RESEARCH ARTICLE

Assessment of Bacterial Load Using 3.8% SDF as an Irrigant in Pulpectomized Primary Molars: A Randomized Controlled Trial

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Abstract

Aim and objective: The aim of the present study was to evaluate the reduction in bacterial loading using 3.8% as an irrigating solution in pulpectomized primary molars.

Study design: A randomized, controlled clinical trial was performed that included primary molars with pulp necrosis. Sixty necrotic canals were included, 30 irrigated with 3.8% SDF (experimental group) and 30 with 1% NaOCl solution (control group); in all cases, two 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 strong significant decrease of bacterial load ($p = < 0.001$). The same occurred in the 3.8% SDF group samples ($p = < 0.001$). When both groups were compared post irrigation, a statistically significant difference was observed in favor of 3.8 % SDF.

Conclusion: 3.8% SDF can be suggested as an alternative irrigant for pulpectomy of necrotic teeth.

Keywords: 3.8% SDF, Endodontic irrigation, NaOCl, Primary molars.

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Introduction

For effective endodontic treatment, an exhaustive cleaning, a precise diagnosis, and a predictable disinfection protocol that utilizes different irrigating solutions and intracanal medicaments are necessary.[1] In spite of noteworthy advancements in all domains of dentistry, the quest for the perfect irrigant solution is still going on in pediatric endodontics.[2]

It is desirable to emphasize irrigants for cleaning primary teeth than removing dentin. Sodium hypochlorite is an extensively used endodontic irrigant, since its antimicrobial and solvent characteristics are adequate at 0.5-5.25% concentration levels for treating tissue,[3] however, it might damage periradicular tissue even if it enters at relatively mild concentrations.[4,5]

Intracanal irrigation using a 3.8% w/v solution of silver diamine fluoride (SDF) has been implemented. It represents a 10-times more diluted variant of the SDF used for performing root canal treatment.[6] Research suggests that 3.8% SDF exerts antibacterial characteristics against an *E. faecalis* biofilm and can be employed as a root canal irrigant to reduce bacteria.[7-10]

In contrast, the results of 3.8% SDF for irrigating primary teeth during endodontic therapy must be evaluated. Consequently, the present research objective was to ascertain and contrast the changes in bacterial activity after using 1% NaOCl and 3.8% SDF solutions for endodontic irrigation of primary teeth. The null hypothesis indicates that there will be an absence of a noteworthy difference among the two solutions with regards to the lower bacterial count.

Materials and Methods

The present research was carried out in collaboration with the Department of Microbiology, Grant Medical College and Hospital, Mumbai, Maharashtra, India and Pediatrics and Preventive Dentistry, Government Dental College and Hospital, Mumbai, Maharashtra, India. The Institutional Ethics Committee gave its approval to the research. A total of 60 necrotic canals of both genders, falling in the age group of 3–7 years, partook in this controlled, randomized clinical testing after acquiring written permission from their parents and/or guardians. The present trial had eligibility criteria of good overall health, a primary molar having necrosis in one or more pulp canals, presence of an abscess, two-thirds or more intact root structure, and adequate tooth structure to hold a dental dam. The trial disqualified children allergic to silver or sodium hypochlorite, systemic illness, pulp floor perforations, extensive mobility, or nonrestorable molar.

Sixty root canals were split in a random manner into two categories, which are as follows:

- Experimental group (30 root canals): 3.8% silver diamine fluoride (FAgamin; Argentina)
- Control group (30 root canals): 1% NaOCl solution

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Disinfected distilled water was used to prepare a 1% NaOCl solution from a 3% concentration level (Neelkanth, India).[11] SDF was similarly diluted to 3.8% from 38% using disinfected distilled water.[12] The pulpectomy process required only one sitting. The sample size was ascertained from a pilot study comprising ten microbiological samples extracted from necrotic root canals (five samples per irrigant); however, these were not included for statistical analysis in this research.

Likewise, reliability and uniformity testing for diagnostic and outcomes assessment was conducted independently; a Kappa test score of 0.90 was obtained.[13] The irrigant for every case was selected randomly from a catalog of computer-generated random numbers. Patient sampling was carried out in a non-probabilistic mode.

Preclinical Laboratory Procedures

Hemin (5 mg L^{-1}) and menadione (1 mg L^{-1}) could retain sampled bacteria vitality; therefore, these substances were augmented using pre-reduced thioglycolate tubes and were employed as growth and movement media.[14]

Isolation and Disinfection of Operative Domain

One pediatric dentist conducted the research who obtained periapical radiographs of the identified teeth using the conventional paralleling method. The patients' oral cavity was disinfected using a 60-second rinse with 0.12% chlorhexidine. Local anesthesia was used for the procedure, and the primary mandibular teeth were numbed using an inferior alveolar nerve block. In contrast, infiltration (palatal and buccal) was used for the primary maxillary teeth. Teeth were cleaned using pumice, and a rubber dam was used to prepare the working area. Petroleum jelly was used on the gingiva of the infected tooth. Provisit (Casaldea, Mexico) was used at the tooth-damn junction to prevent saliva entering the operating area. The tooth crown, dam, operative area, and clamp were disinfected using 30% hydrogen peroxide for 60 seconds. Subsequently, 5.25% NaOCl was used for 60 seconds, followed by inactivation using 10% sodium thiosulfate.[15,16]

Caries tissue was removed using a sterile saline solution cooled number 3 round carbide bur. The operative area and tooth cavity were again sterilized. The roof of the pulp was then extracted using a similar-sized new bur. The root canal was then accessible using a disinfected cotton pellet, which was kept on the base of the pulp chamber to disallow disinfectants from entering tooth canals.

Preparation of Microbiological Samples

Microbiological samples were extracted from tooth canals before irrigation. Three different absorbent paper points were sterilized and used for different canal sizes. These points were inserted in canals for 30 seconds after the canals were accessible. An intraoral periapical radiograph was used preoperatively to determine the workable length of the tooth canals. Some sterile saline solution was used to irrigate the canals, if they were dry before the points were inserted. The paper points used for the roots were placed in a tube containing thioglycolate. Conventional teeth treatment followed sample collection. FlexoFiles (Dentsply, Switzerland) instruments were used with 0.5 mL of the identified irrigant after filing. The canal was wetted and dried one last time after instrumentation and before sealing the tooth. A postirrigation sample was obtained from the canals using another set of three sterilized paper points. Lastly, iodoform paste (Vitapex) was inserted in the canal. An intraoral periapical radiograph was obtained after the operation. Stainless steel crowns (3M) were employed for after-treatment restoration.

Laboratory Procedures

Specimens (post- and pre-irrigations) were transported using 1 mL thioglycolate broth that was inoculated for one day at 37°C. It was followed by plating 1,000 mL broth using Soyabean Caesin Digest Agar medium (HIMEDIA, India), which underwent anaerobic incubation for 3 days at a 37°C temperature. The overall microbial load (per mL) was determined through the Colony Forming Unit (CFU) count on Trypticase Soy Agar comprising 400 g of L-cysteine mL, 0. 5 g of hemin mL, 5% sheep blood, as well as 1 g of menadione mL (Amyl Media, Australia).[17]

Clinical and radiological follow-up was performed on all 120 pulpectomy-treated primary molars at 3 months, 6 months, 9 months, and 12 months. No failures were reported in either group at the end of the 12-month follow-up.

Statistical Analysis

Samples (before and after irrigation) were assessed between and across groups. The Student "t" measure helped ascertain study aspect significance using continuous measurements between the two sets based on metric parameters (independent, two-tailed). Leven's test was conducted to ascertain variance homogeneity.

Results

Children who underwent treatment had a 5-year average age; A total of 120 microbiological samples were gathered, with 60 from the control group (30 pre- and 30 postirrigation) and 60 from the experimental group (30 pre- and 30 postirrigation). Preirrigation baselines were similar concerning bacterial load in necrotic canals across both groups. Preirrigation specimens were identified and contrasted for colony formation characteristics (Table 1).

Experimental group: before versus after irrigation

The pre- and postirrigation samples used for the experiment had mean values of $2.64 \pm 0.31 \times 10^6$ CFU/mL and $2.14 \pm 0.41 \times 10^6$ CFU/mL. Bacterial load (CFU/mL) was significant ($p = < 0.001$) pre- and postirrigation (Fig. 1).

Control group: before versus after irrigation

The control samples before and after irrigation had mean values of $3.40 \pm 0.10 \times 10^6$ CFU/mL and $3.29 \pm 0.05 \times 10^6$ CFU/mL. The changes in bacterial load before and after irrigation (CFU/mL) were statistically significant ($p = < 0.001$) (Fig. 2).

After irrigation: experimental group versus control

Once irrigation was completed, the antimicrobial efficacy of the irrigants was contrasted by quantifying bacteria (CFU/mL). The assessment suggested that the experimental sample was significantly superior to the control sample ($p = 0.300$) (Fig. 3).

Discussion

Pulpectomy helps to reduce premature primary tooth loss. Losses might cause inadequate area for permanent teeth, reduced arch length, impact on premolars, and molar mesial tipping next to the infected primary molar. This process is a conservative approach.[18,19] The objective behind cleaning and shaping of the root canal is the removal of tissue remnants, toxins, and bacteria from the root canal setup. Mechanical processes solely are not adequate for exhaustive canal cleaning. The remaining pulp tissue, dentin particles, and bacteria might remain due to irregular canal structures. Hence, irrigants must complement and aid endodontic treatment. Such solutions must remove dentin particles, break down organic tissue, sterilize the canals, and lubricate the system during the procedure without irritating nearby tissues.[20]

Table 1: Comparison of colony forming units per mL in experimental and control group

Colony forming unit per mL	*Experimental Group*	*Control Group*	*Total*	*p value*
Pre-Irrigation	$2.64 \pm 0.31 \times 10^6$	$3.40 \pm 0.10 \times 10^6$	$3.02 \pm 0.45 \times 10^6$	<0.001*
After Irrigation	$2.14 \pm 0.41 \times 10^6$	$3.29 \pm 0.05 \times 10^6$	$2.72 \pm 0.65 \times 10^6$	<0.001*
difference	0.492	0.107	0.300	–
p value	<0.001*	<0.001*	<0.001*	–

*Statistically significant

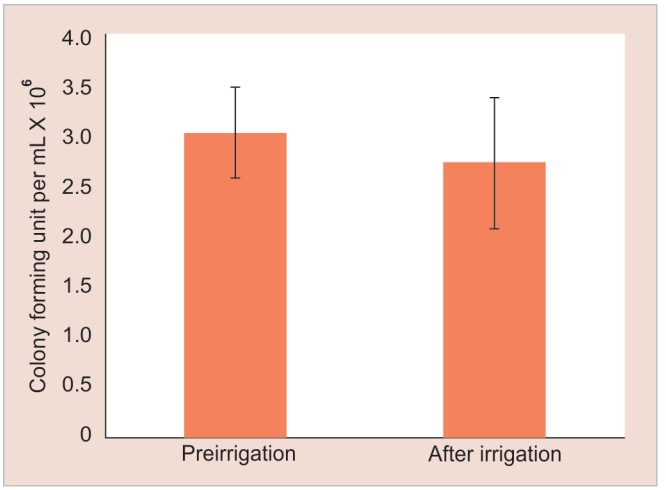

Fig. 1: Comparison of colony forming unit per mL x 10^6 – pre- and postirrigation in experimental group.

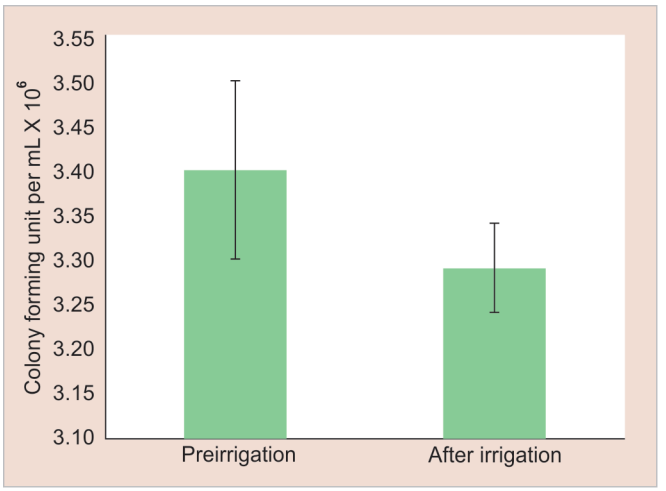

Fig. 2: Comparison of colony forming unit per mL x 10^6 – pre- and postirrigation in control group

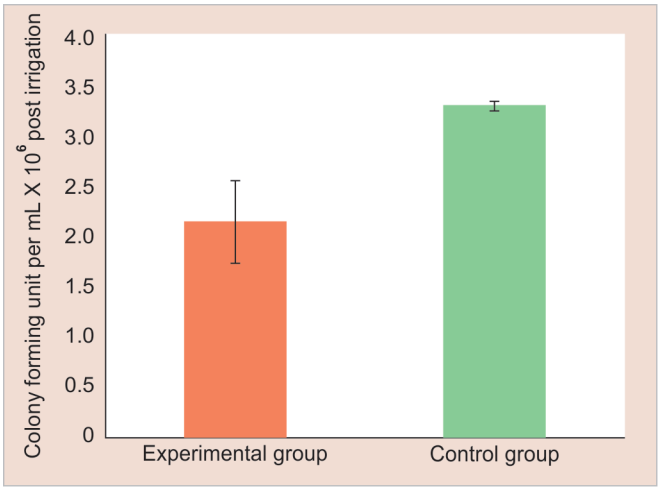

Fig. 3: Comparison of colony forming units per mL x 10^6 pre- and postirrigation in experimental and control group

Henry Dakin was the first person to propose sodium hypochlorite as an antiseptic solution in 1915. In 1920, Crane outlined its usage for root canal debridement as well as sterilization. It has since attained popularity as an effectual root canal irrigant. Typical usage concentration is 0.5–5.25%; however, such concentrations have high hypertonic and alkaline characteristics, corresponding to an 11–13 pH range. It possesses oxidative and proteolytic characteristics that might cause the healthy and necrotic pulp to disintegrate, apart from eliminating microbes. Though NaOCl is an efficacious antibacterial substance, it causes toxicity when the periradicular tissues are exposed to it; moreover, it has an uncomfortable taste and smell.[21,22] Hence, there are continuous efforts to find a sodium hypochlorite substitute.

Records indicate that silver was perhaps used for medicine around 1000 BC. Presently, silver compounds are used for medicinal use in sutures, nitrate, and foil forms. Silver diamine fluoride (SDF) is claimed to be stable at 38% concentration (pH = 8-9) and its concentration can be maintained. Several nations, including Japan and China, have used it to slow dental caries. This compound does not need a reducing substance.[23] Mei et al. asserted that 38% SDF slowed the demineralization process and protected collagen disintegration from demineralized dentin.[24] SDF also possesses cariostatic properties concerning the tooth mineral constituents and itself. It possesses anti-enzymatic properties on the substances formed when $Ag(NH_3)_2F$ and tooth organic tissue react. The reagent has antibacterial characteristics because it reduces enzyme activation and dextran-facilitated agglutination of *Streptococcus mutans* cariogenic strains.[25] SDF is available under the trade names mentioned below: 38% SDF solution, Fluoroplat (NAF Laboratories, Buenos Aires, Argentina), 38% SDF solution, FAgamin, Bv. of the Poles 6136, Córdoba, Argentina, 38% SDF solution, Saforide (Toyo Seiyaku Kasei Co. Ltd., Osaka, Japan), 38% SDF solution, Advantage Arrest (Elevate Oral Care LLC, West Palm Beach, Florida, US), and 38% SDF solution, e-SDF (5 mL bottle, Kids-e-dental Llp, Mumbai, India).

A solution comprising ammoniated silver nitrate has been usually utilized for treating the affected root canal. It has been demonstrated by Tanaka[26] that a liquid solution of AgF has strong antibiotic and protein-coagulating properties, and also has a substantially potent action, which chokes up the dentinal pipettes of root canal wall with respect to the electric resistance. Okamoto et al.[27] discovered that applying SDF solution reduced significantly the number of treatments needed. Hiraishi et al.[12] stated that 3.8% SDF is enough to function as an antimicrobial root canal agent

or interappointment dressing, particularly in locations where potential blackening/browning of dentin by metallic silver is a minor concern. Mathew et al. (2012)[28] discovered that SDF solution as an endodontic irrigant can efficiently eliminate the microbes found in the circumpulpal dentin and canal. Thus, the present research found 3.8% SDF to be better in comparison to 1% NaOCl in bacterial load following pulpectomy process in primary molars. This deduction is confirmed by study carried out by Mathew and colleagues who stated that 3.8% SDF exhibits inhibitory effect on synthesis of bacterial cell wall, cell division, and DNA unwinding, therefore, effectively decreasing microbial load inside the root canal.[28] Noriko et al. investigated the impact of NaOCl and 38% SDF on in vitro *E. faecalis* biofilm and observed 100% effectiveness of 3.8% SDF against *E. faecalis* following a direct 60 minute contact.[9] The review of literature did not find any other research evaluating 3.8% SDF solution as an irrigant in pulpectomy treatments.

The SDF advantage over other irrigants such as sodium hypochlorite is that besides having similar antibacterial characteristics, the interaction between teeth and SDF causes reciprocal formation of fluoroapatite. The quantity of fluoride release is twice of that observed in other fluoridating substances. This increases the antibacterial activity and may lead to increased longevity and enable prevention of reinfection within the root canal. Rise in surface hardness, reduction in permeability, and growth in the root canal's fracture resistance have all been observed when SDF was initially utilized as an adjuvant coupled with lasers in endodontic preparation, since it can safeguard the root dentin from impairment because of laser.[29]

The primary drawback of SDF is black discoloration because of generation of silver phosphate that turns black on decrease in oral environment. Nonetheless, in the present research, this discoloration was concealed by postendodontic recovery in the form of stainless steel crowns. It also results in gingival irritation, which is resolved by itself within 2 days. In the present research, the gingiva was layered with petroleum jelly to prevent this irritation. The SDF is metallic in taste. Thus, in the present research, rubber dam was applied along with provisit to restrain any contact among SDF and saliva. It causes coloring of skin, which is riskless and resolves by itself within few days when the epithelial cells slough off.[29]

Prereduced thioglycolate has been utilized in the present research to carry microbiological samples from septic root canals to the laboratory, as recommended by Carlsson.[14] This mode decreases oxygen, averting the accrual of superoxide radicals which might destroy anaerobic bacteria; furthermore, it comprises tiny quantities of agar, which averts dissemination of oxygen within the medium. A crucial step in endodontic microbiology investigations is the design and execution of a preoperative disinfection procedure of the field, such that the collecting of microbiological samples is conducted in the most hygienic conditions possible, avoiding the polluting that might affect the results.[15] The disinfection protocol utilized in this work was a variation of the one outlined by Ng YL.[30]

1% NaOCl was utilized since research works exhibited insignificant differences in antimicrobial activity for 1%, 2.5%, and 5.25% NaOCl in the affected root canals.[31,32] After 0.5% NaOCl was utilized in higher quantity and with higher irrigation period, it possessed reasonable antibacterial activity.[33] For all concentrations, sodium hypochlorite was effectual in removing resistant endodontically significant microbes such as Bacillus subtilis, *E. faecalis*, *Candida albicans*, *Pseudomonas aeruginosa*, *Staphylococcus aureus,* and *Streptococcus mutans*.[34-35] From a cleansing viewpoint, lesser quantities of NaOCl concentrations nevertheless preserved considerable tissue dissolution ability and were effective in cleansing root canals.[36] Baumgartner and Cuenin[37] demonstrated that each concentration of NaOCl was similarly efficient in removing loose debris and entirely eliminating predentin and pulpal remnants from nonprocessed canal walls. Necrotic pulpal tissues and smear layers were subjected to dissolution equally fine by 5.25%, 2.6%, and 1.3% NaOCl.[38]

Conclusion

More controlled clinical trials are required to support the effectiveness of 3.8% SDF as an irrigant solution, the results reported by this study are highly encouraging in terms of being a suitable and potent alternative for irrigation of endodontic canals of primary teeth.

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