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International Journal of Current Research
Vol. 9, Issue, 04, pp.49259-49262, April, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

COMPARATIVE EVALUATION OF THE ANTIMICROBIAL EFFICACY OF 5% SODIUM HYPOCHLORITE, SUBSONIC ACTIVATED SOLUTION AND DIODE LASER IN ELIMINATION OF *ENTEROCOCCUS FAECALIS* FROM THE ROOT CANAL

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ARTICLE INFO

Article History:
Received 14th January, 2017
Received in revised form
10th February, 2017
Accepted 08th March, 2017
Published online 30th April, 2017

Key words:

Sodium hypochlorite, Endoactivator, Diode laser, *Enterococcus faecalis*.

ABSTRACT

The outcome of root canal treatment is based on efficient disinfection of the root canal system and prevention of reinfection. *Enterococcus faecalis* has been found to be one of the predominant bacteria in root canal failure teeth. The bacteria appear to be highly resistant to available medicaments. Irrigants have been traditionally delivered using a syringe and a needle. The problem with this irrigation technique is inadequate replacement of the irrigant throughout the root canal system. Therefore various new devices like Endoactivator have been introduced. This device enhances hydrodynamic phenomenon by means of the subsonic activation of a passive smooth polymer tip. Another recent method to combat pathogens is the use of lasers. This study was aimed at comparing the antimicrobial efficacy of 5% sodium hypochlorite, subsonic activated solution and diode laser in elimination of *Enterococcus faecalis* from the root canal.

Citation: Dr. Jastinder Singh, Dr. Rajat Bhandari, Dr. Rajiv Tiwari, Dr. Deepika Singla, Dr. Manjot Singh, Dr. Saransh Tuli, Dr. Pardeep Mahajan and Dr. Prashant Monga, 2017. "Comparative evaluation of the antimicrobial efficacy of 5% sodium hypochlorite, subsonic activated solution and diode laser in elimination of *Enterococcus faecalis* from the root canal", *International Journal of Current Research*, 9, (04), 49259-49262.

INTRODUCTION

Normally the dental pulp is sterile and is primarily involved in the production of dentin and in tooth sensibility. But when it becomes infected, the pulp tissue reacts to the invading bacteria in an attempt to eradicate them. If the route of infection is not eradicated by these natural processes or by conservative procedures, then the burden of bacteria invading the complex overcomes the defenses and causes pulpal diseases that range from pulpitis to necrosis, thus causing infection of the pulp chamber and root canal. To remove this infection root canal treatment is done. The main objectives of root canal treatment are thorough shaping and cleaning of pulpal spaces and three dimensional obturation of these spaces with an inert filling material. Currently, many methods are being used to eradicate the pathogens from root canal system prior to obturation. It can be done by irrigating the canal using syringe and needle, use of sonics and ultrasonics with the irrigant and employing other devices like lasers). While cleaning and disinfecting some microorganisms survive harsh, nutrient-limited conditions of the root filled canal. Results of studies in which the microflora of teeth with persistant disease was studied, showed a high prevalence of Enterococci and Streptococci followed by Lactobacilli, Actinomyces species,

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Peptostreptococci, Candida (Siqueira *et al*., 2004). *Enterococcus faecalis* has been found to be one of the most predominant bacteria in teeth with root canal failure and appears to be highly resistant to medicaments used during root canal treatment. This study was done to comparatively evaluate the antimicrobial efficacy of 5% sodium hypochlorite, subsonic activated hypochlorite solution and diode laser in elimination of *Enterococcus faecalis* from the root canal.

MATERIALS AND METHODS

The present in vitro study was undertaken in the Department of Conservative Dentistry and Endodontics, Genesis Institute of Dental Sciences and Research, Ferozepur, Punjab (INDIA). A total of 112 single rooted non carious anterior teeth were selected for this study with completely formed apices and no abnormal root morphology or noticeable defects. The root surfaces of selected teeth were debrided, which were then immersed in a 5% solution of NaOCl for 1 hour and then stored in saline solution until preparation. Each specimen was horizontally sectioned to obtain a root length of 15 mm. Each root canal was preflared using K-Flexofiles (Dentsply) up to #20 and instrument sequence used was Sx instrument at two third of the working length, S1 and S2 at working length minus 1mm. Further F1 (20/.07), F2 (25/.08), F3 (30/.09) were used at working length. Irrigation was performed with a syringe using 33 millilitre of 5% sodium hypochlorite and alternating with 10 millilitre of 10% EDTA; the total irrigation time was 10 minutes per specimen. After drying with paper points, the roots were inspected under the microscope to verify the absence of cracks and canal cleanliness. Each specimen was fixed in a plastic support box. Specimens were placed in envelopes and sterilized with ethylene oxide. Sterilized roots were placed under a laminar flow biohazard cabinet. The sterilized root canals were then infected with 30 microlitre (with the tuberculin syringe) pure culture adjusted previously to optical density 0.15 at 620 nanometre to match the turbidity of 3 x 10^7 CFUs of *Enterococcus faecalis* ATCC 29212. Specimens were further incubated aerobically at 37 degree Celsius for 2 hours to allow penetration of E. faecalis into the root canal dentin.

The 100 samples were randomly subdivided into five groups (n = 20) using a random numbers table.

Group 1: Samples were irrigated for 40 seconds with 2 millilitre of a 5% NaOCl solution at room temperature with a 30-gauge needle syringe 2 millimetre short of the apex. NaOCl was left in the root canal for 15 seconds, before removal with steriler saline solution.

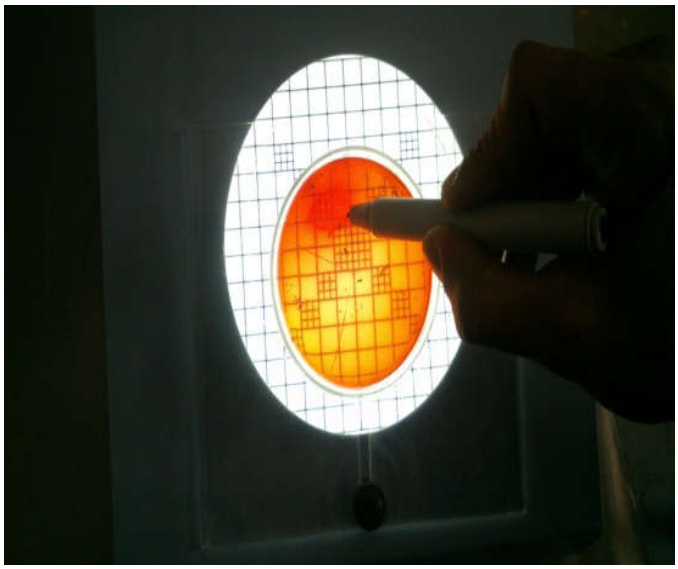
Group 2: Same procedure was followed as group 1 but NaOCl was left in the root canal for 30 seconds, before irrigating with sterile saline solution.

Group 3: Same procedure was followed as group 2 but NaOCl was placed in the root canal and immediately activated subsonically for 15 seconds, the irrigant was then removed with sterile saline solution.

Group 4: Same as group 3 but NaOCl was placed in the root canal and immediately activated subsonically for 30 seconds; the irrigant was then removed with sterile saline solution.

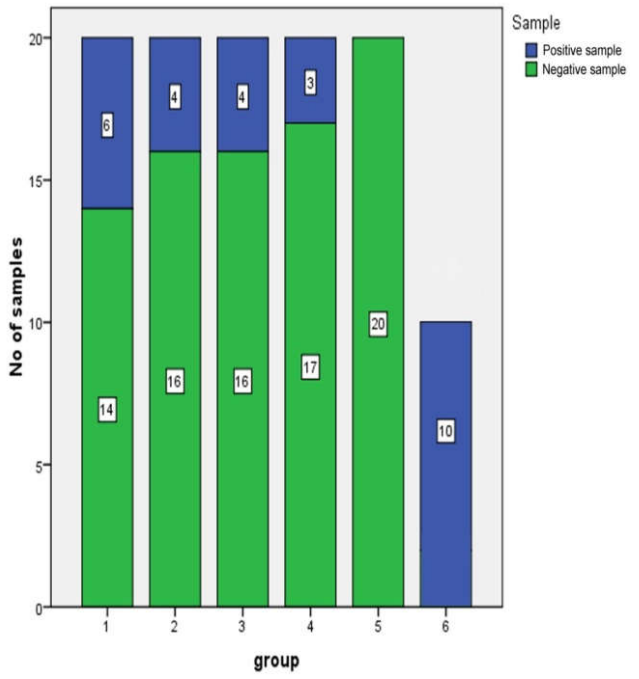
Group 5: Samples were irradiated with diode laser (Elexxion) 810 nanometre at 2 Watts for three consecutive cycles of 10 second activation and 10 second rest. Optical fibre was introduced into the canal and withdrawn in an apical to coronal direction. Root canals were then rinsed with sterile saline solution.

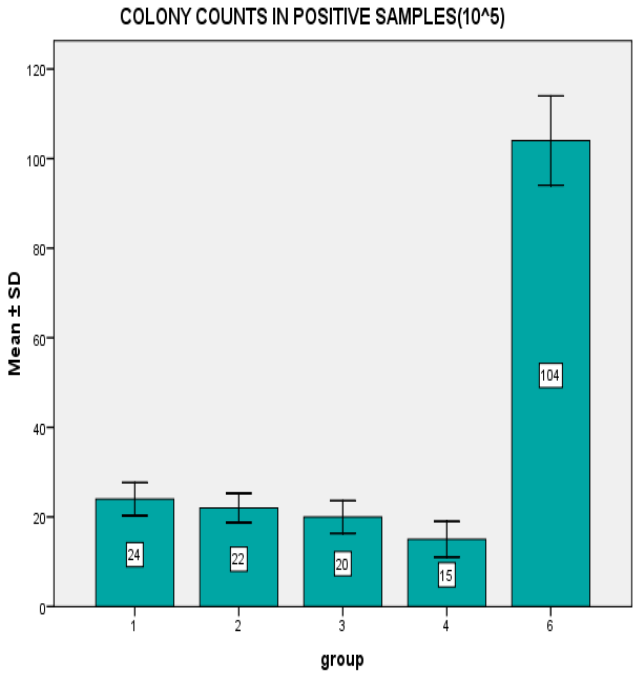
Two additional specimens were used as negative controls and ten additional teeth served as positive controls. Subsequent to each irrigation treatment, the root canals were dried at working length and sampled with sterile paper points. The paper points were transferred to tubes containing 1 millilitre of 0.85% saline solution and vortexed for 1 minute. After 10-fold serial dilutions, aliquots of 0.1 millilitre were plated onto brain-heart infusion medium agar and incubated at 37 degree celsius under aerobic conditions for 24 hours. The colony-forming units (CFUs) grown were counted and then transformed into actual counts based on the known dilution factors.



RESULTS

Results showed that the root canals containing E. faecalis irradiated with diode laser, disinfected the root canals most efficiently followed by sodium hypochlorite agitated with Endoactivator for 30 seconds (EA 30), sodium hypochlorite agitated with Endoactivator for 15 seconds (EA 15), irrigation with sodium hypochlorite for 30 seconds (NaOCl 30), irrigation with sodium hypochlorite for 15 seconds (NaOCl 15) in decreasing order of efficacy.





DISCUSSION

Elimination of microorganisms from the root canal has always been an important part of endodontic therapy. It is established through chemomechanical preparation, irrigation and intracanal medication. Irrigation play a central role in endodontic treatment. But certain microbes show resistance to the irrigants and regimens, one such being *Enterococcus faecalis*, same is the reason for its selection in this study. Enterococci are gram positive, non spore forming and facultative anaerobic cocci. Enterococcal cells are ovoid and can occur singly, in pairs or as short chains. Enterococci can grow at temperatures ranging from 10-45 degree celsius at pH 9.6 and in 6.5% NaOCl. Enterococci possess a number of virulence factors that permit adherence to host cells and cause toxin- mediated damage. Brain Heart Infusion Medium is useful for cultivating a wide variety of microorganisms since it is a highly nutritive medium. This medium was preferred in this study as it is nutritious and well buffered to support the growth of wide variety of organisms including *E. faecalis.* Irrigants act as lubricant and cleaning agent, removing microoganisms, their products associated with tissue degeneration and organic and inorganic remains, thus guaranteeing elimination of tissue and permeability of the canal throughout its length. (Mozo *et al*., 2012) Several irrigants and irrigation systems are available, all of which behave differently and have relative advantages and disadvantages. Common root canal irrigants include sodium hypochlorite, chlorhexidine gluconate, alcohol, hydrogen peroxide and ethylene diamine tetra acetic acid (EDTA). In selecting an irrigant and technique, consideration must be given to their efficacy and safety (Glassman *et al*., 2011). Berber *et al*. 2006, found that 5.25% NaOCl is the most effective irrigant solution among 0.5%, 2.5% and 5.25% concentration of sodium hypochlorite available, So 5% NaOCl was used. The antibacterial effect of NaOCl is time-dependent. For shorter periods of time, the bactericidal effect of NaOCl was found to be lower (Harrison *et al*., 1981, 1990). The bactericidal ability of NaOCl results from formation of hypochlorous acid and sodium hydroxide, when in contact with organic debris. After reaction it gets consumed and become ineffective over a period of time. Group 4 depicted slightly better results with respect to decrease in colony forming units count than group 3 and group 2 better than group 1 in conformity with the findings of Pasqualini *et al*. 2010.

Group 3 and 4 showed greater reduction in colony forming units count than group 1 and 2 which is in agreement with, Pasqualini *et al*. Safety is an important parameter. Among those which produced extrusion of irrigant, EndoActivator extruded statistically significantly less irrigant than Manual, Ultrasonic, and Reinsendo (Desai and Himel, 2009). However some amount of extrusion could occur, which is not the case with the diode lasers. The laser employs wavelength which is within the infrared range. Thin and flexible fibers can be used, which reach the apical third of root canal (Gutknecht *et al*.). Diode laser induce, short-term localized heating of the bacterial microenvironment to lethal temperatures. (Moritz *et al*). Laser light penetrates up to > 1000µm into the dentin, thus has a scope for complete canal sterilization. (Bhatia and Kohli, 2013). Present study depicted that group 5 had significantly greater reduction in colony count as compared to all other groups. Group 5 showed a complete elimination of E. faecalis as established by (Schoop *et al*., 2004)

Conclusion

In the present study it can be concluded that:

(1) All methods were able to reduce the microbial counts though not to the same level.
(2) With the use of Endoactivator for 30 seconds, the results were slightly better as compared to its use for 15 seconds, followed by sodium hypochlorite alone for 30 seconds, followed by sodium hypochlorite for 15 seconds in decreasing order of efficacy.
(3) Superior results were obtained by diode laser as compared to the various root canal disinfection techniques against *Enterococcus faecalis* with complete eradication of micro organism from the root canals.

Conflict of interest

Authors declare no conflict of interest associated with this publication.

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