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A comparative evaluation of antimicrobial efficacy of laser assisted photo dynamic therapy, 1% sodium hypochlorite and 2% chlorhexidine in root canal disinfection of primary teeth: A clinical study

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Abstract

Aim: to evaluate the antimicrobial efficacy of laser assisted photodynamic therapy, sodium hypochlorite (1%) and chlorhexidine (2%) in root canal disinfection of primary teeth**.**

Methods: A total of 75 children aged 4-8 years who required pulpectomy were divided into three groups of 25 each. In Group A 1 % sodium hypochlorite was used as an irrigant, in Group B 2% chlorhexidine was used as an irrigant and in Group C laser assisted Photodynamic therapy(PDT) was used. Pre and post-operative samples were collected using absorbent paper points and subjected to microbiological culture. Total colony forming units (CFU s) were counted at both the periods. Total reduction in number of CFU s in all the three groups was calculated and statistically analysed.

Results: All the three groups showed a significant decrease in bacterial load at the post-operative period with 2 % chlorehexidine showing a maximum antimicrobial efficacy (P<0.001).There was no statistically significant

difference between 1% sodium hypochlorite and laser assisted PDT group.

Conclusion: 2% chlorhexidine showed maximum disinfecting properties among all the groups on both aerobic and anaerobic microorganisms. 1% sodium hypochlorite and laser assisted photodynamic therapy showed comparable disinfecting properties on both aerobic and anaerobic microorganisms.

Clinical Significance: From the present study it can be concluded that laser assisted photodynamic therapy can be used as promising alternative in root canal disinfection of primary teeth.

Keywords: Primary teeth, canal disinfection, chlorhexidine, sodium hypochlorite, laser assisted PDT.

Introduction

The best space maintainer in both primary and mixed dentition is the primary tooth itself. This is not only because of the clinical crown but also due to the presence of roots and periodontium that guide the eruption of succedaneous permanent tooth. Primary teeth also stimulate development of maxilla and help in masticatory process thus preserving the integrity of primary dentition is important for the development of permanent dentition.[1]

The aim of endodontic therapy is to achieve maximum disinfection in the canal before obturation. Endodontic treatment in paediatric patients is challenging because of variations in root anatomy, root resorption, presence of accessory canals along with difficulty faced in achieving patient cooperation.

The use of handheld files and irrigating solutions with disinfectant properties is currently the most common form of endodontic treatment.[2] Sodium hypochlorite (NaOCl) appears to be the most commonly used irrigant in a concentration ranging from 0.5 to 6% (Walker, 1936). The factors contributing to its popularity are low cost, ease of availability and good shelf life, potential bactericidal activity and excellent tissue dissolving action. Chlorhexidine Gluconate is another irrigant widely used in dentistry which is having antiseptic and bactericidal properties. One of the most important properties of chlorhexidine is substantively contributing to its prolonged antimicrobial activity. However, the existence of residual bacteria in many cases can lead to further infection of the root canal, which requires re-treatment or even extraction.[3]

In such scenario laser based Photo dynamic therapy (PDT) seems to be a promising addition to achieve sterilization in infected root canals of deciduous teeth. Photodynamic therapy is based on the concept that nontoxic photo sensitizers can be preferentially localised in certain tissues and subsequently activated by light of the appropriate wavelength to generate singlet

Oxygen and free radicals that are cytotoxic to cells of the target tissue.[4] Very few in vivo studies are available in the literature comparing the antimicrobial efficacy of diode laser, and conventional irrigation systems in paediatric dentistry.

Therefore, the aim of this study is to evaluate the antimicrobial efficacy of laser assisted photodynamic therapy, sodium hypochlorite (1%) and Chlorhexidine (2%) in root canal disinfection of primary teeth.

Methodology

The study was conducted in the Department of Pedodontics and Preventive Dentistry, M.R. Ambedkar Dental College and Hospital, Bengaluru, Karnataka. The samples were selected from patients coming to the department based on inclusion and exclusion criteria. The study was commenced after obtaining approval from the Institutional Ethical Committee and Review Board Based on published literature[4], the sample size for the present study was estimated using the software G Power v. 3.1.9.2 .Considering the effect size to be measured (f) at 40%,

power of the study at 85% and the margin of the error at 5%, the total sample size needed was 72, which was rounded off to 75. Thus, each group comprised of 25 samples [25 subjects x 3 groups = 75 samples].

Inclusion criteria

1. Patients who belonged to Frankel's 3 and Frankel's 4 behavioural rating scale, between the age of 4-8 years of both the sexes with no history of systemic illness and who were not under any medication.
2. Teeth with carious pulp exposure which were indicated for pulpectomy.
3. Teeth with at least 2/3rd of intact root length.
4. Teeth which were restorable.
5. Patients who wilfully gave the assent and for whom the parents or the legal guardian gave the written informed consent to be a part of this study.

Exclusion criteria

1. Teeth with preshedding or abnormal pathologic mobility.
2. Teeth with root resorption more than 1/3rd of the root length.
3. Teeth which showed radiographic signs of inter-radicular involvement ranging from slight thinning of the trabeculae to furcal radiolucency.
4. Non restorable teeth.
5. Teeth which showed perforation of the pulpal floor.
6. Patients with special health care needs.
7. Patients who were not willing or for whom the parents or the legal guardian did not give the written informed consent for the treatment.
8. Patients who were suffering from any other debilitating systemic illness.

Selected sample was randomly allocated to any of the 3 groups by using chit picking method until the sample size of 25 in each group was reached. Pre-operative radiographs were taken to assess the extension of the carious lesion. Before beginning the procedure, all the instruments were autoclaved and non- autoclavable instruments were chemically disinfected. Administration of local anesthesia (2% lignocaine hydrochloride) and isolation of tooth using rubber dam was done. Straight line access cavity preparation was done using a high speed airotor hand piece. Determination of working length was done using Ingle's method. Collection of first sample (S1) was done by introducing the sterile paper points with anatomic diameter compatible to the root canal for 30 seconds, which was transferred to the Eppendorf vial containing normal saline and stored in the refrigerator until sent for microbiological assessment.[4,5]Biomechanical preparation (BMP) was performed in the same appointment using 1% NaOCl as an irrigant in group A. (Vensons India, Sampige road, Bangalore.).[6] using 2% Chlorhexidine as an irrigant in group B. (Stedman pharmaceuticals private Ltd. Alathur, Thiruporur, Tamilnadu).[6] In group C and saline was used followed by LASER assisted Photodynamic therapy for canal disinfetion.[4] Preparation of the photo sensitizer was done by using commercially available 1% Methylene blue dye solution which was diluted to 0.005% solution using distilled water.[7] Commercially available 3% NaOCl solution was diluted (1:3) to 1% concentration using normal saline.[8] In group C, after the completion of biomechanical preparation, the access cavity was filled with methylene blue dye solution using sterile disposable syringe and was allowed to remain in the cavity for 5 minutes. Laser light from diode laser was applied for 30 seconds keeping the tip of the laser unit on each canal orifice of the tooth. (980 nm, 1.2 W power, 200µm uninitiated tip in continuous wave mode .(DenLase-980/7, power output-0.1to 7W, the DENTAL DIODE LASER SYSTEMS, from Daheng New Epoch Technology, China,Inc.(DNET).) Following this, collection of second

sample (S2) was done by introducing sterile paper points with anatomic diameter compatible to the canal for 30 seconds, which was transferred to the eppendorf vial containing normal saline and was sent immediately for microbiological assessment along with first sample (S1)[4,5] Prepared tooth was obturated in the consequent appointment followed by final restoration.

The vials containing the samples in normal saline were thawed at 36°C in a water bath and vortexed for 30–45 seconds. Undiluted sample (200-300µl) was serial diluted and serial dilutions (10^{-1} to 10^{-3}) were plated on blood agar plates (HiMedia Lab Pvt. Ltd., India), by spread plate technique. Blood agar plates were incubated at 37°C and 5%–10% CO_2 atmosphere for 5 days to cultivate aerobic and facultative anaerobic bacteria. Serial dilutions of sample were plated on blood agar plates by pour plate technique. Blood agar plates were incubated anaerobically in anaerobic chamber at 37°C for 5-10 days. Total count was determined and expressed as colony forming unit (CFU) per ml of samples.Total viable bacterial count before and after biomechanical preparation and disinfection in different groups was determined. The values were tabulated and subjected for statistical analysis.

Statistical Analysis

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses. Descriptive analysis of all the explanatory and outcome parameters was done using mean and standard deviation for quantitative variables, frequency and proportions for categorical variables.Kruskal Wallis Test followed by Mann Whitney post hoc Analysis was used to compare the Mean viable bacterial count before and after root canal disinfection procedure. Wilcoxon Signed Rank Test was used to compare the Mean viable bacterial count between before and after root canal disinfection procedure in each study group. The level of significance was set at P<0.05.

Results: Mean CFUs (x 10^2 / ml) of aerobic and anaerobic organisms among the three groups during post-operative period was statistically significant.Comparison of mean difference in CFUs of aerobic and anaerobic organisms between different groups during post-operative period showed a statistically significant difference between Group A (1% NaOCl) and Group B (2% Chlorhexidine) (P<0.001), and between Group B and Group C (Laser assisted PDT) (P<0.001).But, there was no statistically significant difference between Group A (1% NaOCl) and Group C (Laser assisted PDT)(Table 1 and Table 2).

When intra group comparison was made, statistically significant reduction in both aerobic and anaerobic CFUs was seen all the groups.(Figure 1, Figure 2 and Figure 3).

Pre and post-operative colonies on blood agar plates in chlorhexidine group.

(Figure 4a and 4b)

(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI for the Diff.		P-Value
			Lower	Upper	
Group A	Group B	29.68	13.77	45.59	<0.001*
	Group C	5.72	-10.19	21.63	0.74
Group B	Group C	-23.96	-39.87	-8.05	<0.001*

Table 1: Multiple comparison of mean difference in CFUs of Aerobic Organisims b/w groups during Post-operative period using Mann Whitney Post hoc Test

(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI for the Diff.		P-Value
			Lower	Upper	
Group A	Group B	33.76	11.74	55.78	<0.001*
	Group C	-3.08	-25.10	18.94	0.09
Group B	Group C	-36.84	-58.86	-14.82	<0.001*

Table 2: Multiple comparison of mean difference in CFUs of Aerobic Organisims b/w groups during Postoperative period using Mann Whitney Post hoc Test

Discussion

Elimination of microorganisms from infected root canals of primary teeth is a complicated task due to tortuous

canal and complex anatomy. The chances of a favorable outcome with root canal treatment are significantly high if infection is controlled effectively before obturation. Hence irrigating solutions play a key role in the success of endodontic treatment of primary teeth.[9]

According to AAPD guidelines, pulpectomy is indicated in a primary tooth with irreversible pulpitis or necrosis or a tooth treatment planned for pulpotomy in which the radicular pulp exhibits clinical signs of irreversible pulpitis (e.g., excessive hemorrhage that is not controlled with a damp cotton pellet applied for several minutes) or pulp necrosis (e.g., suppuration, purulence). Since instrumentation and irrigation with an inert solution alone cannot adequately reduce the microbial population in a root canal system, disinfection with irrigants such as one percent sodium hypochlorite and/or chlorhexidine is an important step in assuring optimal bacterial decontamination of the canals.[10]

An ideal irrigant should disinfect the root canal, dissolve necrotic tissue, flush out debris, provide lubrication, and have no toxicity. In permanent teeth, the most commonly used irrigants in root canal therapy are sodium hypochlorite (NaOCl), chlorhexidine (CHX), and chelator solutions like ethylene diammine tetra acetic acid (EDTA). The currently recommended irrigants used for pulpectomy in primary teeth are NaOCl and CHX.[11]

The capacity of a 1% NaOCl at 45°C to dissolve human dental pulps is found to be equal to that of a 5.25% solution at 20°C. Though NaOCl is a commonly used root canal irrigant, it has an unpleasant odour and taste; it does not consistently disinfect the root canal system and is toxic when extruded into the periradicular tissues. It can damage permanent tooth follicles, peripheral tissues and oral mucosa. Verma et al., in their study evaluated the effect of two different concentrations of sodium hypochlorite 5% and 1% on healing and postoperative pain after primary endodontic treatment and found that the concentration of NaOCl did not result in a significant difference in the clinical outcome which indicates that 1% NaOCl is as equally efficient as the higher concentrations with lesser toxicity. Therefore, it might be recommended to use 0.5–1% NaOCl for canal irrigation instead of the 5.25% solution. Thus we used 1% NaOCl as one of the irrigants in current study.[9, 6.]

Endodontic literature has shown that chlorhexidine (CHX) is used at 2% concentration as root canal irrigating solution. It reacts with negatively charged groups on the cell surface, thereby showing greater reduction of intracanal bacteria compared with sterile saline solution. This is thus suggested as an effective alternative to saline for pulpectomy of necrotic primary teeth.

Chlorhexidine targets cytoplasmatic membrane cells, thereby causing generalized membrane damage to the phospholipid bilayers. It affects membrane integrity and, depending on its concentration, causes a congealing of the cytoplasm. Despite the advantages of chlorhexidine, its activity is pH dependent and is greatly reduced in the presence of organic matter. Sodium hypochlorite antimicrobial activity, on the other hand, depends on the concentration of undissociated hypochlorous acid (HOCl), which exerts its germicidal effect by oxidative action on sulphydryl (-SH) groups of bacterial enzymes.[9]

According to Gomes *et al.,* .2001[12] and Vianna *et al,*. 2004[13] when tested in vitro, both substances exhibited a comparable potential antibacterial effect. But it remains unclear whether CHX is effective during chemo mechanical preparation because of its inability to dissolve organic tissues (Okino *et al,*. 2004)[14]. So, we used higher concentatrion i.e.2% CHX in one of the experimental groups for irrigation.[15]

Considering the weaknesses of common irrigants in root canal treatment, in recent years new methods such as

lasers have been introduced in order to effectively clean the root canal system. Among different types of lasers, diode laser is the most desirable type due to the properties such as high penetration depth into the dentinal tubules and good antibacterial effect.[16]

Blue dyes, especially toluidine blue and methylene blue, used with a 632.8 nm wavelength laser have shown significant results in the reduction of several in vivo bacterial and fungi cultures. The chemical reactions occur among the photosensitizer, light and substrate. By maintaining the same dose but varying the intensity or exposure time, different results can be obtained, and the effects can also depend on the photosensitizer's absorption and concentration. The efficacy of this therapy depends on the photosensitizer selectivity and its retention, the electromagnetic radiation intensity that reaches the treatment region, the optical tissue properties, the activator photons absorption efficiency, the molecular excitation energy transfer and the molecule oxidant effect.[7]

Methylene blue was used as the photosensitizer in this study because of its superior hydrophilicity along with its low molecular weight and positive charge, which allows passage across protein- protein channels in the outer membrane of Gram negative bacteria. Methylene blue whose intravenous administration is Food and Drug Administration (FDA) approved, predominantly interacts with the anionic macromolecule lipopolysacharide and results in the generation of dimers, which participate in the photosensitization process.[4]

A culture dependent approach was used, as it is one of the most reliable methods of detecting viable bacteria, particularly when samples were taken immediately after antimicrobial treatment where viability may not be ascertained by most culture-independent methods.[16] The aerobic and anaerobic colonies were counted manually to get the total CFUs .Similar microbiological protocol was used in the study conducted by Prabhakar *et al*, Pinherio *et al.*,[7,4]

In the current study, it was shown that chlorhexidine had maximum antibacterial efficacy compared to 1% sodium hypochlorite and laser assisted photodynamic therapy. Similar to studies done by Ruiz-Esparza *et al.*, [17], Louwakul et *al.*, [11],and Jolly et *al.*, [18] respectively who assessed the efficacy of 2% chlorhexidine gluconate compared to saline solution. Ruiz-Esparza *et al.*,[17] and Jolly *et al.*,[18] both reported a significant decrease, in terms of CFU/mL, in favour of chlorhexidine and Louwakul *et al.*, [11] showed overall success rates of 100%, 97%, and 93% at 6, 12, and 18 months, respectively when compared with normal saline. However, 2% concentration of chlorhexidine is generally recommended in endodontic literature for the purpose of root canal irrigation. In the present study, chlorhexidine demonstrated a broad spectrum antimicrobial action, which is similar to the results of the previous studies **Mohammadi Z *et al.*, Afzal** *et al.*, and **Haapasalo** *et al.*,[19,20,21.]

Siqueria *et al.*, conducted an in vitro study comparing the antimicrobial efficacy of different concentrations of NaOCl i.e. 1% , 2.5%, and 5.25% with saline and reported that all NaOCl solutions were significantly more effective than saline solution in reducing the number of bacterial cells within the root canal ($p < 0.05$).[22]A study by Singhal *et al.*, also reported that 1% NaOCl was more effective irrigant for debris removal in deciduous root canals when compared with NaOCl gel (1%) and Carislov.[8] Oliveira BP conducted an in vitro study to evaluate the efficacy of photodynamic therapy (PDT) and sodium hypochlorite (NaOCl 5.25% and 1%) and different combinations of both in root canal disinfection, and found that 1% NaOCl and 1% NaOCl + PDT exhibited similar antimicrobial effects.[23]. These studies are in accordance with the present

study as we also found significant results in 1% NaOCl group (Figure 1).

When laser assisted PDT was considered, comparison of mean CFUs (x 10^2 / ml) of aerobic and anaerobic organisms between pre-operative and post-operative period showed a statistically significant difference. (P<0.001)/9 (Figure 3)A study conducted by Attiguppe *et al.,* to compare the efficacy of latest advancements in disinfection techniques using diode laser(810 nm,) showed similar results (p-value<0.001) which are in accordance with the current study.[24]Ashofteh *et al.,*[25]observed a 97.56% reduction in the amount of bacteria using an 830-nm diode laser with output power of 1.5 W and claimed that diode laser can be considered as an alternative technique for root canal disinfection. In another study, Rahimi *et al.,* [26] reported that laser is less effective in root canal disinfection compared to combined use of laser and NaOCl; hence, using laser in combination with root canal irrigants was recommended. In a similar study, de Souza *et al.,* [27] concluded that laser irradiation following chemomechanical irrigation was more effective than NaOCl irrigation alone in root canal disinfection and elimination of *E. faecalis.* All these studies are in accordance with the current study where a statistically significant reduction in the CFUs was observed in the laser assisted PDT group.[16] (Figure 3).

Tennet *et al.,* reported in their study that treating root canals with sodium hypochlorite (NaOCl 3%), PDT and a combination of NaOCl (3%) irrigation and PDT caused a significant reduction of *E. faecalis* in the root canals which is similar to the results of the current study [28]

A study by Alrikaby et al., reported that Nd: YAG laser 1064 nm is less effective (if used alone) as a bactericidal agent in comparison to NaOCl 5.25% which is not in agreement with the present study. This can be attributed to concentration of NaOCl (1%) used and nonuse of photosensitizing agent by the researches.[29]

Sohrabi *et al.,* compared 5.25% NaOCl solution and diode laser in reducing the *E.faecalis* and concluded that diode laser could be considered as a complementary disinfection method in root canal treatment which is in accordance with the current study.[16]

Vianna *et al.,* compared microbial reduction after chemo mechanical preparation of human root canals containing necrotic pulp tissue when using two endodontic irrigating reagents, sodium hypochlorite (2.5% NaOCl) or chlorhexidine gel (2% CHX) which showed that using 2.5% NaOCl showed better results than 2% CHX . But in our study since we have used lower concentration of NaOCl (1%) as higher concentration has deleterious effects on periapical area of primary teeth as previously mentioned, chlorhexidine used in concentration of 2% was found to be better .[15]

The present study showed a statistically significant decrease in total CFUs when laser group was considered and these results are in agreement with the two similar studies conducted by *Pinherio et al.,* and *Prabhakar et al.,* where laser was used as an adjunct along with 1% NaOCl. Hence it can be stated that laser assisited PDT is recommended as adjunct therapy for microbial reduction in deciduous teeth with necrotic pulp. [7,4]

In the current study 2% CHX showed maximum antibacterial efficacy over 1% NaOCl and laser assisted PDT. This is in accordance with a similar study conducted by Walia *et al.,*[30]. Hence, diode laser irradiation may be a possible complimentary to existing protocols for disinfecting the root canal system.

The present study is one of the few studies which compared the conventional irrigation protocols with laser assisted photodynamic therapy in vivo in primary teeth. It was found that all the three irrigation protocols showed

good antibacterial efficacy. Regardless the type of organisms i.e aerobic or anaerobic, CHX proved to be much more efficient (Figure 2)when compared to NaOCl and laser assisted PDT. Promising results are shown in our study for laser assisted PDT group and hence it can be advocated as an alternative disinfection protocol in primary teeth.

However, further research with a larger sample size, longer follow ups and with different irrigants at varied concentrations under controlled conditions should be carried out to ascertain as to which is the best disinfection protocol for primary teeth.

Conclusion

From this study the following conclusions can be drawn:

1. 2% chlorhexidine showed maximum disinfecting properties among all the groups on both aerobic and anaerobic microorganisms.

2. 1% sodium hypochlorite and laser assisted photodynamic therapy showed comparable disinfecting properties on both aerobic and anaerobic microorganisms.

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Legend Figure

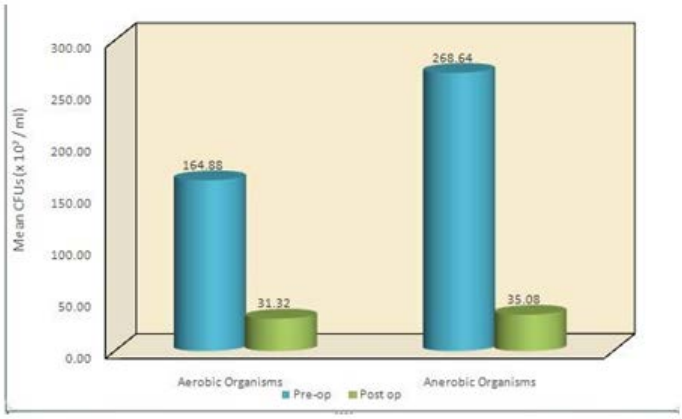

Figure 1: Figure 1: Mean CFUs (x 10^2 / ml) of Aerobic and Anaerobic organisms between Pre-operative and Post-operative period in Group A (1% NaOCl).

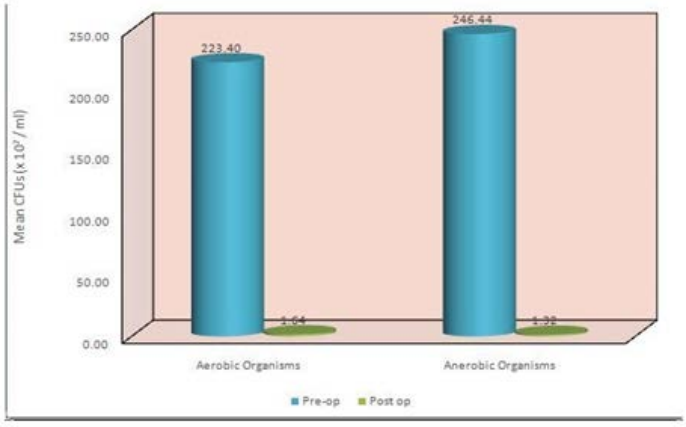

Figure 2: Mean CFUs (x 10^2 / ml) of Aerobic and Anaerobic organisms between Pre-op and Post-operative period in Group B (2% Chlorhexidine).

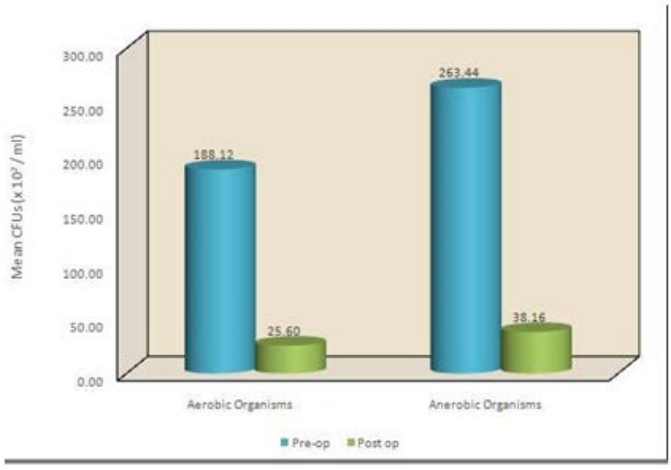

Figure 3: Mean CFUs (x 10^2 / ml) of aerobic and anaerobic organisms between pre-operative and postoperative period in Group C (Laser assisted PDT).

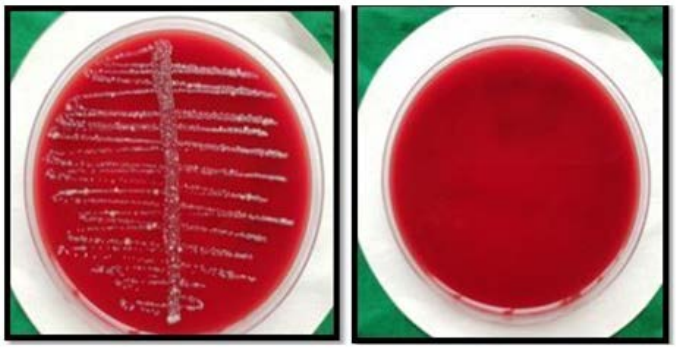

Figure 4 (a) Figure 4 (b)

Pre-operative and Post-operative colonies in Chlorhexidine group