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Original Article

Dentistry Section

Effect of Diode Laser on Bacteria Beyond the Apex in Relation to the Size of the Apical Preparation – An In-Vitro Study

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ABSTRACT

Introduction: Microorganisms causing periapical infection are usually difficult to eradicate after conventional endodontic treatment or even in retreatment resulting in poor outcomes. So the purpose of the study was to assess whether disinfection of root canal with laser had any effect on bacteria in the periapex region.

Aim: The aim of the present study was to evaluate the effects of a diode laser when activated in root canals with varying apical diameters, on the bacteria present beyond the apex of the teeth.

Materials and Methods: Total 30 intact single rooted teeth were taken and decoronated to standardize the root to a length of 12mm. They were divided into three groups depending on last file size used for instrumentation at apex i.e., size 30, 40 and 50 respectively. The samples were then mounted on test tubes such that roots of teeth were in contact with fresh broth of *Escherichia coli* (ATCC 25922) and left for incubation. Later a diode laser (Ezlase 940, Biolase) was used for disinfection of root canals of the samples. Following this the bacterial inoculums from each test tube were cultured and CFU were obtained from which the mean log values were obtained. Statistical analysis was done using Kruskal Wallis ANOVA test to compare mean CFU in three groups. Mann-Whitney U test with Bonferroni correction was used to compare inter-group differences.

Results: There was statistically significant difference in mean log values of CFU in all the three study groups. Inter-group comparisons showed that, Group A had significantly lower mean CFUs than Group B and C respectively.

Conclusion: The study showed that intracanal irradiation with diode laser had an effect on the bacteria present beyond the apex, and it was influenced by the size of the apical preparation i.e., smaller apical size led to a greater reduction in the bacterial count.

Keywords: Apical diameter, Apical periodontitis, *Escherichia coli*, Root canal disinfection

INTRODUCTION

Bacterial infections are primarily responsible for the development of endodontic diseases. Once endodontic infections progress beyond the root canal, the time required for disinfection and subsequent healing is prolonged. In some treated cases, the periapical infections persist and progress, requiring invasive strategies such as periapical surgery or extraction.

Our understanding of the mechanisms of endodontic and periapical infections is evolving, along with the modes of eliminating them. No individual irrigant or instrumentation technique is capable of completely disinfecting the root canal space. However, combining different approaches can possibly have a synergistic effect. Lasers have the potential to be used in such approaches. Various laser systems have been investigated for their role in root canal disinfection following instrumentation, either alone, or in association with a photosensitizer. In vitro studies have established the effective antibacterial activity of lasers on dentin, and within the root canals [1-5].

Since there are very few studies [6, 7] in literature assessing efficacy of laser disinfection of root canal on bacteria present beyond the root apex, the present study aimed to evaluate the effect of a diode laser when activated in root canals with varying apical diameters, on the bacteria present beyond the apex of the teeth. The null hypothesis was that the size of the apical preparation would not have a significant effect on the antibacterial activity of the diode laser.

MATERIALS AND METHODS

Total 30 intact single rooted mandibular premolars extracted for orthodontic reasons were collected from the Department of Oral and Maxillofacial Surgery, Sri Sai College of Dental Surgery, at Vikarabad and used for the study. They were decoronated with the help of diamond disc to obtain an average root length of 12mm. The samples were randomly divided into three groups of 10 each, namely–

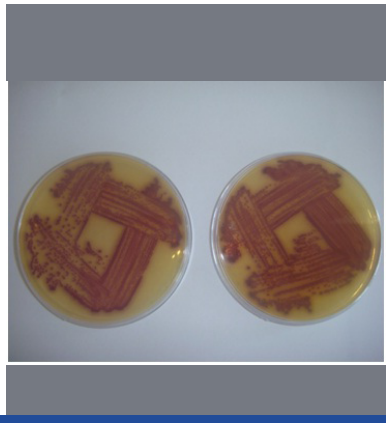
- Group A – canal instrumented with K files to size 30 at the apex.
- Group B – canal instrumented with K files to size 40 at the apex.
- Group C – canal instrumented with K files to size 50 at the apex.

The instrumentation was done using step back procedure with K files and the canals were irrigated thoroughly with saline during and in between instrumentation. The samples were then autoclaved for 15min at 121°C. They were then mounted on test tubes with the help of putty impression material and cyanoacrylate glue, and the entire setup was again autoclaved [Table/Fig-1].

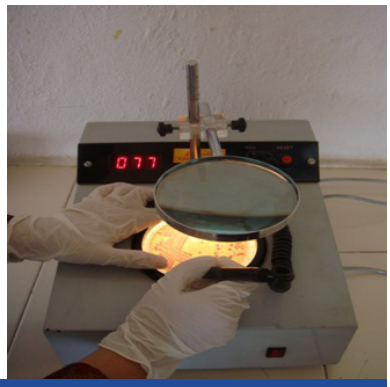
Fresh broth of *Escherichia coli* (ATCC 25922) at 0.5 McFarland units (1.5 * 10^8 CFU/ml) was prepared [Table/Fig-2]. The broth was then inoculated into the test tubes, such that the apices of the teeth were in contact with the culture medium and left for incubation for 24 hours at 37 °C [Table/Fig-1].

A diode laser (Ezlase 940, Biolase) with a 200µm fibreoptic endodontic tip was used. The laser has a maximum output of 7 Watts at an operating wavelength of 940nm [Table/Fig-3]. The laser setting used in this study was 1.5 Watts power, at 20 Hz frequency, in the continuous mode [2,3]. The irradiation protocol followed for this study was a 5sec irradiation followed by a 20sec interval, which constituted one lasing cycle. Four such cycles were performed for each tooth. The tip was positioned at the apex, followed by activation during which it was dragged coronally, in a helicoidal movement [1].

Following the test procedure, 10µl of the bacterial inoculum from each test tube was streaked on to the culture plates and incubated for 24hrs at 37ºC, and colony counting was done [Table/Fig-4] using a colony counter. The mean log values were calculated for each group from the CFU values obtained.



[Table/Fig-1]: Mounted samples with apices touching the broth.
[Table/Fig-2]: *Escherichia coli* (ATCC 25922)



[Table/Fig-3]: Ezlase 940 unit. **[Table/Fig-4]:** Colony counter.

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS version 14. A p-value of <0.05 was considered statistically significant. CFU were log transformed and then compared among the three groups with Kruskal Wallis ANOVA test. Mann-Whitney U test with Bonferroni correction was used to compare inter-group differences.

RESULTS

There was a statistically significant difference in the mean log Colony Forming Units (CFUs) among the three study groups (p=0.001). The mean log CFU of Group A was 11.26, compared to those of Group B and Group C (12.46 and 12.56 respectively). Inter-group comparisons showed that, Group A had significantly lower mean CFUs than Group B and C (p=0.004 and 0.004) respectively. No significant difference was seen between Group B and C as shown in [Table/Fig-5].

	Group						p-value	Post-hoc comparison (p-value corrected for alpha)
	A		B		C			
	Mean	SD	Mean	SD	Mean	SD		
Log CFU	11.26	.14	12.46	.09	12.56	.04	0.001; Sig	A Vs B (0.004); Sig
								A Vs C (0.004); Sig
								B Vs C(0.025); NS

[Table/Fig-5]: Mean CFU values were log transformed, p-value was obtained with Kruskal Wallis ANOVA test. Mann-Whitney U test with Bonferroni correction was used to compare inter-group differences.

DISCUSSION

Root canal disinfection is crucial for the success of endodontic treatment. However, once bacteria gain access to the periapical tissues, it becomes increasingly difficult to achieve a sterile environment which is imperative to healing. A clinical investigation conducted by Riccuci et al., to evaluate human periapical lesions using specimens obtained from 50 untreated extracted teeth revealed that 18 of 50 teeth had bacteria in the periapical tissues [8]. In such cases, the success of nonsurgical endodontic treatment is limited due to the inability in eliminating bacteria beyond the apex.

Since the invention of lasers, these devices have been used for various applications in diverse fields, including dentistry. In endodontics, lasers have been investigated for their role in root canal treatment, especially intracanal disinfection. Safety concerns regarding the use of lasers in such treatment, especially regarding the possibility of tissue damage due to temperature rise in the root canal during laser irradiation have been raised. However, in vitro studies evaluating the temperature rise on the external root surface during intracanal irradiation using diode lasers have recorded temperature rise in the range of 1-7°C [1,9,10]. Since the threshold bone necrosis temperature is 47°C for 1min, this temperature rise is below the danger mark. This has cleared the way for the widespread clinical use of lasers for root canal treatment.

However, most studies conducted so far have focused on their ability to achieve disinfection of the canal system [1-5], whereas there have been few studies investigating their effect on disinfection in the periapical areas [6,7] hence, the study was planned.

The laser used in this study was Ezlase 940 (Biolase), a Ga-As-Al diode laser. The output power can be adjusted in the range of 0.5 to 7 Watts and the pulse and interval duration can be adjusted between 0.05ms to 5ms. A diode laser was chosen for this study as it has been shown to be a tissue-friendly laser, showing lesser temperature rise compared to other lasers [3]. The irradiation protocol followed for this study was as described by Gutknecht et al., [2] with one difference – the fiber optic tip was positioned at the apex, instead of 1-2mm from the apex, so as to begin irradiation at apex as followed by Nagayoshi et al., [7].

The samples were mounted in such a way as to ensure that the apices would be in touch with the bacterial broth in the test tubes. The bacterial strain used was *Escherichia coli* (ATCC 25922), which is a gram negative bacillus, which has been found in root canals of teeth with post treatment apical periodontitis [11].

The null hypothesis was rejected, as the result showed that when root canals with a narrower apical diameter were irradiated with the tip at the apex, a larger bacterial kill was recorded. The colony counts in the broth increased with an increase in the apical diameter. This could be because of the higher energy density achieved because of the narrow apical diameter of Group A compared to the other experimental groups.

While the instrumentation technique goes against Grossman's philosophy of confining instruments within the root canal, violating the apical constriction up to K file size 30 was advocated to encourage drainage in cases of acute periapical abscess [12]. An orthograde approach to the periapical lesion has already been introduced in the form of the Apexum technique [13]. The diameter of the NiTi ablator in this device is 0.18mm, and case reports

published detailing the clinical use of this device have noted that the apex of the treated teeth have been instrumented with a 35mm K file, or a profile instrument of size 30 with a 4% taper (Metzger et al.,) [14,15]. Our results showed that lower bacterial counts were associated with the apices of the smallest diameters. Consequently, if validated by further studies, periapical disinfection might be achieved in a non-invasive fashion without the need of compromising tooth structure. The methodology specified above could be an alternative to surgical treatment if validated by further studies.

LIMITATION

This study assessed the antibacterial efficacy against the *Escherichia coli* bacterium which was in a planktonic state; however, recent studies have shown the role of biofilms in established periapical infections. Since the predominant bacterial species in canals of post treatment failure cases is *Enterococcus faecalis*, this bacterium and its biofilm should be assessed in future studies [16]. While a diode laser was used in this study, other lasers that are being used for instrumentation of canals, such as Er:YAG, Er:YSGG and Nd:YAG could be investigated in a similar manner.

CONCLUSION

- The study showed that the antimicrobial efficacy of diode laser irradiation at the apex was influenced by the size of the apical preparation, i.e., smaller apical size led to a greater reduction in the bacterial count.
- Other studies can be done evaluating the effects of intracanal laser disinfection on extraradicular biofilms.
- Different types of lasers could also be compared for their efficacy to disinfect periapical lesions.

If further validated by additional studies, laser irradiation could be used to ensure effective periapical disinfection

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