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Efficacy of Root Canal Irrigants against Self-inducted Streptococci – An Exvivo Study

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Abstract **Objective:** To compare antibacterial efficacy of various root canal irrigants against self-inducted streptococci in purposely sterilized root canals. **Material and Method:** Sixty (60) extracted mandibular & maxillary molars were collected and stored in saline at ambient temperature. The teeth were deroofed & their wider canals (distal canal in mandibular & palatal canal in maxillary molars) were selected for preparation. Working lengths of specimens was recorded by inserting K file# 20into the canals. All teeth were washed thoroughly with normal saline& autoclaved. Apical widening of canals was done using K-file # 30 with a step back technique. Streptococci were inducted in the sterile canals and their colonies were counted. **Result:** There was apparent difference in the antibacterial efficiency between 1% NaOCl, 0.12% chlorhexidine, 1% Povidone Iodine, NaCl, NaOCl with NaCl as final rinse NaOCl with 0.12% chlorhexidine as final rinse. The best results were achieved with 1 % NaOCl with Chlorhexidine as final irrigant. **Conclusion:** 1% NaOCl can be safely used by undergraduate dental students as main root canal irrigant with 0.12 % chlorhexidine as a final rinse.

Keywords: *irrigating solution for RCT, intracanal medication, NaOCl in endodontics, root canal irrigants*

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1. Introduction

Success of endodontic treatment is directly influenced by the elimination of microorganisms from infected root canals [1]. Majority of microbes from the canals are eliminated using hand & rotary files. Mechanical action of instruments alone is unable toacceptably clean a root canaldue to the intricacy of the internal tooth morphology marked by the presence ofapical deltas, lateral canals, accessory canals, etc. and because direct contact between the instruments and all the walls of the root canal system is not possible [2].

Irrigating solutions are therefore,essential during root canal preparation because they perform multiple functions to help clean the root canal; lubricate the files, flush out debris, kill bacteria, and dissolve infected dentine without damaging the periapical tissues. The selection of the ideal irrigant depends on its antibacterial action and lack of toxicity to periapical tissues.

Sodium hypochlorite (NaOCl) is an oxidizing and hydrolyzing agent [3]. It is bactericidal and has been used as an endodontic irrigant since early twentieth century [4]. As an endodontic irrigant, NaOCl solution is relatively cheap, tissue dissolving and virucidal [5].

For two decades, chlorhexidine gluconate (CHX) owing to its potent antibacterial activity has been widely used in periodontics to chemically control the biofilm [6]. Its use in endodontics has been proposed both as an irrigant and as an intracanal medication. It inhibits the gram-positive and gram-negative microorganisms commonly found in endodontic infections. Its efficacy is based on the interaction between the positive charge of the molecule and the negatively charged phosphate groups on the bacterial cell wall, which allows the CHX molecule to penetrate pathogenic bacteria [7].

Iodine (I_2) is a strong oxidizing agent. It reacts with free sulfhydryl groups of bacterial enzymes. Its use is advocated as it has been shown to be an antiseptic against a broad range of micro-organisms, hypoallergenic, with low toxicity and has a lesserpropensity for dentin staining [8].

Saline (NaCl) provides gross debridement & lubrication. A few investigators have supported isotonic saline solution as an irrigation solution to minimize tissue irritation & inflammation. In isotonic concentration, saline produces no documented tissue damage & has been confirmed to flush debris from the canal. It has no bactericidal action and therefore doesn't effectively reduce bacterial load. Irrigation with saline alone declines chemical destruction of microbiologic matter & dissolution of mechanically inaccessible tissue [9].

Major primary root infections are multimicrobial, dominated by obligate anaerobic bacteria. The most frequently isolated microorganisms before root canal

treatment include Gram-negative anaerobic rods, Gram-positive anaerobic cocci, Gram-positive anaerobic and facultative rods, Lactobacillus species and various Gram-positive facultative Streptococci [10]. The obligate anaerobes can be effortlessly eliminated during root canal preparation but facultative microbes like Streptococci, Enterococci, and Lactobacilli, once established, are more expected to persist after chemomechanical canal preparation and root canal medication [11].

Many studies have been done in this regard with varying results which cause confusion in users' minds, especially undergraduate dental students. To remove this ambiguity and to give a standard protocol to be followed by all the students in endodontic OPD, this exvivo study was performed to comparethe efficacy of various irrigants which are cheap and commonly available for use in endodontic practices. The irrigant with the best cleansing ability would be recommended for students' to use while performing a procedure in the endodontic clinics of the institution.

2. Material and Method

1.0 % NaOCl, 0.12% Chlorhexidine, 1% Iodine, NaCl, and colonies of streptococci were used in this experimental study.

Sixty freshly extracted human mandibular and maxillary molars stored in saline at room temperature were taken. Teeth were deroofed by using straight fissure diamond bur # 21. Distal canal in the mandibular molars and the palatal canals in the maxillary molars were selected as they are wider, straighter and easily accessible canals. Working lengths of the specimens were recorded by using radiographs taken with # 20 K file inserted in the canals. Apically, canals were enlarged up to size 30 K file with step-back technique. Rest of the radicular flaring was done using size 50 K file.

Rating of the commonly found root canal bacteria was carried out first, by incubating the paper points taken from the unprepared infected canals as sample; in the Blood Agar Culture Medium for 24 hours at 37°C. Microscopic reading revealed the presence of Streptococci colonies in abundance.

All teeth were washed using normal saline and autoclaved at 121°C under 15 lbs pressure for 15mins.

Inclusion Criteria: Distalcanals of extracted mandibular molars and palatal canals of maxillary molars were selected for experiment.

Exclusion Criteria: Root treated teeth, wisdom molars, teeth with dilacerations or morphological anomalies, teeth with open apices.

Considering Streptococci as potential pathogen, colonies were cultured in Blood Agar and the suspension was inducted in each sterile specimen by employing the pipette of 10 µl.

All sixty teeth were allowed for incubation for 48 hours at 37°C and divided equally into six groups of 10 each(5 mandibular,5 maxillary molars) designated as Group 'A','B','C','D','E' & 'F'.

2.1. Preparation of Group A Samples

In this group 1.0 % NaOCl was used as a root canal irrigant. Throughout the instrumentation, 5 ml of NaOCl solution was used for irrigation.Removal of excessive moisture from the canals was carried out by absorbent paper points and a sterile point of 30# was retained in the irrigated canal up to the determined working length for 15 seconds. These points were transferred into sterile test tubes, used to inoculate the bacteria in plates of 5% Blood Agar and incubated for 48 hours at 37°C. Colony counter was used finally for results.

2.2. Preparation of Group B, C, D, E Samples

Irrigant used for group B was 0.12 % CHX, for group C 1 % povidone Iodine, for group D NaCl, for group E NaOCl with final rinsing of CHX andfor group F NaOCl with final rinsing of NaCl.

Rest of the procedure was same as performed for group A.

3. Results

A one way analysis of variance was conducted to evaluate the efficacy of root canal irrigants against self-inducted streptococci (N=60). The independent variables included six groups where Group A (M=2.88±0.21, n=10) was 1% NaOCl, Group B (M=8.9±0.16, n=10) was 0.12% Chlorhexidine, Group C (M=17.92±0.17, n=10) was 1% Iodine, Group D (M=32.33±0.67, n=10) was NaCl, Group E (M=0.27±0.42, n=10) was 1% NaOCl + 0.12 Chlorhexidine, and Group F (M=1.14±0.21, n=10) was 1% NaOCl + NaCl. Table 1 shows the mean number of bacterial colonies present after irrigation had been performed.

Table 1. Mean number of colonies found with each irrigant used

Group	Mean No. of Colonies
Group A (1%NaOCl)	2.88±0.21
Group B (0.12%CHx)	8.90 ±0.16
Group C (1%Iodine)	17.92±0.17
Group D (NaCl)	32.33±0.67
Group E (1% NaOCl+0.12%CHx)	0.27±0.42
Group F (1%NaOCl+NaCl)	1.14±0.21

The assumption of normality was evaluated using histograms and found tenable for all groups. The ANOVA was significant, F (5, 54) = 12346.22, p = 0.0005, $\eta 2$ = 0.13. Post Hoc comparisons to evaluate pairwise differences among group means were conducted using the Tukey HSD test since equal variances were tenable. The tests revealed significant pairwise differences between the mean scores of all the irrigants used, p = 0.0005.

Out of the 6 solutions used to irrigate the root canal, 1% NaOCl along with a final flushing of the canal with 3 ml 0.12% CHx possessed greatest antibacterial efficacy against microbes, followed by 1%NaOCl with a final flushing of 3 ml NaCl. Contrarily, NaCl, when used alone, was the least effective irrigant. Figure 1 shows a descriptive graphical representation of the results.

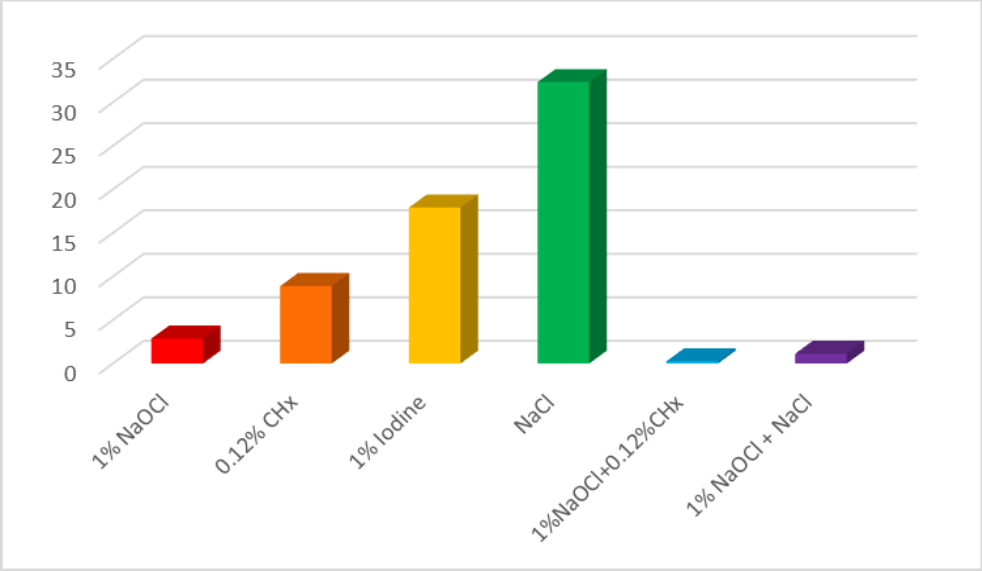

Figure 1. Graphical representation of the results with 1%NaOCl + 0.12%CHx

4. Discussion

The root canal preparation is performed with a goal to attain precise scrubbing and overall decontamination of the canals systems but anatomical variation in canal system obstructs the canal instrumentation which consequently leads to failure [12]. To avoid the procedural failure six type of irrigating solutions were used to reach and disinfect the intracanal areas which were inaccessible to mechanical debridement. Many other solutions are also marketed for this purpose. As during clinical sessions they are plenteously used by the learning students, only those solutions were used which were easily, cheaply and uninterruptedly available in bulk quantity from the market.

Irrigation of root canals with NaOCl in concentrations ranging from 1 % to 5.25 % is acommonly followed practice. In this study the lowest percentage of NaOCl solution was used keeping in mind two reasons. Firstly, dilute solutions cause fewer complications in case any accident occurs by an operator, especially when an operator is a beginner as higher concentrations increase chances of occurring any emergency. Secondly, reports of at least two studies show that there is no difference in cleansing ability of 0.5 % NaOCl or 5.0 % NaOCl [13,14]. Moreover 1 % NaOCl solutions is good enough to dissolve canal debris [15]. Minimal number of bacterial colonies were found when 1 % NaOCl was used for canal irrigation but still the canal was not free from the microbial growth. This finding is in agreement with results of another study where canals were rendered totally sterile with 1-5 % NaOCl [16]. The reason behind this difference may be the time factor for which the solution is kept inside the canal as time also plays vital role in canal disinfection [17].

CHX is not promoted to be used as the main irrigant in root canal cases as it doesn't dissolve tissue remnants in the canal [18] and it is found less efficient in the canal as compare to NaOCl. In this regard results of this study match the findings of another study [19]. CHX is the most capable agent to employ as a final irrigant as it has an affinity to bond to mineralized tissues [20], and once bound to a surface, gives prolonged antimicrobial activity. This might have been the logical reason that after NaOCl irrigation final wash with CHX rendered the canals bacteria free.

Although I_2 is less cytotoxic and irritating to living tissues than NaOCl and CHX [21] but its efficacy according to findings of this study was not better than NaOCl and CHX mixture. Moreover, I_2 has a much greater potential to cause an allergic reaction [22] as compare to NaOCl and CHX [23].

It is therefore safe for undergraduate dental students to use 1 % NaOCl for copious irrigation during the root canal procedure as of all the solutions used in this study; itseemsto be the best lonely used solution. It fulfils more requirements of an ideal endodontic irrigant than any other identified substance and used in combination with CHX as final flush serves the purpose better than using it alone [24].

More studies with different combinations and leaving irrigants inside the canal for variable time and with or without warming NaOCl should be performed.

5. Conclusion

The undergraduate dental students can safelyuse 1 % NaOCl as main irrigating solution during a root canal treatment and at end of the procedure 3 ml of CHX wash as final irrigation solution. Normal saline may also be used in place of CHX.

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