Evaluation of the bactericidal effect of strong acid electrolysed water against *Enterococcus faecalis* and *Porphyromonas gingivalis* in primary molar root canal: An *in vivo* study

***Ashwin B Abraham[1], Roopa K B[2], Poornima P[3], Mallikarjuna K[2], Nagaveni N B[2], Bharath K P[4]**

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Abstract

Introduction: Strong acid electrolysed water (SAEW), a root canal irrigant, has shown promise against resistant microorganisms like *Enterococcus faecalis (E. faecalis)* and *Porphyromonas gingivalis (P. gingivalis).*

Objective: To compare *in vivo* antimicrobial efficacy of 3% sodium hypochlorite and SAEW against *E. faecalis* and *P. gingivalis* in primary teeth.

Method: Seventy primary maxillary and mandibular posterior teeth of 4-10 year old children were irrigated either with 3% sodium hypochlorite (n=35) or SAEW (n=35). Paper point samples were collected at baseline, after chemo-mechanical preparation and 48 hours after irrigation during pulpectomy. Presence of *E. faecalis* and *P. gingivalis* in the three samples were evaluated using culture.

Results: When compared to SAEW, the antimicrobial efficacy of 3% sodium hypochlorite was not significantly different ($p>0.05$).

Conclusions: SAEW and 3% sodium hypochlorite are equally efficient irrigants against *E. faecalis* and *P. gingivalis* in necrotic primary molar teeth.

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(Key words: *Porphyromonas gingivalis*, *Enterococcus faecalis*, primary teeth, sodium hypochlorite, strong acid electrolysed water)

[1]*Postgraduate Student,* [2]*Professor,* [3]*Professor and Head,* [4]*Reader, Department of Pedodontics and Preventive Dentistry, College of Dental Sciences, Davangere, Karnataka, India*

**Correspondence:* drashwinabraham@gmail.com

https://orcid.org/0000-0003-0096-3029

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Introduction

Chronically infected deciduous root canals have always been a haven for anaerobic bacteria like *Enterococcus faecalis* (*E. faecalis*) and *Porphyromonas gingivalis* (*P. gingivalis*)[1,2]. A perfect root canal irrigant should be biocompatible, anti-bacterial, provide efficient lubrication for instrumentation and have debridement capability. Till today, we are using sodium hypochlorite (NaOCl) as an effective irrigant, even though aware of its toxic disadvantages[3,4]. The food industry reaped the benefits of electrolysed water, which is safe, effective, provides disinfection on contact and is also bactericidal against *E. faecalis* biofilms just like NaOCl. Strong acid electrolysed water (SAEW) has been investigated as a potential root canal irrigant in the recent past[3]. From what we know, there has not been any *in vivo* study on the effect of acidic electrolysed water on infected primary root canal flora.

Objectives

To assess the bactericidal ability of SAEW and 3% NaOCl against *E. faecalis* and *P. gingivalis* in infected deciduous molar root canals.

Method

This *in vivo* study was undertaken on 70 deciduous molar teeth in systemically healthy, 4 to 10 year[5] old children needing pulpectomy treatment. They were chosen randomly from the outpatient section in the Department of Paediatric and Preventive Dentistry, College of Dental Sciences, Karnataka, India.

Inclusion criteria consisted of systemically well children with periapical or furcal radiolucency[6], at least two carious primary molars indicated for pulpectomy, root resorption less than one third[2], sufficient tooth structure to support rubber dam[6] and primary posterior teeth with pulp necrosis.

Exclusion criteria included teeth with excessive mobility, traumatic injuries, too much root resorption, unhealthy periodontium, developmental anomalies and children who consumed antibiotics within the last three months[6].

Sample collection: After adequate local anaesthesia and scaling, rubber dam isolation (Figure 1) of the teeth was achieved and the tooth was wiped with 10% povidone iodine. After five minutes, isopropyl alcohol was rubbed on the tooth surface[5]. Slow and high speed burs were used to

excavate the dental caries. High speed, diamond, round bur (BR-31) flushed with sterile water was used to gain access into the pulp chamber. Chamber irrigation was done with 0.9% saline and disinfection with 3% NaOCl.

Figure 1: Pre-operative view

Widest canals were chosen for sample collection, distal and palatal canal in mandibular and maxillary molars respectively[2]. For the first microbiological sample (Sample 1), saline irrigation was followed by introduction of a number 20 Hedstroem file in a gentle filing motion, 1mm short of the apex approximately after cross checking the length on diagnostic radiograph. Sterile paper points were introduced into the canals for 60 seconds (Figure 2).

Figure 2: ***Sample collection***

These paper points were introduced into the aseptic Eppendorf tube containing reduced transport media. Instrumentation was then executed with Hedstroem files till size 40 in an alternating back and forth rotation motion. For each group, 2ml of respective irrigant in a 26-gauge sterile needle and syringe was used[5]. Irrigant used for group A (35 teeth) was 3% NaOCl. In group B (35 teeth) customized SAEW was used. After instrumentation of the canals, they were irrigated with 0.9% sterile saline. The second microbiological sample (Sample 2) was obtained by leaving a sterile paper point analogous to the canal diameter for 60 seconds. These paper points were transferred into sterile Eppendorf tubes with reduced transport media. Subsequently the opened cavity was sealed with an aseptic cotton pellet in the chamber and Glass ionomer cement (GIC) (Figure 3)[5], after instrumentation, irrigation and drying. Any high points were reduced if needed.

Figure 3: ***Post-operative glass ionomer cement (GIC) placement***

To assess the antimicrobial potential of the remnant irrigant in the canal, the patients were summoned after 48 hours[5,6]. The pulp chambers were exposed under rubber dam isolation and with the high speed diamond round bur (BR-31). The cotton pellet was removed from the chamber. The third sample (Sample 3) was obtained by placing a sterile paper point in the canal for 60 seconds and transferring it to the reduced transport media in the Eppendorf tube to send it to the clinical microbiology laboratory for culturing within 48 hours. This was followed by completion of the pulpectomy procedure.

Ethical issues: Ethical approval was obtained from the Institutional Ethics Committee of the College of Dental Sciences, Davangere, Karnataka, India (Ref. CODS/IEC/1810/2016-2017). Written informed consent was taken from the guardian or parent of the child before his or her inclusion in the study.

Statistical analysis: Friedman's test was used for intragroup comparison of the colony count from the pre-irrigation, post-instrumentation and post-48 hours of irrigation samples within both groups (Tables 1 and 3). Mann-Whitney U test was done for intergroup analogy (Tables 2 and 4). Wilcoxon matched pairs test was done for analogy of sample values in group I (Table 3). IBM Statistical Package for the Social Sciences (SPSS) version 24 was used for statistical analysis. The statistical significance level was fixed at 5% ($p<0.05$).

Table 1: ***Comparison of E. faecalis colony count at different time intervals in each study group***

Group	Sample	Number	Mean (SD)	Range	Median (Q1-Q3)	Friedman test	
						Chi square	p-value
I	1	35	16.86 (37.08)	0 - 150	0 (0 – 10)	0.75	0.69
	2	35	16.57 (38.57)	0 - 150	0 (0 – 0)		
	3	35	10.63 (37.40)	0 - 200	0 (0 – 0)		
II	1	35	21.29 (42.83)	0 - 150	0 (0 – 10)	2.90	0.24
	2	35	19.80 (50.14)	0 - 200	0 (0 – 0)		
	3	35	09.0 (27.26)	0 - 126	0 (0 – 1)		

*p<0.05 statistically significant

Table 2: ***Comparison of E. faecalis colony count between the study groups at each time interval***

Sample	Group	Number	Mean (SD)	Range	Median (Q1-Q3)	Mann Whitney U test	
						U statistic	p-value
1	I	35	16.86 (37.08)	0 - 150	0 (0 – 10)	579.50	0.63
	II	35	21.29 (42.83)	0 - 150	0 (0 – 10)		
2	I	35	16.57 (38.57)	0 - 150	0 (0 – 0)	579.50	0.81
	II	35	19.80 (50.14)	0 - 200	0 (0 – 0)		
3	I	35	10.63 (37.40)	0 - 200	0 (0 – 0)	583.50	0.64
	II	35	09.0 (27.26)	0 - 126	0 (0 – 1)		

Table 3: Comparison of *P. gingivalis* colony count at different time intervals in each study groups.

Group	Sample	Number	Mean (SD)	Range	Median (Q1-Q3)	Friedman test		Wilcoxon sign rank test (p-value) Comparing samples		
						Chi square	p-value	2-1	3-1	3-2
I	1	35	32.60 (53.96)	0-150	0 (0–100)	6.24	0.04*	0.25	0.01*	0.04*
	2	35	21.71 (49.85)	0-200	0 (0–20)					
	3	35	08.66 (30.02)	0-150	0 (0-0)					
II	1	35	54.86 (76.86)	0-250	0 (0–100)	5.16	0.08			
	2	35	29.63 (58.86)	0-200	0 (0–30)					
	3	35	19.20 (41.57)	0-150	0 (0–10)					

**p<0.05 statistically significant*

Table 4: ***Comparison of P. gingivalis colony count between the study groups at each time interval***

Sample	Group	Number	Mean (SD)	Range	Median (Q1-Q3)	Mann Whitney U test	
						U statistic	p-value
1	I	35	32.60 (53.96)	0 - 150	0 (0 – 100)	546.00	0.38
	II	35	54.86 (76.86)	0 - 250	0 (0 – 100)		
2	I	35	21.71 (49.85)	0 - 200	0 (0 – 20)	576.50	0.62
	II	35	29.63 (58.86)	0 - 200	0 (0 – 30)		
3	I	35	08.66 (30.02)	0 - 150	0 (0 – 0)	506.50	0.11
	II	35	19.20 (41.57)	0 - 150	0 (0 – 10)		

**p<0.05 statistically significant*

Results

In the present randomized *in vivo* split mouth study, no statistically significant differences were found between the groups. Statistical evaluation showed decreased colony numbers in samples from pre-irrigation, post-irrigation and post-48 hours for each organism in groups I and II. A statistically vital difference was noted in the reduction of *P. gingivalis* colonies in the group I from pre-irrigation to post-48 hours of irrigation ($p<0.05$) (Table 3). No significant disparity was noted between group I and group II (Tables 2 & 4).

Discussion

Bacterial synergism in the infected root canals may cause resistance to antimicrobial irrigating agents[7]. It is also clear that the gold standard irrigant NaOCl has toxic side effects[3,4]. Thus an alternative irrigating agent is the need of the hour in paediatric dentistry[6]. Studies show that microbes in permanent root canals are similar to that of primary

teeth[5]. *P. gingivalis* and the resistant *E. faecalis* were among the most frequent organisms in deciduous root canal infection[1,8]. Laboratory studies have been done on planktonic cultures of bacterial strains, but they least resemble the biofilm formation on the wall of infected root canals[9]. These facts encouraged us to go ahead with our study using an *in vivo* technique.

In our study, samples were obtained from the single largest necrotic root canal to narrow down the microbial growth evaluation to a single ecological condition[10,11]. To determine the antimicrobial effectiveness of any remaining irrigant in the canals after obtaining initial samples, the pulp chambers were sealed with cotton and Glass Ionomer cement for next 48 hours. The effect on the bacteria inaccessible to the irrigant during the initial irrigation could be evaluated[12]. *E. faecalis* and *P. gingivalis* were found to be surviving post irrigation according to Gomes *et al*.[13] Also, Oncag *et al*[5] suggest that *E. faecalis* count reduced more after 48 hours, thus backing the idea of collecting samples two days after the day of initial irrigation. Culturing makes it possible to quantify all major bacteria in samples and broad range prototype. Thus in our study it was decided to choose the culturing technique to determine and detect the presence of *E. faecalis* and *P. gingivalis*[14]. Root canal samples were transported for culturing in a reduced transport medium which reduced oxygen and prevented superoxide radicals which would kill anaerobic bacteria included in our study, especially *P. gingivalis* which is an anaerobic strain, unlike *E. faecalis* which is a facultative anaerobe[8].

E. faecalis was selected as an experimental species as it was an easily cultivable, non-fastidious organism associated with resistant cases of apical infection after treatment[14]. Antibacterial effects of new drugs and irrigants opposing *E. faecalis* are of interest as the organism is often isolated from treated root canals[4,9]. Black pigmented anaerobic rods like *P. gingivalis* have been associated with the signs of acute peri-radicular inflammation. Their characteristics create a disorder in the host immune response against it, thus potentiating tissue destruction[11]. Their resistance against the biomechanical irrigation and instrumentation may be the key reason they are found in treated canals, keeping an ability to reinstate the infection. *P. gingivalis* has been found in deciduous root canals which is similar to studies on adults, synergistically aiding in infection[7]. *E. faecalis* and *P. gingivalis* species were isolated often from toddlers and preschoolers with necrotic pulp, but could not be explained with ease as there is a scarcity of studies highlighting associations in deciduous teeth[7]. Bacterial associations between black pigmented bacteria and Enterococcus species was noted[15]. Thus we selected these two strains in our study.

NaOCl in higher concentrations has shown weakening consequences on the teeth[16]. A compromise in the dentinal micro hardness and modulus of elasticity was seen more when exposed to higher concentration of 5.25% NaOCl than a lesser concentration[17]. Concentrations close to 3% was found to be effective against, smear layer, predentin, debris, remaining pulp tissue, along with important organisms like *E. faecalis*, and *P. gingivalis*[18,19,20]. Hence a low effective concentration of 3%[21,22] was chosen as control. Strong acid electrolyzed water (SAEW) or electrochemically activated water or oxidative potential water has been slowly finding its role in this arena. The idea of electrolyzed water found its origin in Russia, where it was used for regenerating and decontaminating water. Initially, it was used in sterilizing instrument in medical institutes followed by areas of agriculture or livestock management[23]. It is hazardous for bacteria but safe to the human tissue.

Resistance to a thousand times is noticed in naturally formed biofilms than broth grown counterparts. *E. faecalis* biofilms have been detected in infected root canals[16]. According to Marias JT *et al*[12] electrolysed water is efficient against biofilms formed in the dental unit waterline. Anolyte solution has been effective against *E. faecalis* according to Cheng X, *et al*[3] and *P. gingivalis* according to Lee SH. *et al*[14]. Thus we used electrolysed water as an irrigant against *E. faecalis* and *P. gingivalis* in the experimental group. Our study exhibits that NaOCl and SAEW are efficient against *E. faecalis* and *P. gingivalis* counts in an infected deciduous tooth. Bacterial colony count was highest from the pre-irrigation sample, it reduced in the post-irrigation sample and the least colony count was found from the samples taken after 48 hours. This reflects the effectiveness of NaOCl and SAEW against the aforementioned organisms in a positive manner. SAEW is able to have a degrading effect on the existence of *E. faecalis* and *P. gingivalis*. On intergroup comparison no statistically significant difference was noted among the groups. This is because both these irrigants are exerting similar effectiveness in lowering the bacterial count from the first pre-irrigation sample to the last sample taken after 48 hours.

When *P. gingivalis* colony counts were compared, a declining trend was noticed in the NaOCl as well as SAEW group. The comparison of the colonies in the NaOCl group showed statistically significant difference. A noteworthy reduction was noted in *P. gingivalis* samples taken before and after irrigation

when compared to the post 48 hour sample in the NaOCl group. Dhariwal *et al*[21] mentions in his culture study that obligatory anaerobes like *P. gingivalis* are more sensitive to NaOCl than facultative anaerobes like *E. faecalis*. Stojanovic *et al*[24] through his molecular study states that such gram negative organisms are eliminated with low concentration of NaOCl. Sakamoto *et al*[25] through their culture study shows that *P. gingivalis* failed to thrive well after detecting positive growth in the first samples were taken.

Similar to our results, Cheng X, *et al*[3], Zan R, *et al*[26] and Gomi K, *et al*[27] found SAEW to be equally effective as NaOCl against *E. faecalis* and had a better smell than NaOCl. On the contrary, Mena-Mendivil *et al*[28] through their *in vitro* study showed that NaOCl gave better results than electrolyzed water against *E. faecalis* and *P. gingivalis*. Our results are also similar to studies by Lee SH, *et al*[12], Chen CJ, *et al*[29], Mena-Mendivil ED, *et al*[28], and Kim SB[30] which show that electrolysed water is effective against *P. gingivalis*. In our study, *E. faecalis* was seen in 45.7% of the cases while *P. gingivalis* was seen in 54.3% of cases of primary molars. *E. faecalis* was found by Chandwani M, *et al*[31] in 30%, Cogulu D, *et al*[1] in 16%, Oncag O, *et al* in 63%[32] and Siqueira JF Jr, *et al*[33] in 7.5% in primary root canals. *P. gingivalis* was detected by Cogulu D, *et al*[1] in 16%, Gomes GB, *et al*[2] in 100%, Yang QB, *et al*[34] in 13.9% and Fabris As, *et al*[7] in 49% in the primary root canal. Still, a significant amount of bacteria prevailed following irrigation. A rise in resistance against antimicrobial irrigants used in paediatric dental practice seems to enhance due to the synergism of *E. faecalis* and *P. gingivalis* strains[7]. Hence, the biocompatible and safe SAEW seems promising as an ideal irrigant for deciduous root canals, especially for pediatric dental patients.

The strength in this study lies in the fact that this is an *in vivo* study which took bacterial samples from the oral conditions. Through this study, a safer antibacterial alternative for root canal irrigation was observed in SAEW, especially for paediatric dental practice. The limitations of the study are that it had a small sample size and that patients were not evaluated with follow up after completion of pulpectomy. Since our study is among the very few studies done on primary molars using SAEW, more research with a larger sample and follow up is needed before coming to a definite conclusion.

Conclusions

1. Sodium hypochlorite is an efficient irrigating agent against *E. faecalis* and *P. gingivalis* in infected deciduous molar root canals. However it showed better declining effect on *P. gingivalis* colony count.
2. Strong acid electrolysed water is an efficient irrigating agent against *E. faecalis* and *P. gingivalis* in deciduous molar root canals.
3. On comparison of the two irrigants for their efficacy against *E. faecalis* and *P. gingivalis,* results showed both to be equally effective.

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