Reduction in Bacterial Loading Using MTAD as an Irrigant in Pulpectomized Primary Teeth

Farhin K* / Viral PM** / Thejokrishna P*** / Sajjad M****

***Objective**: The aim of the present study was to evaluate the reduction in bacterial loading uing MTAD as an irrigating solution in pulpectomized primary teeth. **Study design**: A randomized, controlled clinical trial was performed that included primary teeth with pulp necrosis. Sixty necrotic canals were included, 30 irrigated with MTAD (experimental group) and 30 with 1% Na0Cl solution (control group); in all cases, 2 microbiological samples from within the canals were taken with sterile paper points, the first after the canal opening and before the first irrigation, and the second after instrumentation and final irrigation, before obturation. All samples were evaluated by Agar plate method. **Results**: The results were statistically analyzed by student 't' test. After analyzing samples before and after irrigation in the control group (NaOCl), we found a significant decrease of bacterial load (p = < 0.001). The same occurred in the MTAD group samples (p = < 0.001). When both groups were compared post irrigation, a statistically significant difference was observed in favor of MTAD. **Conclusion**: MTAD can be suggested as an alternative irrigant for pulpectomy of necrotic teeth.*

Key words: pulpectomy, MTAD, 1% Na0Cl

INTRODUCTION

One of the fundamental steps in a pulpectomy treatment in primary teeth when compared to permanent teeth (root canal therapy), is the bio – disinfection of the pulp root canal system by means of mechanical instrumentation and profuse irrigation. the rationale to irrigate is a) removal of the remains of inflamed or infected pulp tissues, shavings of dentin debris (smear layer), blood exudates, food and medications, b) instituting detergent, antiseptic and bleaching action and c) hydration & lubrication of the canal walls during instrumentation.[1-4]

Mainly, the perceived difficulty of behavior management and uncertainty about the effects of root canal filling material and instrumentation on the succedaneous teeth, anatomic situations like the often complicated, curved and tortuous root canals and closeness of the advancing tooth buds, make the treatment more difficult.[5] Despite the outstanding advancement reached in all fields of dental research, search for the ideal irrigant solution still challenges endodontics and thereafter great effort has been focused on assessing the potential of different substances for root canal irrigation.[6] Conventional root canal treatment includes mechanical instrumentation in combination with antimicrobial and tissue solvent irrigation to dissolve and dislodge debris, and create a clean environment compatible with periapical health. In the case of primary teeth, extensive dentin removal is probably undesirable, placing greater emphasis on irrigants for cleansing. Sodium hypochlorite is most widely recommended endodontic irrigant because of its excellent tissue solvent and antimicrobial properties in a concentration between 0.5% and 5.25%,[7] but it is known to cause serious damage when allowed to enter periradicular tissues even in small amounts.[8,9]

On the other hand , *MTAD* (a mixture of tetracycline isomer, an acid and a detergent) has been introduced by Torabinejad and Johnson[10] as the disinfection of the root canal system.[11] It is an aqueous solution of 3% doxycycline, a broad spectrum antibiotic; 4.25% citric acid, a demineralizing agent; 0.5% polysorbate 80 detergent (Tween 80).[12] Studies have reported MTAD to be clinically effective[13] and biocompatible when used as an endodontic irrigant.[14]

Because of these properties, MTAD is **considered as intracanal irrigant** in the endodontic treatment of permanent teeth, although its potential for use in pulpectomized primary teeth has not been completely evaluated. Therefore, the aim of this study is to evaluate the reduction in bacterial loading using MTAD as an irrigating solution in pulpectomized primary teeth.

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MATERIAL AND METHOD

The present study was carried out in the Department of Pedodontics and Preventive Dentistry, Terna Dental College, Navi Mumbai, India. The study was approved by the ethics committee of the same institution. This controlled, randomized clinical trial included 60 necrotic canals from both sexes between 3 and 7 years old after obtaining a written consent from their respective parents/ guardians.

Inclusion criteria were as follows

- Patients in good general health
- Primary teeth (posterior) containing at least one necrotic pulp canal, abscess, or sinus tract.
- Carious lesion without direct exposure to the oral environment
- Presence of radiolucent area in furcation or periapical region.
- At least two thirds of root remaining.
- Sufficient tooth structure to support a rubber dam.

Patients who had received antibiotics up to 2 weeks prior to the sampling or having any systemic compromise, non restorable teeth, perforated pulpal floor, excessive mobility, or pathological root resorption were excluded. Sixty canal treatment were performed in necrotic primary teeth: 30 belonged to the experimental group, which were irrigated with MTAD (Dentsply Tulsa Dental, Johnson City, TN), and 30 to the control group, which were irrigated with 1% NaOCl solution. Commercially available 3% NaOCl solution was diluted 1:3 to 1% concentration using tap water.[15] All treatments were performed in a single visit. Sample size was calculated on the basis of a pilot study, consisting of 10 microbiological samples taken from necrotic primary root canals (5 corresponding to each irrigating solution), which were not included in the statistical analysis. Likewise, consistency and reliability tests for the diagnostic and results evaluator were carried out in an independent manner by means of an unweighted Kappa test, which resulted in a score of 0.90.[16] The sampling of patients was realized non probabilistically and the irrigant selected for each case was made from a list of random numbers generated from computers.

Pre reduced thioglycolate tubes, supplemented with hemin (5mg L-1)and menadione (1mg L-1) Oxiod LTD, Basingstoke, Hampshire, UK], were used as transport and growth media owing to either capacity to maintain the vitality of sampled bacteria.[17]

Isolation and operative field disinfection

The study procedure was performed by a single pediatric dentist, periapical radiographs of the selected teeth were taken using a standard paralleling technique. After antisepsis of the oral cavity wherein patients were asked **to rinse with 0.12% chlorhexidine for 60 seconds**, local anesthesia was induced using an inferior alveolar nerve block for the primary mandibular teeth and infiltration [buccal and palatal] for the primary maxillary teeth. Each treated tooth was cleaned with pumice and isolated with a rubber dam. Provisit (Casa Idea,Mexico) was placed along the tooth rubber dam interface to prevent leakage of saliva into the operative field. To disinfect the operative field, we followed the following protocol ,[18,19] briefly the tooth crown, surrounding rubber dam, and clamp were swabbed with 30% H_2O_2, followed by 5.25% NaOCl for 1 minute each; both solutions were inactivated with 10% sodium thiosulfate.

The gross carious tissue was removed with a sterile round number 3 carbide bur cooled with sterile saline solution. The cavity and field were again disinfected as above. Then the pulpal roof was removed using a new bur of the same size, a sterile cotton pellet was placed on the floor of the pulp chamber to prevent penetration of disinfectants into the canals and the root canal was accessed.

Once the canals were exposed and after the canal's length was estimated using the preoperative periapical radiograph, the first microbiological sample was obtained from inside the canal (pre irrigation), then 3 sterile absorbent paper points of a size compatible with the root canal diameter were sequentially placed for 30 seconds. If the canal was dry, then a small amount of sterile saline was used to wet the canal before the points were inserted. The retrieved paper points were immediately placed into the tube with thioglycolate. After sample collection, all teeth were treated conventionally. The usual instrumentation was done with FlexoFiles (Dentsply, Switzerland), together with one irrigation of 0.5ml of the selected solution between each file. At the end of the instrumentation and before obturating, the canal was irrigated for the last time and dried. At that time, a second microbiological sample was taken from the same canal, as previously described, with another 3 paper points. Finally, the canal was filled with an iodoform paste (Vitapex) and a postoperative intraoral periapical radiograph was made.

Laboratory procedures

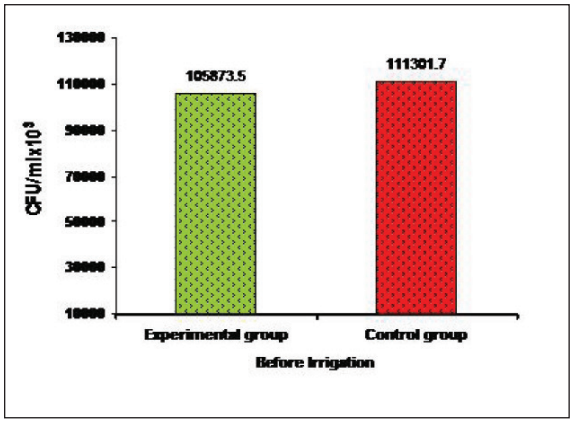
1ml of broth was plated on Soyabean Caesin Digest Agar medium [HIMEDIA^R] and subjected to anaerobic incubation at 37^0 C for 72 hours. The total microbial load per milliliter was determined by measurement of the number of CFU on Trypticase soy agar (Oxoid) containing 1 µg of menadione ml^{-1}, 0.5 µg of hemin ml^{-1}, 400 µg of L-cysteine ml^{-1}, and 5% horse blood (Amyl Media, Kings Langley, New South Wales, Australia).[20]

Intra group and inter group comparisons [pre irrigation sample versus post irrigation samples] were performed. Student ' t' test (paired) has been used to analyze the data regarding microbial load before and after irrigation, whereas unpaired 't' test has been used to compare the microbial load between experimental and control group.[21]

RESULTS

In all, 60 canals, treatment were performed on the pediatric patients, whose average age was 5 years. Of these, 120 microbiological samples were obtained: 60 from the experimental group (30 pre and 30 post irrigation) and 60 from the control group (30 pre and 30 post irrigation). Basal conditions (pre irrigation) were similar in both groups in relation to the amount of bacteria present in necrotic canals. The number of colony forming units [CFU]/ mL from the pre irrigation samples were quantified and compared. Analysis exhibited a' p 'value of 0.912 (Student unpaired 't' test), indicating that there was no statistical difference between the groups at the beginning of the study (the samples were homogenous before debridement). (Fig 1)

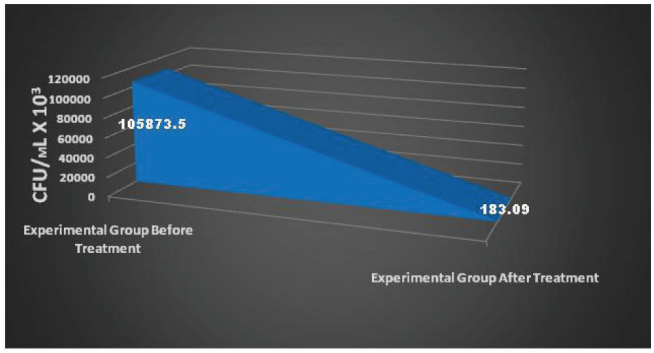
Figure 1: Comparison of microbial load in experimental and control group before irrigation.



Experimental group: before versus after irrigation

In the pre irrigation and post irrigation samples corresponding to the experimental group, a mean of 105873.5 ± 11.82 X 10^3 CFU/mL and 183.09 ± 59.14 X10^3 CFU/mL was reported respectively. The difference between bacterial counts, or CFU/mL , before and after irrigation was statistically significant [p = < 0.001 , Student paired 't' test]. (Fig 2).

Figure 2 : Comparison of bacterial quantification before and after irrigation in experimental group.



Control group: before versus after irrigation

In the pre irrigation and post irrigation samples corresponding to the experimental group, a mean of 111301.7±10.80 X 10^3 CFU/mL and 354.25±18.73 X 10^3 CFU/mL was reported respectively. The difference between bacterial counts, or CFU/mL , before and after irrigation was statistically significant [p = < 0.001 , Student paired 't' test]. (Fig 3).

After irrigation: experimental versus control

Finally, the antimicrobial ability of both irrigating solutions employed in the study was compared through bacterial quantification, or CFU/mL, after irrigation. Analysis showed a significant difference in favor of the experimental group. (p = 0.082, Student unpaired 't' test]) (Fig 4).

Figure 3 : Comparison of bacterial quantification before and after irrigation in control group.

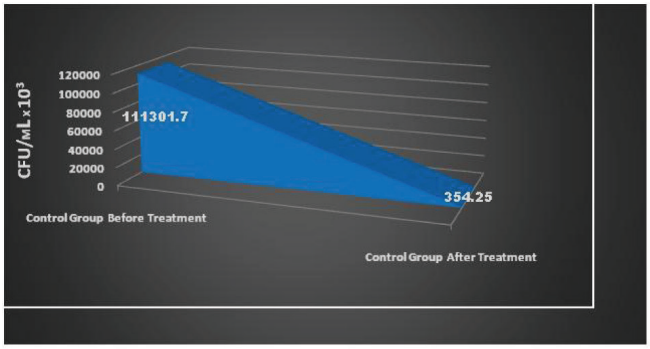
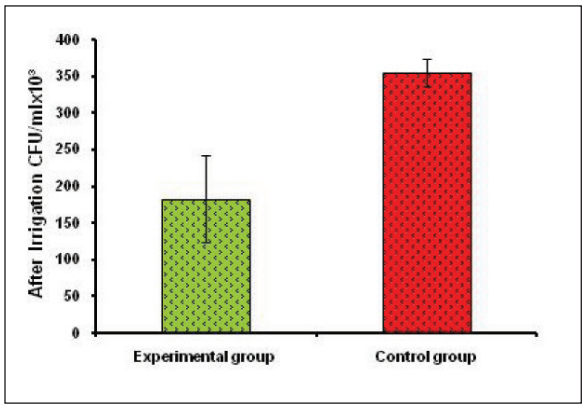


Figure 4 : Comparison of microbial load in experimental and control group after irrigation.



DISCUSSION

One of the fundamental steps in a pulpectomy treatment in primary teeth is the reduction of the pathogenic bacterial load to the minimum level within the root canals.[22] Hence the clinician must pay particular attention to the biochemical preparation of the complex pulp canal system characteristic of primary teeth to reduce the bacteria and their byproducts to a minimum, thus increasing the chances of a successful pulpectomy. **Facultative anaerobic microorganisms such as *E faecalis, S aureus* and *C albicans* are considered highly resistant species and therefore possible causes of failed root canal treatments.[23]**

In the present study, we found MTAD, with a greater reduction of intracanal bacterial loading when compared to 1% NaOCl solution when used as an irrigant. This result is similar to study conducted by Torabinejad and asscoaites in permanent dentition.[24,25] Several studies have compared the properties of currently used endodontic irrigants. Most of these studies have been carried out on permanent teeth. There has been no study reported regarding the antimicrobial effect of MTAD on primary root canals on pubmed search.

Sodium hypochlorite is till date, the most commonly employed root canal irrigant. The antimicrobial activity of NaOCl is by the release of hypochlorus acid and its oxidative action on sulfhydryl groups of bacterial enzymes thereby disrupting the metabolism of the microorganisms. Although it is an effective antibacterial agent, NaOCl is toxic when extruded to the periraicular tissues and has unpleasant odor and taste[26]. Therefore, research for new irrigant continues.

MTAD is an aqueous solution of 3% doxycycline, a broad spectrum antibiotic; 4.25% citric acid, a demineralizing agent; 0.5%

polysorbate 80 detergent (Tween 80).[12] The antimicrobial efficacy of MTAD is because of anticollagenous activity of Doxycycline, its low pH =2.0, ability to be released gradually over the time and its action facilitated by citric acid which removes the organic and inorganic substances. Tween 80 reduces the surface tension on the dentinal tubules and allows deeper penetration of Doxycycline into the tubules.[27] Not only this MTAD has the capacity to kill E. Faecalis after a mere exposure of 5 minutes. Thus making it an ideal endodontic irrigant. Hence MTAD was found superior when compared to NaOCl solution as irrigant with respect to reduction in bacterial load after treatment. However some studies reported limited use of it in primary teeth due to fear of discoloration to permanent tooth buds.[28] However more studies are required to confirm the same.

Pre reduced thioglycolate has been used to transfer microbiological samples from infected root canals to the laboratory, as suggested by Carlsson[29]. This medium reduces oxygen, preventing the accumulation of superoxide radicals that would kill anaerobic bacteria; also, it contains small amounts of agar that prevent diffusion of oxygen into the medium. An essential step in endodontic microbiology studies is the design and implementation of a preoperative disinfection protocol of the field, so that the taking of microbiological samples is carried out in the most aseptic possible conditions, avoiding the contamination that might confuse the results.[30] The disinfection protocol used in this study was a modification of the one described by Ng YL[31]. **1% NaOCl was used since studies showed negligible differences in antibacterial activity among 5.25%, 2.5% and 1 % NaOCl in infected root canals.[32,33] Even 0.5% NaOCl, when used in larger volumes and with longer irrigation times, possesses good bactericidal activity.[33] Sodium hypochlorite at all concentrations was effective in eliminating resistant endodontically relevant microbes including Candida albicans, Pseudomonas aeruginosa, E faecalis, Bacillus subtilis, Streptococcus mutans and Staphylococcus aureus.[34-36] From a cleansing perspective, lower concentrations of NaOCl still retained substantial tissue dissolution capacity and are effective in cleaning root canals.[37] Baumgartner and Cuenin[38] showed that all NaOCl concentrations were equally effective in flushing out loose debris and completely removing pulpal remnants and predentin from non instrumented canal walls. Smear layers and necrotic pulpal tissues were dissolved almost equally well by 1.3%, 2.6% and 5.25% NaOCl.[39]**

The present study demonstrates that MTAD reduced substantially the bacterial load in the root canals of primary teeth. However, more randomized controlled clinical trials are necessary to evaluate the clinical and radiological evolution of necrotic primary teeth treated with pulpectomy and irrigated with MTAD, efficacy of this irrigant and the success of this procedure in primary teeth.

CONCLUSION

Although more controlled clinical studies are needed to support the effectiveness of MTAD as an irrigant solution, the results exhibited in this study are highly promising in terms of being a feasible alternative for irrigating after pulpectomy of necrotic primary teeth.

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