

Comparative Evaluation of Isthmus Cleaning Ability of Photodynamic Therapy Versus Sonically and Ultrasonically Activated Sodium Hypochlorite: An in Vivo Study

Bhupesh Gandhi^{1*} Atiksha Sharma² Gyan Prakash³ Chinmay Vyas⁴ Arka Jyoti Chakraborty⁵
Kavil Veetil Swarna⁶

¹Senior Lecturer, Department of Conservative Dentistry and Endodontics, Surendera Dental College and Research Institute, Sri Ganganagar, Rajasthan, India.

²PG Student, Department of Conservative Dentistry and Endodontics, Maharaja Ganga Singh Dental College & Research Centre, Sri Ganganagar, Rajasthan, India.

³Senior Lecturer, Department of Conservative Dentistry and Endodontics, Vyas Dental College, Jodhpur, Rajasthan, India.

⁴Senior Lecturer, Department of Conservative and Endodontics, Yogita Dental College, Khed, Maharashtra, India.

⁵PG Student, Department of Conservative Dentistry and Endodontics, Maharaja Ganga Singh Dental College & Research Centre, Sri Ganganagar, Rajasthan, India.

⁶PG Student, Department of Conservative Dentistry and Endodontics, Maharaja Ganga Singh Dental College & Research Centre, Sri Ganganagar, Rajasthan, India.

ABSTRACT

Aim: Comparative evaluation of isthmus cleaning ability of photodynamic therapy versus sonically and ultrasonically activated sodium hypochlorite: *an in vivo study*.

Materials and Methods: A total of eighty-eight non vital teeth were selected. After biomechanical preparation teeth were randomly divided into 4 groups for final irrigation and disinfection. Group1 - Control group (n=22): No irrigant activation. Group 2 - Sonic irrigation group (n=22). Group 3- Passive ultrasonic irrigation group (n=22). Group 4- Photoactivated disinfection group. The sterile cotton pellet was placed in the pulp chamber and the pulp chamber was sealed with temporary cement followed by immediate extraction. Teeth were stored in formalin and sent for histological preparation. Sectioning off each group's slides was performed and stained. Blinded and mounted sections of all the groups were evaluated using a stereomicroscope at 40X/100X/400X magnification.

Result: Mean cleanliness values of the isthmus at different levels (1,3 and 5mm) levels evaluated and comparison of isthmus cleanliness were evaluated. There were significant differences between cleanliness in all groups at different levels. The highest cleanliness was found in group 3 (ultrasonic) ($p < .000$).

Conclusion: From the results of this dissertation, it was concluded that the poultra tips gave the better result.

Keywords: Sodium hypochlorite, isthmus, endoactivator, ultrasonics, fotosan.

INTRODUCTION

The Endodontic therapy aims at removing all vital or necrotic tissue, microorganisms and their by-products from the root canal system.¹ The intricate nature of root canal anatomy has complicated the complete debridement of all areas of the root canal.

Isthmuses, fins, webs, anastomoses, and other irregularities within the root canal often harbour tissue, microbes and debris following instrumentation. Root canal irrigation is a fundamental component for successful endodontic

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*Correspondence Dr. Bhupesh Gandhi.

Department of Conservative Dentistry and Endodontics, Surendera Dental College and Research Institute, Rajasthan, India.

Email: bhupesh.gandhi30@gmail.com

treatment.² The contemporary nickel-titanium instruments only act on the central body of the canal, leaving canal fins, isthmi, and cul-de-sacs and approximately 35% of the canal untouched even after completion of the preparation. Previous studies have suggested that the cleaning of the root canal space is more dependent on the type of irrigation than on the method of instrumentation.³⁻⁸ The presence of apical ramifications, lateral canals and apical deltas makes it complicated to achieve this goal.⁹ Conventional irrigation methods produce less effective results apically.⁷ To obtain optimal results, an irrigation method must also be effective at the working length (WL). Functions of an irrigant include physical flushing action to remove debris, dissolution of tissue remnants, removal of smear layer and inactivation of intracanal bacterial biofilms and their endotoxins.¹ No presently available single irrigant is able to achieve these desired goals. Hence there is a need to activate them to improve their efficacy. Cavitation and acoustic streaming of the irrigant contribute to the biologic, chemical activity for maximum effectiveness. In the present study endoactivator, ultrasonics and PAD (fotosan) were used to investigate the effect of these activators on endodontic pathogens in vivo conditions.

MATERIAL AND METHOD

Source of data

Patients with eighty-eight non vital teeth participated in this study. Two rooted premolars and mandibular molars of both male and female patient of the age range of 15-50 years, with informed consent selected for the study. Patients were recruited from the regular pool of patients presenting to the Department of Conservative Dentistry and Endodontics, in accordance with specific inclusion and exclusion criteria's.

Methodology

METHOD OF COLLECTION OF DATA

The present study was conducted on patients reporting to the Department of Conservative Dentistry and Endodontics, Jodhpur Dental College General Hospital, Jodhpur. The Human Subjects Review Committee of the Jodhpur National University approved the study and written informed consent was obtained from each subject.

The subjects were in good health as determined by written and oral questioning.

ENDODONTIC PROCEDURE:

A total of eighty-eight (88) teeth were selected according to the above-mentioned criteria for the study taking into account 10% dropouts if present. Preoperative radiographic examination was made before proceeding with the actual procedure. Clinical examination by cold testing (Endo ice, Coltene Whalident, Germany) and heat testing with gutta-percha sticks (Prime Dent Pvt. Ltd Mumbai), Electric pulp Tester (Parkell) and percussion testing of all the teeth were carried out. Nonvital teeth as diagnosed clinically and radiographically were selected for the study. Prior to initiation of the study, random six-digit numbers were recorded on a master code list corresponding to the experimental groups. The master code list was randomly assigned to subjects allotted to a particular group. This master code list was consulted only after completion of the study to eliminate operator bias.

CLINICAL PROTOCOL

Each tooth to be treated was anaesthetized and isolated with a rubber dam. Pulp chambers were opened under aseptic conditions with distilled water-cooled high-speed diamond points. Working length was estimated by an apex locator (Root ZX mini, J Morita J MFG. Corp. Kyoto, Japan) followed by confirmation with an intraoral periapical radiograph. Mesial Canals of mandibular molars and buccal and palatal canals of maxillary premolars were enlarged to appropriate size X2 with rotary NiTi files-Protaper Next and EDTA (RC Help, Prime Dental Products Ltd, Thane) following manufacturer's instructions. Prior to the use of rotary files, root canals were explored with size 15 K-files to avoid overloading of NiTi instrument tips with unexpected canal curvatures or excessive wall contact. Irrigation was done with 3% sodium hypochlorite (NaOCl) (V-Concept, Vishal Dentocare, India) with 30-gauge side-vented irrigation needles (R C Twents irrigation needles, Prime Dental Products Pvt Ltd, Mulund Mumbai). The needles were placed 1 mm short of the working length. On completion of cleaning and shaping the teeth were randomly divided into 4 groups for final irrigation and disinfection protocol:

Group1 - Control group (n=22): No irrigant activation.

Group 2 - Sonic irrigation group (n=22): Agitation with EndoActivator

Group 3- Passive ultrasonic irrigation group (n=22): Agitation with ProUltra tips.

Group 4- Photoactivated disinfection group (n=22): Activation with FotoSan

Group 1 consisted of 22 teeth in which no additional irrigant activation was done and acted as a control group. After completing the process, canal were dried.

Group 2 consisted of 22 teeth which were agitated with a sonic device (EndoActivator, DentsplyMaillefer, Ballaigue, Switzerland) at 10000 CPM for 20 seconds.

Group 3 consisted of 22 teeth which were agitated with ProUltra tips (Dentsply, Tulsa Dental Specialities) along with ultrasonic unit (SatelacActeongroup, Merignac, France) for 1 minute per canal. The energized ultrasonic needle was used continuously for 1 min per canal.

Group 4 consisted of 22 teeth in which canals were filled with 2.5ml of photosensitizer – Toluidine Blue –0.01% and irradiated with FotoSan (CMS Dental Copenhagen, Denmark), 630 nm, 2000 mW/cm² for 30 seconds just short of binding.

After completion of the final disinfection protocol a sterile cotton pellet was placed in the pulp chamber, and the pulp chamber was sealed with temporary cement (Orafil G, PrevestDenpro Limited, Jammu, India). This was followed by immediate extraction of the teeth. The canal was irrigated with 1ml 3% NaOCl to remove any possible pieces of temporary filling material or blood. Any remaining pulp tissue was fixed by irrigation of the canal with 1 ml of 10% formalin. 0.5 mm vertical groove was placed on the buccal surface of the mesial root of mandibular root with # ½ round bur. Teeth were stored in a 20 ml vial of 10% formalin till the teeth were sent for histological processing and labelled with a random six-digit number .

HISTOLOGIC PREPARATION

Following fixation for 1 month, all teeth were decalcified in an aqueous solution of equal parts 50% formic acid and 20% sodium citrate for 10 days. Teeth were then dehydrated. This was followed by placement of the specimen in an embedding boat containing Paraffin wax. This was followed by sectioning of the specimen (5-micron sections). Sections were collected until the apical foramen was located. Starting at the 1mm level from the apical foramen, slides were prepared of 4 sections every 0.2mm until the 3mm level was reached. Slides were stained and the best, technically error-free section was chosen from each stained slide for evaluation.

METHOD OF EVALUATION

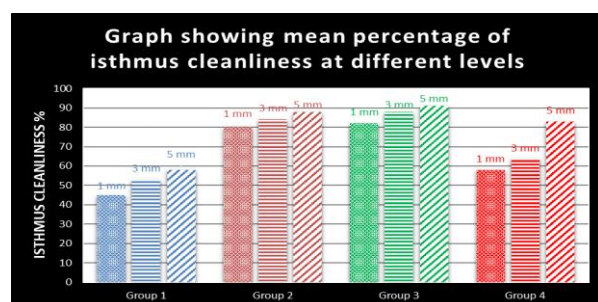
Blinded and mounted sections of all the groups were evaluated using a stereomicroscope (Laurence and Mayo). Necrotic debris in the isthmus area was calculated at 40X magnification. Percentage of remaining debris was calculated by dividing the area of necrotic debris present to a total area of the isthmus. The percentage of debris removal was determined by subtracting the percentage of remaining debris from 100%. The following structures were identified by the following colours: gram-negative organisms pink to red; gram-positive organisms-blue; nuclei-red; filaments of Nocardia and Actinomyces- blue. These colours helped in identifying bacteria from tissue, but the type of bacteria present in each section was not recorded. The isthmuses between canals were traced separately from the primary root canals, and their total area and the area of their remaining. Bacterial biofilm/necrotic debris recorded as described above. Tracing both the mesiobuccal and mesiolingual canals differentiated the boundaries between the canals and the isthmuses.

RESULT

Mean cleanliness values of the isthmus at different levels (1, 3 and 5mm) are shown in table1 and comparison of isthmus cleanliness is shown in graph 1 and figure 1-4. There were significant differences between cleanliness in all groups at different levels. There was a higher cleanliness at 5 mm level. The highest cleanliness was found in group 3 (PUI) (p < .000).

Table1: Mean percentage of isthmus cleanliness at three different levels:

Levels	Numbers	Group 1	Group 2	Group 3	Group 4
1 mm	22	45.3±35.6	80.3±26.4	82.8±17.8	58.5±30.5
3 mm	22	53.5±27.0	84.6±17.0	88.1±14.3	64.5±27.5
5 mm	22	58.6±30.2	88.1±18.5	91.5±14.9	83.0±27.5



Graph 1: Mean percentage value of isthmus cleanliness in all groups at 1,3,5 mm levels.

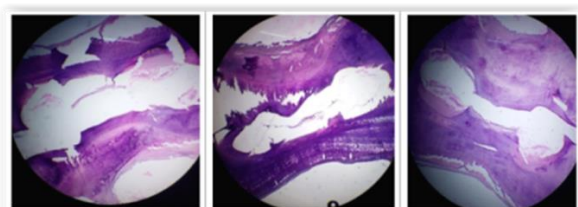


Fig 1: Group 1(Control) Microscopic view of isthmus cleanliness at 1, 3, 5mm levels respectively (P< .000).

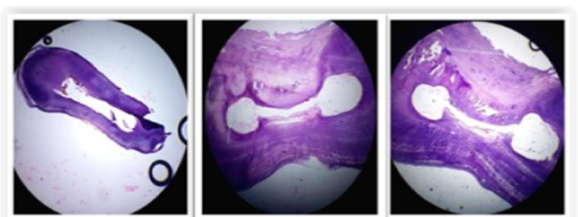


Fig 2: Group 2(Sonic irrigation):Microscopic view of isthmus cleanliness at 1, 3, 5mm levels respectively (P< .000).

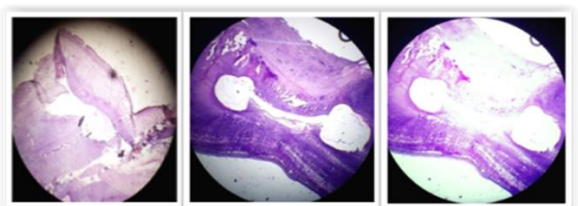


Fig 3: Group 3(Passive ultrasonic irrigation) Microscopic view of isthmus cleanliness at 1, 3, 5mm levels, respectively (P< .000).

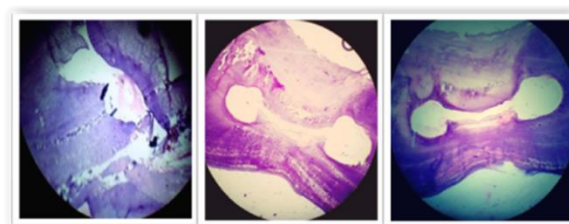


Fig 4: Group 4 (Photoactivated disinfection) Microscopic view of isthmus cleanliness at 1, 3, 5mm levels respectively (P< .000)

Statistical Analysis

Annova test was applied to find out the association of different groups with respect to debris removal ability .

Comparisons between the groups were carried out by **Post Hoc Test** .

P-Value <.05 shows a significant difference.

DISCUSSION

An isthmus is a narrow ribbon-shaped communication between two root canals which contains pulpal tissues. Isthmus has been found in mesial as well as in distal roots of mandibular and maxillary molars. The incidence of the isthmus is higher in 3-5 mm apical area.⁹ In our studies isthmus prevalence in the mesial roots was found to be 58% (1 mm level), 73% (3 mm level) and 88% (5 mm level). Higher prevalence of isthmus cleanliness was found at 3 and 5 mm apical level as compared to 1 mm level. Partial isthmus and no isthmus were found in some cases. A partial isthmus was classified as a narrow projection of one root canal opening toward the second in the same root section but not merging. An important implication of isthmus is the presence of tissues and microbial debris. Therefore isthmus cleaning is an important aspect. The flushing action of irrigating solutions can be enhanced by different irrigation systems. In this research, the irrigation systems used were ultrasonic (PUI), EndoActivator and Fotosan. Plain hypochlorite was used for irrigation which showed the least cleanliness. we used the 3 % concentration of NaOCl in the thesis to prevent the cytotoxicity and harmful effect beyond the apex and according to some articles, the concentration used along with agitation techniques was 3%. 3 % concentration of

NaOCl has been described as complete removal of the smear layer with agitation devices.

Chemical disinfectants penetrate only by 100 μ into the dentine, as indicated by Berutti *et al.*¹⁰ This could be the reason for the lower antibacterial efficiency and isthmus cleanliness in this study in group 1. Hence it was the reason that isthmus cleanliness was less at 1mm level as compared to 3 and 5mm levels. In this thesis, we used EndoActivator, PUI and Fotosan. More significant contact of the irrigation solution with the walls is possible by activated streaming. The EndoActivator device is a form of active irrigation and was found by Burleson *et al.*¹¹ When cavitation bubbles are produced by acoustic waves, they eventually collapse and the energy released is transferred to the root canal and the walls, detaching debris found within, which could be the reason for the reduction of bacteria within the isthmuses in the present study. Ultrasound creates a higher speed and flow volume of the irrigant in the canal and isthmus area during irrigation, thereby eliminating more debris, producing less apical packing, a better access of the chemical product to accessory canals and even the flush effect produced by ultrasound but not manual irrigation. Although PUI and Endoactivator both have streaming action still, PUI was good in isthmus cleanliness than the Endoactivator. Because sonic irrigation is somewhat different from ultrasonic irrigation because it operates at a lower frequency. For sonic application, the frequencies range from 1 to 10 kHz, which is lower as compared to ultrasonics (25-30 kHz). PAD is not only effective against bacteria, but also against other micro-organisms including viruses, fungi, and protozoa.^{5,12} Many studies have shown its efficacy in endodontics.

In 2006 Williams *et al.* measured the antibacterial action of PAD on different endodontic bacteria (*Fusobacterium nucleatum*, *Peptostreptococcus micros*, *Prevotella intermedia* and *Streptococcus intermedius*) in planktonic suspension and in root canals, and concluded that PAD kills endodontic bacteria at statistically significant levels.¹³

Methylene blue alone (PS) fully eliminated all bacterial species with the exception of *E. faecalis* (53% killing); methylene blue in combination with red light (PAD) was able to eliminate 97% of *E. faecalis* biofilm bacteria in root canals using an

optical fibre with multiple cylindrical diffusers that uniformly distributed light at 360 degrees. FotoSan is one of the most recent PAD devices introduced in endodontics, so there are a limited number of works on it in the literature as of today.

In contrast with manufacturer's instructions and with the results reported by Schlafer *et al.*, our study demonstrated that FotoSan used for a longer time (90 seconds vs 30 seconds) showed significantly higher bacterial reduction percentages *in vitro*. In our study, NaOCl is still the most effective irrigating solution in killing endodontic pathogens than the fotosan. In this thesis agitation of NaOCl with PUI gave the best result because the PUI has greater frequency and oscillations compared to the EndoActivator. The action of sodium hypochlorite to dissolve organic material was increased when passive ultrasonic irrigation was performed, due to the agitation itself, and the temperature increase promoted by the ultrasonic waves.¹⁴ In our dissertation, ultrasonic agitation gave the best cleaning ability among the other groups. Control group gave the least cleanliness. According to this dissertation, there may be the following reasons: Less in-depth penetration of needle was there in control group so there was less flushing action of debris through the needle so more debris at 1mm level. We used 3% NaOCl which is sufficiently compelling when agitated. More the concentration with agitation may harm the tissues so to prevent the tissue injury by agitation heat of NaOCl it was decided to use 3%. And in the control group, there was no agitation. So the drawback of this concentration was less isthmus cleanliness. Though 5.25% concentration of NaOCl is considered as an ideal irrigant in the control group but we used 3 % to prevent any accidental damage risk by 5.25%.¹⁵

It was also observed in some samples that there was no significant difference between isthmus cleanliness due to narrow isthmus. So some samples of each group showed the almost same cleanliness at 1 and 3 mm levels. At last, it is concluded that it is substantiated in many types of research showed that 1-minute activation of NaOCl is enough for removal of smear layer. And the same value of time was used by us in the ultrasonic group so that may be another important reason for us that we got the best cleanliness in the ultrasonic group ($P < .000$).

CONCLUSION

It was observed that in this dissertation, the ultrasonic gave better cleanliness among all the groups due to high frequency and higher acoustic streamings. Although Fotosan is a new invention but still it gave less cleanliness as compared to ultrasonic. Though with the help of Fotosan activation of methylene blue dye was done rather than the sodium hypochlorite so that was the reason for less isthmus cleanliness of group 4 (photoactivated disinfection). After microscopic evaluation, it was found that there was no significant difference at 1mm levels of all groups but higher significant difference was observed at 3 mm and 5 mm levels. From the results of this dissertation, it was concluded that the poultra tips gave a better result (isthmus cleanliness) at 3 and 5mm levels after canal preparation as compared to other groups. Clinically PUI can be useful as a successful additional irrigation procedure and 1-minute use of ultrasonically activated irrigation has been shown to improve the canal and isthmus cleanliness in terms of necrotic debris removal. This data can only be noted as anecdotal, and further study would be required to confirm the results obtained and expand on these observations.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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