

Reduction in Bacterial Loading using Papacarie and Carisolv as an Irrigant in Pulpectomized Primary Molars – A Preliminary Report

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Objective: The aim of the present study was to evaluate the reduction in bacterial loading using Papacarie and Carisolv as an irrigating solution in pulpectomized primary molars. **Study design:** A controlled, randomized clinical trial involving 120 necrotic canals from both genders between 3 and 7 years old children were included, 30 irrigated with Papacarie [group I], Carisolv [group II], 1% NaOCl gel [group III] and 1% NaOCl solution [group IV] each; 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 ANOVA. After analyzing samples before and after irrigation in all the groups, a strong significant decrease in bacterial load [$p = < 0.001$] was found with Papacarie and Carisolv. **Conclusion:** Papacarie and Carisolv can be suggested as an alternative irrigant for pulpectomy of necrotic teeth.

Keywords : Carisolv, Papacarie, 1% NaOCl, pulpectomized primary molars.

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INTRODUCTION

The aim of pulpectomy procedure is to eliminate infection and substrate from the root canal system.¹⁻³ Pulpectomy procedure still proves to be complicated and has remained controversial for a number of reasons in the pediatric population. 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.⁴

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 deciduous teeth, extensive dentine 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 concentrations between 0.5 and 5.25%,⁵ but it is known to cause serious damage when allowed to enter periradicular tissues even in small amounts.⁶⁻⁷ Hence the search for an ideal irrigant for primary teeth still continues.

Carisolv™ (MediTeam, Goteborg, Sweden) is a well researched product which is advocated for chemo mechanical removal of infected carious dentine. A preliminary study showed that Carisolv has the potential to clean immature canals although it was less

effective than undiluted house hold bleach.^{1,8} In 2003, a research project in Brazil led to development of a new formula that was commercially known as Papacarie (Formula & Ac, Sao Paulo, Brazil) [a word that means ‘eating caries’]. It is basically composed of papain, chloramines, toluidine blue salts, thickening vehicle which are responsible for the Papacarie’s bactericide, bacteriostatic and anti inflammatory characteristics. Chemomechanical agents – Cariolv (MediTeam, Goteborg, Sweden) and Papacarie (Formula & Ac, Sao Paulo, Brazil) promotes the concept of conserving healthy tooth structures and giving comfort, solace and instilling a positive attitude towards dental treatment, justifying its use in the specialty of public health dentistry.⁹⁻¹¹

However the effect of Carisolv™ (MediTeam, Goteborg, Sweden) and Papacarie (Formula & Ac, Sao Paulo, Brazil) as endodontic irrigant in primary teeth needs to be investigated. Hence the present study was conducted with the aim to evaluate and compare the reduction in bacterial load after the use of Papacarie, Carisolv, 1% NaOCl gel and solution as endodontic irrigant in primary teeth. The null hypothesis was that there would be no significant difference in these irrigants with respect to bacterial count.

MATERIALS AND METHOD

The present study was carried out in the Department of Pediatric and Preventive Dentistry, Government Dental College and Hospital Mumbai, India, in collaboration with Department of Microbiology, Grant Medical College and Hospital, Mumbai, India. The study was approved by the Institutional ethics committee [GMC 1890/2017] This controlled, randomized clinical trial involved 120 necrotic canals from both sexes between 3 and 7 years old after obtaining a written consent from their respective parents/ guardians.

Inclusion criteria

Patients in good general health. Primary molar teeth 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.

Exclusion Criteria

Patients who had received antibiotics up to 2 weeks prior to the sampling. Patients having any systemic disease. Non restorable teeth, perforated pulpal floor, excessive mobility, or pathological root resorption. Patients with history of allergy to sodium hypochlorite.

One hundred and sixty canals were randomly divided into following four groups:

1. Group I [30 root canals] : Carisolv™ (MediTeam, Goteborg, Sweden)
2. Group II [30 root canals]: Papacarie® (Formula & Ac, Sao Paulo, Brazil)
3. Group III [30 root canals]: 1% NaOCl gel.
4. Group IV [30 root canals]: 1% NaOCl solution.

Commercially available 3% NaOCl solution [Neelkanth, India] was diluted to 1% concentration using sterilized distilled water.¹² 1% NaOCl gel was prepared freshly, as it is not commercially available, a thickening agent, methylcellulose (Sigma Aldrich) was

added to 1% sodium hypochlorite solution to prepare the sodium hypochlorite gel. The sodium hypochlorite solution was added drop by drop to methylcellulose powder and mixed in a mortar and pestle until uniform consistency of the gel was obtained.¹³ The pulpectomy procedure was performed in a single visit. Sample size was calculated on the basis of a pilot study, consisting of 20 microbiological samples taken from necrotic primary root canals [5 corresponding to each irrigating solution], which was not included in the statistical analysis of the present study. Likewise, consistency and reliability tests for the diagnostic and results evaluator was carried out in an independent manner by means of an unweighted Kappa test, which resulted in a score of 0.90.¹⁴ 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.

Preclinical laboratory procedures

Pre reduced thioglycolate tubes, supplemented with hemin [5mg L-1] and menadione [1mg L-1] [Oxiod LTD, Basingstoke, Hampshire, UK], was used as transport and growth media owing to capacity to maintain the vitality of sampled bacteria.¹⁵

Isolation and operative field disinfection

The study procedure was performed by a single pediatric dentist, periapical radiographs of the selected teeth were made using a standard parallel technique. After antiseptis 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. Petroleum jelly was applied on the gingiva of the concerned tooth. Provisit (CasaIdea, Mexico) was placed along the tooth rubber dam interface to prevent leakage of saliva into the operative field. To disinfect the operative field, the following protocol, was followed—the tooth crown, surrounding rubber dam, and clamp were swabbed with 30% H₂O₂, followed by 5.25% NaOCl for 1 minute each; both solutions were inactivated with 10% sodium thiosulfate.¹⁶⁻¹⁷

The gross carious tissue was removed with a sterile round carbide bur (No. 3) 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 were accessed.

Collection of microbiological samples

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 irrigant solution between each file. Irrigants were introduced into the canals in each group, with a 25-gauge

endodontic needle (Miraject, Hager Werken), attached to a Luer-Loc syringe. 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. Stainless steel crowns (3M) were used as post endodontic restorations.

Laboratory procedures

Pre and post irrigation samples were transported in 1ml of thioglycolate broth. This broth was incubated at 37° C for 24 hours. 1µl of broth was plated on Soyabean Caesin Digest Agar medium (HIMEDIA[®]) and subjected to anaerobic incubation at 37° 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% sheep blood (Amyl Media, Kings Langley, New South Wales, Australia).¹⁸

All 120 pulpectomy treated primary molars were subjected to 3 months, 6 months, 9 months and 12 months follow up for clinical and radiological follow up. Criteria’s used for clinical follow up were– history of pain, tenderness to palpation/percussion, pathological mobility, intra or extra oral swelling and/or intra or extra oral sinus. Criteria’s for radiological follow up were – presence or absence of radiolucencies in bifurcation or apical area, integrity of lamina dura, pathological internal or external resorption and/or pulp canal obliteration. At the end of 12 months follow up, no failures were reported in either of the groups based on above mentioned clinical and radiological follow up.

Statistical Analysis

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and on categorical measurements in number (%). Significance was assessed at 5 % level of significance. Analysis of variance (ANOVA) has been used to find the significance of study parameters between four groups.

RESULTS

In all one hundred and twenty canals were treated on pediatric patients, whose average age was 4 years. Of these, 240 microbiological samples were obtained: 60 from Papacarie (30 pre and 30- post irrigation), 60 from the Carisolv group (30 –pre and 30-post irrigation), 60 from 1% NaOCl gel group and 60 from 1% NaOCl solution group. Basal conditions (pre irrigation) were similar in all groups in relation to the amount of bacteria present in necrotic canals. The number of colony forming units from the pre irrigation samples were quantified and compared.(Table 1).

Group I Papacarie : before versus after irrigation

In the pre irrigation and post irrigation samples corresponding to the experimental group, a mean of 3.08± 0.20 X 10⁶ CFU/mL and 2.71 ± 0.31X10⁶ CFU/mL was reported respectively. The difference between bacterial counts, or CFU/mL, before and after irrigation was statistically significant (p = < 0.001). (Graph 1).

Group II Carisolv : before versus after irrigation

In the pre irrigation and post irrigation samples corresponding to the experimental group, a mean of 2.81± 0.32 X 10⁶ CFU/mL and 2.43 ± 0.17X10⁶ CFU/mL was reported respectively. The difference between bacterial counts, or CFU/mL, before and after irrigation was statistically significant (p = < 0.001). (Graph 1).

Group III 1% NaOCl gel : before versus after irrigation

In the pre irrigation and post irrigation samples corresponding to the experimental group, a mean of 3.39± 0.12 X 10⁶ CFU/mL and 2.97 ± 0.15 X10⁶ CFU/mL was reported respectively. The difference between bacterial counts, or CFU/mL, before and after irrigation was statistically significant (p = < 0.001]. (Graph 1).

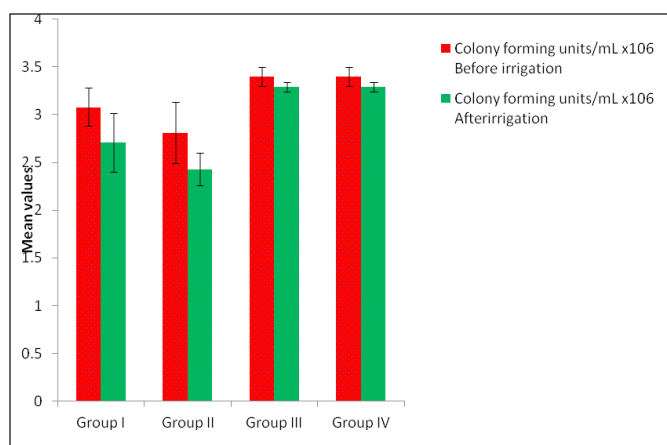
Group IV 1% NaOCl solution : before versus after irrigation

In the pre irrigation and post irrigation samples corresponding to the control group, a mean of 3.40 ± 0.10 X 10⁶ CFU/mL and 3.29 ± 0.05 X10⁶ CFU/mL was reported respectively. The difference between bacterial counts, or CFU/mL, before and after irrigation was statistically significant [p = < 0.001]. (Graph 1).

Table 1: Comparison (log)Colony forming units x 10⁶ in four groups studied

	Group I	Group II	Group III	Group IV	Total	P value
Colony forming units/mL x10 ⁶ Before irrigation	3.08±0.20	2.81±0.32	3.40±0.10	3.40±0.10	3.17±0.32	<0.001**
Colony forming units/mL x10 ⁶ After irrigation	2.71±0.31	2.43±0.17	3.29±0.05	3.29±0.05	2.93±0.42	<0.001**
Colony forming units/mL x10 ⁶ Count difference	0.37±0.20	0.37±0.24	0.11±0.10	0.11±0.10	0.24±0.22	<0.001**

Results are expressed in log



Graph 1 : Comparison (log) Colony forming units x 10⁶ in four groups studied

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.¹⁹ 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. In the present study a strong statistical significant difference was found between Papacarie, Carisolv and NaOCl with respect to reduction in bacterial count post irrigation.

The present study is the first to evaluate Papacarie Formula & Ac, Sao Paulo, Brazil) as an endodontic irrigant in pulpectomized primary molars. **Papacarie** Formula & Ac, Sao Paulo, Brazil) consists of papain, chloramines, toluidine blue salts, thickening vehicle which are responsible for the Papacarie's bactericide, bacteriostatic and anti inflammatory characteristics.¹¹ Papain is a proteolytic enzyme. Similarly to the human pepsin, papain acts as a debrillant anti – inflammatory agent which does no damage the healthy tissue and accelerates the cicatricial process. Papain comes from the latex of the leaves and fruits of the green adult papaya.

Carica papaya, for instance, is cultivated in tropical regions such as Brazil, India, South Africa and Hawaii, and is largely used in food, beverage and drug industries. It acts only on carious tissue which lacks the plasmatic protease inhibitor alpha -1 – antitrypsin, but its proteolytic action is inhibited on healthy tissue, which contains this substance.

Chloramine is a compound composed of chlorine and ammonia having bactericidal and disinfectant properties. Widely used as an irrigating solution for radicular canals in order to chemically soften the root carious dentin. These factors could be the probably reasons for high efficiency shown by Papacarie when used as irrigant with respect to reduction in bacterial count post irrigation in the present study. The degraded portion of the carious dentin collagen is chlorinated by the chloramines and is easily removed with excavator.²⁰ Toluidine blue is a photosensitive pigment that fixes into bacterial membrane.

Carisolv™ (MediTeam, Goteborg, Sweden was introduced by Ericson and available in two syringes, one containing 0.5% NaOCl

and the other containing 0.1 M amino acids, gel sub stance, sodium chlorite, sodium hydroxide and a color indicator (erythrocin). When these components are mixed together, the amino acids bind with chlorine to form high pH chloramine which is a potent disinfectant with tissue solvent activity. Carisolv also contains 0.5 % NaOCl that provides an excellent non specific proteolytic and antimicrobial properties.²¹ These factors could be the probably reason for high efficiency shown by Carisolv when used a irrigant in pulpectomized primary molars with respect to reduced bacterial count post irrigation.

Kilani *et al* ascertained that Carisolv is more effective than physiologic saline in removing debris from the non instrumented walls of root canals. Carisolv cleaned canals more effectively than phosphate buffer saline but never reached the consistent high levels of cleanliness achieved with strong sodium hypochlorite, even after long incubation times and ultrasonic agitation.¹ Parul and associates reported that 1% NaOCl solution, 1% NaOCl gel, and Carisolv had comparable activity at coronal third and middle third of root canals of primary teeth. At the apical third, NaOCl solution cleaned canals better than NaOCl gel and Carisolv. Carisolv cleaned debris better than NaOCl gel at the apical third.²² Shabnam and associates concluded that Carisolv and Papacarie both are equally efficient as an antibacterial gel against *S mutans* and *Lactobacillus* present on carious primary dentin.²³ Lager *et al* reported that the composition of Carisolv (contains NaOCl as the main ingredient) is responsible for its antibacterial activity. In accordance with our data, this has been proved by Silva *et al*²⁴⁻²⁶ Korb,²⁷ Motta *et al*²⁸ The studies of Motta²⁹ and Matsumoto³⁰ inferred Papacarie as an excellent option for achieving significant reduction in total bacteria and *Streptococcus mutans*.

Pre reduced thioglycolate had been used in the present study to transfer microbiological samples from infected root canals to the laboratory, as suggested by Carlsson¹⁵. 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.¹⁶ The disinfection protocol used in this study was a modification of the one described by Ng.¹⁷

1% NaOCl gel was used in the present study since Papacarie and Carisolv both exists in gel form. 1% NaOCl was used since studies showed negligible differences in antibacterial activity among 5.25%, 2.5% and 1 % NaOCl in infected root canals.^{24,25} Even 0.5% NaOCl, when used in larger volumes and with longer irrigation times, possesses good bactericidal activity.²⁶ 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*.²⁷⁻²⁹ From a cleansing perspective, lower concentrations of NaOCl still retained substantial tissue dissolution capacity and are effective in cleaning root canals. Baumgartner and Cuenin³⁰ 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.

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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