RESEARCH ARTICLE

Comparison of the Effect of Diode Laser Irradiation and Fluoride Varnish on Salivary *Streptococcus mutans* Bacterial Colonies Counts: A Randomized Controlled Clinical Trial

Soheila Darmiani[1], Masoud Yousefi[2], Masoome Saeedi Rad[3]

Abstract

Aim: The aim of this study was to assess antibacterial effects of diode laser irradiation and fluoride varnish on *Streptococcus* (S) *mutans* bacterial colonies counts in saliva.

Materials and Methods: This was a randomized controlled clinical trial research, in which 36 healthy caries-free schoolchildren aged 7–10 years referred to Birjand University dental school were selected randomly. None of the subjects had used any fluoride products before sampling. They divided into three groups: Group A: Diode laser irradiation, Group B: Fluoride varnish (FV) + diode laser irradiation, and Group C: FV. From each child, the baseline unstimulated saliva samples were obtained, transferred to the mitis salivarius agar (MSA) culture media and assessed for *S. mutans* colonies counts. The follow-up unstimulated saliva samples were collected one day after the treatments. Then the number of colony-forming units per milliliter were counted and analyzed statistically using Wilcoxon, Mann Whitney U and Kruskal-Wallis tests ($p \leq 0.05$).

Results: No adverse events were reported. Salivary count of *S. mutans* significantly decreased in all groups. The highest and lowest number of the colonies of *S. mutans* in treated groups was observed in group I and group II, respectively. *S. mutans* was not completely eliminated by any of the treatments.

Conclusion: Considering the limitations of this study, antimicrobial efficacy of fluoride varnish + diode laser was higher than that of FV or diode laser alone. So this laser in combination with fluoride varnish may be useful in prevention of dental caries and antimicrobial treatment protocols.

Clinical trial registry: IRCTID: IRCT 201,706 181,7756N20

Keywords: Colony-forming units assay, Laser therapy, Saliva, Sodium fluoride, *Streptococcus mutans*.

International Journal of Clinical Pediatric Dentistry (2022): 10.5005/jp-journals-10005-2166

Introduction

The most common chronic infectious childhood disease is tooth caries. Epidemiological studies reported the caries prevalence was more than 80%.[1] High concentrations of organic acids can demineralize the underlying surface. *Streptococcus* (S.) mutans has been implicated as the main microorganism responsible for caries because of its ability to use carbohydrates for energy production, relatively more numbers in dental plaque and tolerance to low pH environments. A particularly aggressive form of dental caries is severe early childhood caries that affecting young children and is strongly related to *S. mutans*.[2,3]

Flouride, fissure sealants, antimicrobial agents, dietary control etc. are some methods to prevent dental caries. Fluoride is a chemical agent that can reduce plaque levels.[4] Modes of fluoride application include the following: fluoride dentifrice/mouthrinse, water fluoridation, professional fluoride application, and anticariogenic materials such as glass ionomer cement. Strong evidence exists on the caries control through therapeutic interventions like fluoride.[4-6] Fluoride is an important factor in dental decays prevention due to its effect on hard dental tissues like: enamel and dentine. Fluoride can reduce acid formation in some dental plaque bacterial species such as *S. mutans*, too.[1,6]

Diode laser has thermal and photodisruptive effects which has an antibacterial effects.[7] During laser irradiation, cell death might not occur immediately but sublethal damage included destruction of cell wall integrity and possibly the accumulation of denatured protein, inhibited the cell growth.[8] The aim of this study was to test antibacterial effects of diode laser irradiation and fluoride varnish on *S. mutans* bacterial colonies counts in saliva.

[1]Department of Endodontics, School of Dentistry, Birjand University of Medical Sciences, Birjand, Islamic Republic of Iran

[2]School of Medicine, Infectious Diseases Research Center, Birjand University of Medical Sciences, Birjand, Islamic Republic of Iran

[3]Dentist, Khaf, Razavai Khorasan, Islamic Republic of Iran

Corresponding Author: Soheila Darmiani, Department of Endodontics, School of Dentistry, Birjand University of Medical Sciences, Birjand, Islamic Republic of Iran, Phone: +98 9370554413, e-mail: soheiladarmiani@yahoo.com

How to cite this article: Darmiani S, Yousefi M, Rad MS. Comparison of the Effect of Diode Laser Irradiation and Fluoride Varnish on Salivary *Streptococcus mutans* Bacterial Colonies Counts: A Randomized Controlled Clinical Trial. Int J Clin Pediatr Dent 2022;15(S-2):S239–S241.

Source of support: Nil

Conflict of interest: None

Materials and Methods

This *in vivo*, randomized clinical trial (Clinical trial registry: IRCT 201,706 181,7756N20) was conducted among children after obtaining approval from Institutional Ethical Committee (Reference No:ir.bums.rec.1396.80). A voluntary informed consent was obtained from the parents prior to treatment and assent from all the children. All children between 7 and 10 years of age, referring

to the Department of Pedodontics Dentistry, Birjand Dental School, Iran, were examined. Visual and tactile examination of their teeth was done and bitewing radiographs were taken to assess the interproximal caries.

Exclusion criteria for selection:

- Medically compromised children or those who were on any medication
- Physical limitations
- Presence of any caries
- Presence of any intraoral infections

On the basis of the results obtained, the healthy caries-free patients were selected.

Sample Size

Cohen's method (type I error of 0.05/power of 80%) was used to calculate the sample size. In each group, the sample size was 12. The children were allocated into three groups randomly by lottery method.

The study groups were as follows:

- Group I: Diode laser irradiation applied.
- Group II: FV and diode laser irradiation applied.
- Group III: FV applied.

Saliva Collection

The children were asked to refrain from eating, drinking or tooth-brushing at least 2 hours prior to saliva collection. The unstimulated saliva was collected between 8 and 11 am. Collection of sample was done by suction method using sterile syringes. Oral hygiene instructions were explained to the children.

Diode Laser Application

A commercially available diode laser (Biolase epic10, USA) of 940–nm wavelength and a power of 5W was used in a continuous mode. The laser beam was delivered through a bleaching tip as a transmission element. The laser energy was calibrated with a power meter (Coherent; Morita Mfg. Corp., Tokyo, Japan). Distilled water cooling (30 mL/minute) to reduce the thermal effect of diode laser was applied. The laser was held perpendicular to the irradiated surface (dorsal surface of tongue, buccal, and lingual/palatal surfaces of teeth) with 1 to 2 mm distance. To prevent energy loss, the irradiation was cut 1 mm after irradiating each subject. Total time irradiation was 30 seconds.

FV Application Procedure

FV application was done for each child with the applicator tips using paint on brush technique. Prior to the application of the varnish, oral prophylaxis was done for the teeth. Teeth were dried and isolated with cotton rolls, saliva ejector, and dried with a gentle blow of air for 30 seconds. Approximately 0.1 mL of FV (Bifluorid 12, VOCO GmbH, Germany) was applied to all the teeth of children . The varnish was allowed to dry for 1 minute .Children were instructed not to rinse, eat/drink for for 1 hour after the application of the varnish and not to brush till the next morning. At the end of one day, saliva samples were collected again.

Microbiological Procedure

In this study, the saliva samples were vortex mixed and diluted in a 1:10 ratio with an isotonic saline solution. Mitis salivarius agar (MSA) (Merck Co., Germany) supplemented with 0.2 units/mL of bacitracin was prepared in the Microbiology Department of Birjand University of Medical Sciences and was used for the selective isolation of *S. mutans*. Using a standard titanium 0.001 mL titanium loop, the saliva samples were inoculated under sterile conditions on MSA media. The plates were incubated at 37°C with 10% carbon dioxide (CO_2) for 24–48 hours. The number of colony-forming units of *S. mutans* with typical colony appearance in saliva (CFU/mL) was determined using a colony counter. The identification of *S. mutans* was performed based on colony morphological characteristics on MSA media and confirmed by Gram staining and standard biochemical tests such as catalase test and fermentation of mannitol and sorbitol (Merck Co., Germany).

Statistical Analysis

Analyses were done using SPSS software version (version 25, Chicago, IL, USA). The mean ± standard deviation (SD) of *S. mutans* colony in each group were assessed and compared with the pretreatment baseline amount. Bacterial count changes were recorded. Probability values of $p < 0.05$ were set as the reference for statistically significant results.

Results

Total 36 boys completed the study. The mean age of them in the study was 7/5 ± 1/05 years. Table 1 shows the *S. mutans* count in samples of children saliva in FV, laser, and FV+ laser groups before and after treatment. Decrease in bacterial count was seen significantly on day 1. There were statistically significant differences between groups. In the FV and laser groups, reduction percent in *S. mutans* colony count was more than that in the FV and laser groups.

Discussion

S. mutans is a facultatively anaerobic, gram-positive *coccus* commonly found in the human oral cavity and is a significant contributor to tooth decay. It is able to acquire new properties allowing for the expression of pathogenicity determinants determining its virulence in specific environmental situations. It adheres to a solid surface, so it is capable of colonizing the oral cavity and forming bacterial biofilm. Additional properties of *S. mutans* is it's ability to survive in an acidic conditions and specific interaction with other microorganisms colonizing this ecosystem.[2,9] *S. mutans* cell wall is composed of highly cross-linked murein. *S. mutans* is relatively resistant to laser irradiation due to its tough cell wall. In this study, *S. mutans* were selected because they

Table 1: Comparison of *S. mutans* colony count (Mean ± SD (103 CFU/mL)) in three groups (n = 12)

p value	*FV*	*FV + Diode laser*	*Diode laser*	
< 0/001	7/8 ±3/8	3/2 ±2/8	10/05 ± 6/6	Before treatment
< 0/001	3/4 ±1/2	1/1 ± 1/1	6/8 ±4/7	After treatment
< 0/001	57/07	65/58	35/45	% of change
	0/002	0/003	0/003	*p* value

were the predominant bacteria in tooth decays[3,10] and were broadly used to evaluate the bactericidal effect of restorative materials.

One of the first media to be developed as a selective medium for culturing *S.* species in general is MSA.[2,4] In this study, MSA was used for the selective isolation of *S. mutans,* too.

An advantage of the current study was the consideration of the difference of CFU at baseline, meaning the difference may cause mistales in comparing two treatments by over/under estimating.

Various formulations of antimicrobial materials including mouthwashes, toothpastes, and gels are available. Recently, antimicrobials have been incorporated into different sustained-release systems such as varnishes. Fluoride varnishes were originally developed to prolong the contact time between fluoride and enamel. They adhere to the tooth surface for longer times in a thin layer, and prevent immediate loss of fluoride after application. The effect of varnishes on microorganisms, especially on cariogenic bacteria, has been well shown through different studies.[11,12] So in this study antimicrobial effect of fluoride varnish versus laser was assessed.

Laser light has been used in dentistry in antimicrobial therapy. Studies show that laser application can effectively reduce or eliminate pathogenic microorganisms like enterococcus faecalis, *S. mutans* etc. Different lasers have been used in related studies, but the commonly used lasers in dentistry in clinical trials including our study is diode laser.[13,14] Diode lasers are more cost-effective and user-friendly and easily transferable compared to other laser types. They are also battery operable. The possible mechanisms about the antibacterial effect of diode laser are thermal and photodisruptive effects.[7] The interaction of laser light and biological tissues is determined by various factors like: wavelength and absorption in tissue, mode of operation, energy or power output, repetition rate, application method of the laser, time of application and etc.

The operator can avoid any thermal damage on dentin by using water cooling or selecting the suitable power output.[15] Inappropriate use of laser parameters could lead to side effects such as burnt dentine. Therefore in this study the 5W was selected as the highest power to proceed the evaluation of bactericidal effect. In this study, the continuous movement of laser tip in combination with distilled water cooling could reduce the thermal effect and simultaneously reach high bactericidal efficiency. In Lee's study, *S. mutans* began to notably lose their normal cell morphology after receiving 3W of diode laser irradiation[8]; therefore in this study power of 5W was used.

Bargrizan et al. and Fekrazad et al. showed that laser was not effective for elimination of microorganisms.[13,14] In our study, reduction in bacterial count in the laser group was 35.45% which confirms the results of previous studies.

Considering the significant role of *S. mutans* in development of dental caries, our results showed that FV in combination with laser irradiation could be used as an adjunct to decrease the prevalence of caries. Future clinical studies with larger sample sizes, different light sources, and optical parameters are required to obtain results that are accurate. Further studies are also required to evaluate the effect of repeat of treatments with longer follow-ups of patients to suggest a preventive and effective protocol for children.

Conclusion

The FV in combination with diode laser irradiation can reduce the quantity of *S. mutans*. It can be an adjunct tool to prevent dental caries.

Acknowledgments

This study was supported by a grant from Birjand University of Medical Sciences, project no.11. Dr Bita Bijari is gratefully acknowledged for the statistical analysis of the manuscript.

Compliance with Ethical Standards

The study design was approved by the Ethics Committee of Birjand University (acceptance number: ir.bums.rec.1396.80)

References

1. Philip N, Suneja B, Walsh L. Beyond Streptococcus mutans: clinical implications of the evolving dental caries aetiological paradigms and its associated microbiome. Br Dental J 2018;224(4):219–225. DOI: 10.1038/sj.bdj.2018.81
2. Ahmed MI, Sabahalkheir AH, Aldebasi YH, et al. Antibacterial influence of Omega diode laser exposure durations on Streptococcus mutans. J Microbiol Antimicrob 2011;3(6):136–141. DOI: 10.5897/JMA.9000005
3. Feng L, Yan Q, Zhang B, et al. Ratiometric fluorescent probe for sensing Streptococcus mutans glucosyltransferase, a key factor in the formation of dental caries. Chem Commun 2019;55(24):3548–3551. DOI: 10.1039/C9CC00440H
4. Somaraj V, Shenoy RP, Panchmal GS, et al. Effect of herbal and fluoride mouth rinses on streptococcus mutans and dental caries among 12–15-year-old school children: a randomized controlled trial. Int J Den 2017;2017:5654373. DOI: 10.1155/2017/5654373
5. Walsh T, Worthington HV, Glenny AM, et al. Fluoride toothpastes of different concentrations for preventing dental caries. Cochrane Database Syst Rev 2019;3(3):CD007868. DOI: 10.1002/14651858.CD007868.pub3
6. de Sousa FS, Dos Santos AP, Nadanovsky P, et al. Fluoride varnish and dental caries in preschoolers: a systematic review and meta-analysis. Caries Res 2019;53(5):502–513. DOI: 10.1159/000499639
7. Khosravi JR, Moradinejad P. Investigation of bactericidal effect of low level laser of gallium-aluminum-arsenide on cariogenic species on streptococci and lactobacillus. J Med Sci 2008;8(6):579–582. DOI: 10.3923/jms.2008.579.582
8. Lee BS, Lin YW, Chia JS, et al. Bactericidal effects of diode laser on Streptococcus mutans after irradiation through different thickness of dentin. Lasers Surg Med 2006;38(1):62–69. DOI: 10.1002/lsm.20279
9. Baker JL, Edlund A. Exploiting the oral microbiome to prevent tooth decay: has evolution already provided the best tools? Front Microbiol 2019;9:3323. DOI: 10.3389/fmicb.2018.03323
10. Delfani S, Mohammadrezaei-Khorramabadi R, Ghamari S, et al. Systematic review for phytotherapy in Streptococcus Mutans. J Pharm Sci 2017;9(5):552–561.
11. Jacob B, Nivedhitha MS.Comparative assessment of the antibacterial efficacy of natural products and chlorhexidine mouthwash against Streptococcus mutans: a systematic review. J Clin Diagnostic Res 2018;12(12)1–7.
12. Erdem AP, Sepet E, Kulekci G, et al. Effects of two fluoride varnishes and one fluoride/chlorhexidine varnish on Streptococcus mutans and Streptococcus sobrinus biofilm formation in vitro. Int J Med Sci 2012;9(2):129–136. DOI: 10.7150/ijms.3637
13. Bargrizan M, Fekrazad R, Goudarzi N, et al. Effects of antibacterial photodynamic therapy on salivary mutans streptococci in 5-to 6-year-olds with severe early childhood caries. Lasers Med Sci 2019;34(3):433–440. DOI: 10.1007/s10103-018-2650-2
14. Fekrazad R, Bargrizan M, Sajad S. Evaluation of the effect of photo activated disinfection with Radachlorin against streptococcus mutans: an in vitro study Photodiagn Photodyn Ther 2011;8(3):249–253. DOI: 10.1016/j.pdpdt.2011.03.337
15. Takasaki AA, Aoki A, Mizutani K, et al. Application of antimicrobial photodynamic therapy in periodontal and perimplant diseases. Periodontol 2000 2009;51:109–140. DOI: 10.1111/j.1600-0757.2009.00302.x