micrometers into the dentinal walls. The normal irrigants cannot surpass the curved and complicated canals of primary teeth and hence it affects the complete elimination of bacteria from root canals. 3 The Diode laser is seen to be able to penetrate more than 1000 micrometers into the periluminal dentin.4 Moreover, the action of chelating substances in the chemical irrigants facilitates the penetration of laser light to a depth of 1mm into the dentin. It has been seen to have low interaction with water and hydroxyapatite in permanent teeth but has decontaminant effect in the root canals. Thus, Diode laser was seen to enhance the disinfection during endodontic treatment of permanent teeth. The effect of Diode laser on the tooth is influenced by the wavelength, duration of exposure, irradiation mode, power density and tissue type. The conduction of heat to the tissue surrounding the tooth is also affected by the presence of air or water during irradiation. However, the thermal effect induced by Diode laser application in dental hard tissues is considered very limited.5 The bactericidal properties of Diode laser is mainly through its thermal properties and is proven instudies6.One of the main advantages of using Diode laser is that the bacteria cannot develop resistance to laser. Vast majority of studies based on Diode lasers are done impermanent teeth whereas only few studies have been done in primary root canals. The search is on for a method that can eliminate bacteria effectively, so as to reduce endodontic failures in children. This Invivo study aims to evaluate and compare the colony count and the predominant microbial flora in the root canals of primary teeth after disinfecting with Diode laser.

Materials and methods

Materials used.

1. Primary Teeth
2. Diode Laser with Endo Tips
3. 1% Naocl
4. Gates Glidden Drills.
5. Kedo S Files.
6. Distilled Water.
7. Bhi Broth Medium
8. Blood Agar Plate

Figure 1: Armamentarium

Figure 2: Kedos Files

Figure 3: Diode Laser

Inclusion Criteria

- Primary teeth indicated for pulpectomy.
- Primary teeth with at least 2/3rd root length
- Children of parents who gave their consent.

Exclusion Criteria

- Teeth with root caries
- Teeth fractured, grossly decayed or resorbed with
- root length less than 2/3rd root
- Children with physical, mental or developmental
- anomalies.
- Teeth with developmental anomalies.
- Children with systemic disease.

Methodology

Ethical clearance was obtained from the Institutional Committee of Mahe Institute of Dental Sciences and Hospital. Parents of children included for the study were explained about the procedure and parental consent was obtained from them. The participants include a total of 10 children, aged 4-7 years, with dentoalveolar abscess and who were not on antibiotics were chosen for the study. Local anaesthesia was administered and rubber dam application was done. Access opening of primary root were done using No.160 round bur. The remaining pulp tissue was removed with a no.10 size K file. Initial sample was taken from the root canals on a sterile paper point kept in the canal for 30 secs and transferred to Brain Heart Infusion medium (BHI) and incubated for 24 hours at 37 degree C, for determining base line load values of microbial flora in the root canals.

A) Group A: Biomechanical preparation was done on all the teeth using Kedo S rotary files, and canals were disinfected using 1% NaOCl. The canals were washed with saline. Samples were taken, from the root canals, with the help of paper points kept in the canal for 30 secs and transferred to BHI medium for incubation for 24 hours and the colony count was obtained with a digital colony counter.

B) Group B: The canals were then treated with Diode laser of 1.5 watt for 3 secs. Samples were taken on a paper point kept in the canal for 30 secs, and transferred to BHI medium for incubation at 37 degree Celsius for 24 hrs. The colony count was assessed using a digital colony counter.

Turbidity was seen in all the samples. The prominent colonies of all the groups were streaked on the Blood agar plates and the bacteria present were identified using Vitek machine using Malditoff software.

Figure 4: Vitek Machine

Statistical Analysis

All statistical procedures were performed using IBM Statistical Package for Social Sciences (SPSS) 22.0. All quantitative variables expressed in mean and standard Deviation. Shapiro-Wilk test were used for testing the normality assumption of the data. One Way ANOVA was used to compare the difference in bacterial count across the groups. Post hoc test was done to identify the significantly differing pairs. Probability value p <0.05 was considered statistically significant.

NULL Hypothesis(H0)

No significant difference exists in mean bacterial count among control, conventional BMP or Diode laser.

Alternate hypothesis (HA)

There is significant difference in mean bacterial count among control, conventional BMP or Diode laser.

Result

Bacterial Profile: Prior to BMP, around 60% of the samples showed presence of 1 or 2 of Limisilactobacillus fermentum, a gram positive normal microbial flora, E Faecalis, a gram positive pathogenic bacteria , and Acinetobacter Juni and Neisseria mucosa , both of which are gram negative pathogenic organisms, inside the root canals. After BMP, there was not much reduction in pathogens in the specimens checked. In just 2 cases however, there was presence of Streptococcus mitis and Limisilactobacillus fermentum. Presence of pathogenic bacteria's like Aneurin bacillus aneurinilyticus, Streptococcus mitis, E. faecalis, Acinetobacter Juni and Streptococcus cristatus was also noticed. After Diode laser treatment 20 % of the samples had shown a completely sterilized canals and the rest 80% had shown a healthy pathogenic microflora including streptococcus oral is, streptococcus mitis and E. fecalis. Table 1 shows the predominant bacteria found before biomechanical procedure, after BMP and after Diode laser treatment.

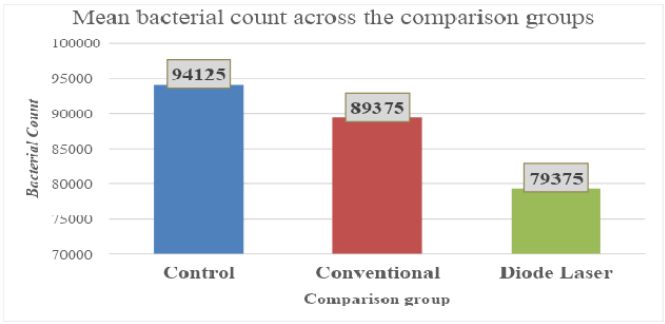
Table 1: Viable organisms after treatment.

S.No.	CONTROL	AFTER BIOMECHANICAL PREPERATION(Group A)	AFTER LASER TREATMENT(Group B)
1	Limisilactobacillus fermentum (gram positive)	Limisilactobacillus fermentum	Streptococcus mitis (gram positive)
	Acenetobacter juni (gram negative)		
2	Acenetobacter juni	Acenetobacter juni	Streptococcus vestibularis (gram positive)
3	Limisilactobacillus fermentum Aneurinibacillus aneurinilyticus(gram negative)	Aneurinibacillus aneurinilyticus	Streptococcus vestibularis
4	Neisseria mucosa (gram negative)	Limisilactobacillus fermentum	No Viable organisms
5	Enterococcus faecalis (gram positive)	Enterococcus fecalis	Enterococcus faecalis (gram positive)
6	Lactococcus lactis	Streptococcus cristatus (gram positive)	Streptococcus mitis (gram positive)
7	Corynebacterium tuberculostearicum (gram positive) Streptococcus mitis	Streptococcus mitis	Streptococcus mitis
8	Streptococcus mitis Streptococcus cristatus	Streptococcus mitis, Streptococcus cristatus	Streptococcus mitis

Figure 5: On Blood Agar Medium Initial Colonies Formed

Figure 6: Colony formed after conventional preparation.

Figure 7: Colony formed after laser treatment.



Graph 1: Mean Bacterial count across the comparison groups.

Follow up	No of samples, n (N=24)	Mean	Standard Deviation	Minimum	Maximum
Control	8	94125.00	3482.097	88000	98000
Conventional(Group A)	8	89375.00	5262.740	82000	96000
Diode laser(Group B)	8	79375.00	6345.696	72000	90000

Table 2: Distribution of bacterial count after each procedure.

Comparison of bacterial count across the follow up Shapiro-Wilk test statistics shows that the data is normally distributed in each group. Hence One-way Anova test was used to test if there is a significant difference between the mean bacterial count across the groups. An evident reduction of bacterial count by 5.05 % (4,750) after conventional BMP and a further reduction by 11.19 % (10,000) was noticed after diode laser. Overall, there was a reduction of 15.67% (14750) after the diode laser treatment, compared to the bacterial count at baseline. One Way Anova statistics gives a highly significant p value of <0.001 ad hence the null hypothesis was rejected, and alternate hypothesis was accepted, indicating that the reduction in bacterial count between the control, conventional and diode laser is significantly different at a highly significant p value of less than 0.001.

Table 3: One-way ANOVA test for comparison of bacterial count at each follow up.

Follow up	No of samples, n (N=24)	Mean	Standard Deviation	95% Confidence Interval for Mean		P value
				Lower Bound	Upper Bound	
Control	8	94125.00	3482.097	91213.89	97036.11	<0.001
Conventional	8	89375.00	5262.740	84975.24	93774.76	
Diode laser	8	79375.00	6345.696	74069.87	84680.13	

Post hoc test to identify significantly different Pairs

A Tukey post hoc test was done to specifically identify the pairs which are significantly different to each other. Post hoc test show that there is no significant difference between the control and Group A. While the mean count is significantly different for both between the control-Group B (p=0.001) as well as the Group A and Group B samples.

Discussion

Due to the complex intracanal anatomy of the root canals of primary teeth, successful endodontic treatment of primary teeth still remains a challenge. The complex anatomy and the age of the child poses limitations for the syringe/needle irrigation technique. So, new adjunctive antibacterial therapeutic strategies targeting residual microorganisms will be helpful in the success of root canal treatment in primary teeth. The thorough

disinfection of root canals of primary teeth enhances the healing rates, especially those with periapical involvement. Microorganisms are seen as deep as 1100 micrometers into the periluminal dentin 2,3. Conventional biomechanical procedures (BMP) are not able to reach these depths, leaving considerable pathogenic bacteria remaining in the root canals causing secondary infection. The majority of the samples for the study were taken from abscessed tooth. Dental abscess was thought to contain huge amount of pathogenic bacteria, predominantly obligate and facultative anaerobes. Streptococci, commonly implicated in dental abscesses, is a facultative anaerobe belonging to therians group, and includes mitis, oral is, salivary, sanguinis and mutans group7. In this study streptococci have shown resistant to Laser treatment and was the predominant one found in 50 % of the samples. This agrees with the study by Cohen et al , who reported Streptococci represented over 70% of the microbiota in teeth indicated for extraction8. They are present in the RC associated with primary endodontic infections which is similar to our study9.Other organisms detected are Aneurinibacillusaneurinilyticus, Limisilactobacillus fermentum, Streptococcus mitis, E. faecalis, Neisseria mucosa, Acinetobacter Juni and Streptococcus cristatus. This agrees with Toyoshima et al. who reported that in root canals of primary teeth with necrotic pulp and periapical lesions submitted to retreatment there is a polymicrobial infection with predominance of anaerobic microorganisms, similar to the microbiota of permanent teeth10. In the present study, the root canals of primary teeth were instrumented with Kedo S rotary files. The bacterial count educed by only 40% after the BMP procedure. In studies done by Üreyen Kaya B et al on permanent teeth, found that Instrumentation may reduce bacterial load, but it does not provide a bacteria free root canal11. In our study we found that the bacterial count was reduced considerably with the laser application. There was reduction of bacterial count by 5.05 % (4,750) after BMP and further reduced by 11.19 % (10,000) after laser treatment. Overall, there was a reduction of 15.67% (14750) after the diode laser treatment compared to the bacterial count at baseline. This was statistically significant($p<0.001$). It agrees with the study done by Prabhakar et al in 2013, where they attained 99.9% reduction of bacteria, after laser treatment12. In our study the laser was applied in the root canals only for 3 secs. We had taken abscessed teeth for our study and since there could be resorption of bone in the periapical region of the teeth and due to the presence, the permanent successors there, when the patients complained of pain at the end of 3 secs, we had to stop the laser at that point. E. faecalis, frequently found in cases of therapy resistant infection 13was very resistant to removal from the root canals in our study, with the application of the diode laser for 3 secs. According to Schoop et al., Diode lasers at 1 W for 20 secs are efficacious for E. fecalis in permanent teeth; It is resistant to heat.13 Gutknecht et al described that an application of the laser in permanent teeth below 1 W is less important in endodontics because neither is the smear layer completely removed nor are the dentinal tubules sealed. With settings of 1.25 W–1.5 W significant changes on the root canal surface were determined in permanent teeth viz; complete removal of organic material and occlusion of dentinal tubules14According to Zahra Bahrololoomi et al., Diode laser with a power output of 1.5 W, is effective in reduction of E. faecalis bacterial count without damaging periodontal structures in primary teeth15. Due

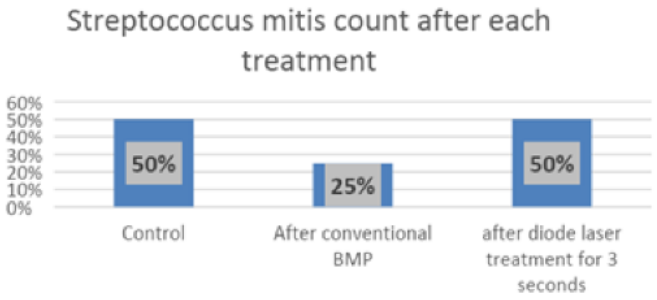
to the presence of the permanent tooth bud under the primary teeth, we did not raise the wattage beyond 1 W. In the present study, Diode Laser application of 1W for 3 secs was seen not to affect the gram positive organisms like E coli, and Streptococcus mitis (found in 2 samples), whereas gram negative organisms were shown to be sensitive to Diode laser. The resistance of gram-positive organisms to laser treatment may be due to the exposure time used in this study, that was less (3 secs). The resistance of Strep. Mitis and Enterococcus Faecalis to this dosage of Laser application is disturbing since these organisms can cause secondary infection and failure of the root canal treatment. Very less Invivo studies have been done on the efficacy of diode lasers on the root canal disinfection, in both permanent and deciduous teeth. The results of this study show that the 980 nm diode laser with 1W for 3 secs can eliminate gram negative organisms, the major colonisers of root canals of primary teeth that has immigrated into the deeper layers of the root canal dentin.

Table 4: Predominant viable bacteria after laser treatment

Viable organism after diode laser treatment	Bacterial Count of viable bacteria	percentage
Enterococcus fecalis	1	14.29%
Streptococcus mitis	4	57.14%
Streptococcus vestibularis	2	29%

Table 5: Gram Staining

Gram staining character	Viable organism in control(n)	Viable organism in control (%)	Viable organism in after Conventional BMP (n)	Viable organism in after Conventional BMP (%)	Viable organism after diode laser treatment for 3 seconds(n)	Viable organism after diode laser treatment for 3 seconds (%)
Gram (- ve)	3	27%	2	20%	0	0%
Gram(+ve)	8	73%	8	80%	7	100%
Total viable organisms cultured	11		10		7	



Graph 2: Streptococcus mitis count after each treatment

Conclusion

The diode laser treatment of primary root canals was seen to considerably reduce the gram-negative pathogenic organisms and can be applied by all means as a support in endodontic treatment, thus increasing the success rate. However, gram positive anaerobes usually found in the root canal of infected primary teeth were resistant. This should be further validated by performing clinical studies with increased sample size. The acceptance of laser technology by clinicians in primary teeth still remains limited.

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