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ANTIMICROBIAL EFFICACY OF OZONATED WATER AND COMPARE THE ANTIMICROBIAL ACTIVITY OF DIFFERENT IRRIGANT SOLUTIONS AGAINST E.FAECALIS AND E.COLI

Dental Science

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ABSTRACT

Aim: To determine the antimicrobial efficacy of ozonated water and compare the antimicrobial activity of different irrigant solutions against E.faecalis and E.coli.

Material and Method: After incubation, 21 contaminated roots were divided into 3 groups according to the irrigation regimen used i.e. Group A: Ozonated Water (7 teeth), Group B: 3% NaOCl (7 teeth) and Group C: Saline (7 teeth). All the teeth were handled with sterile gloves and sterile tweezers to prevent contamination. A sterile 5 mL syringe with 26 gauge needle was used to deliver irrigant into the canal. All experimental teeth were then flushed with distilled water to prevent potential carry-over of irrigants.

Results: Results indicated that Group C showed highly significant difference from group A and Group B indicating that Group A and Group B were effective against both E.faecalis and E.coli. Results also show that there was no significant difference in efficacy of Group A and Group B.

Conclusion: The results found that ozonated water has proved to be an alternative antimicrobial agent.

KEYWORDS

E.faecalis, E.coli, Ozonated Water

INTRODUCTION:

Enterococcus faecalis is a facultative anaerobe and a persistent organism found in a high percentage of root canal failures and it is able to survive in the root canal as a single organism or as a major component of the flora. The prevalence of E. faecalis in primary endodontic infection is 40% and in persistent endodontic infection 24 to 77%[1].

Instrumentation has a key role in the cascade of treatment procedures to eradicate microbes in the root canal system. Thus, root canal treatment of teeth with pulp necrosis and periapical lesions should not only sacrifice bacteria, but also remove the dead cells and/or promote the inactivation of the lipid A (the toxic portion of the endotoxin)[2].

Several irrigating solutions, such as Chlorhexidine (CHX) and sodium hypochlorite, are used during endodontic treatment[3]. The effectiveness of irrigation depends on the working mechanism(s) of the irrigant and the ability to bring the irrigant in contact with the microorganisms and tissue debris in the root canal. Broadwater et al[4] in 1973 reported that ozone at low concentration, 0.1 ppm, is sufficient to inactivate bacterial cells including their spores. Ozone dissociates readily back into oxygen (O_2), thus liberating so called singlet oxygen (O_1), which is a strong oxidizing agent. It is this particular reactivity that provides the starting point for ozone's therapeutic effects on the body.

Although ozonated water is a powerful antimicrobial agent against bacteria, fungi, protozoa and viruses, less attention has been paid to the antibacterial activity of ozonated water in bacterial biofilm and hence in root canal infection[5]. Therefore the aim of this study was to determine the antimicrobial efficacy of ozonated water and compare the antimicrobial activity of different irrigant solutions against E.faecalis and E.coli.

Materials and method: This study was conducted in the Department of Pedodontics. The microbiological media used were Brain Heart Infusion Broth, Brain Heart Infusion Agar Media and Macconkey Agar Media

Inclusion Criteria For Selection Of Teeth

1.Single rooted anterior teeth
2.Teeth With complete root formation

Exclusion Criteria

1.Teeth With open apices
2.Calcified canals
3.Multi rooted teeth

Preparation Of Samples

- 21 extracted non-carious, single rooted human incisors which were extracted for periodontal reasons were used in this study.
- Calculus and tissue tags were removed using hand and Ultrasonic scaling.
- The teeth were soaked in 5% NaOCl for 30 minutes to remove any remaining residual loose tissue and debris from the root surface.
- The teeth were stored in gauze soaked sterile saline till use to prevent dehydration.
- All the teeth were marked and then sectioned 14 mm from the apex with a carborundum disc using a low speed straight hand piece, so as to standardize roots of all the teeth approximately to the same length.

Instrumentation

- An ISO #15 K file was used to determine the working length.
- The root segments were mounted in wax bases for ease of instrumentation.
- All root canals were instrumented, using the stepback technique and the circumferential filing motion, to K file #60.
- During cleaning and shaping, sterile distilled water was used after each instrument size.
- The segments were then removed from the wax bases.

Sealing Of Root Apices

·Finally, the canals were flushed with 5 mL of distilled water to remove any debris.
·The root apices were sealed with type II GIC and coated with two coats of nail varnish to prevent bacterial leakage.

Inoculation

·The bacterial strains used in this study are Enterococcus faecalis (MTCC 439) and Escherichia coli (ATCC 25922).
·The primary culture was raised by inoculating Enterococcus faecalis (MTCC 439) and Escherichia coli (ATCC 25922) in the Brain heart infusion (BHI) broth, after incubation at 37°C for 24 hrs.
·The canals of the experimental teeth were cautiously inoculated using a micropipette with 20 µL of the freshly prepared suspension of both the organisms and sterile #15 K file was used to carry the bacterial suspension to the entire root canal length.
·The teeth were then incubated at 37°C for 72 hours.

Root Canal Irrigation

After incubation, 21 contaminated roots were divided into 3 groups according to the irrigation regimen used i.e. Group A: Ozonated Water (7 teeth), Group B: 3% NaOCl (7 teeth) and Group C: Saline (7 teeth). All the teeth were handled with sterile gloves and sterile tweezers to prevent contamination. A sterile 5 mL syringe with 26

gauge needle was used to deliver irrigant into the canal. All experimental teeth were then flushed with distilled water to prevent potential carry-over of irrigants.

COLONY FORMING UNIT /ml=

Number of colonies obtained X Dilution Factor

Volume of sample inoculated

RESULTS:

Table 1: Pre And Post Irrigation Comparison Within Group Using Paired T-Test

Group Name			N	Mean	SD	P-Value
GroupA	Pair1	E.faecalisPre-Irrigation	10	9.19	3.25	0.005
		E.faecalisPost-irrigation	10	1.60	1.56	
	Pair2	EcoliPre-Irrigation	10	4.29	4.09	0.02
		EcoliPost-irrigation	10	0.89	1.00	
GroupB	Pair1	E.faecalisPre-Irrigation	10	2.00	0.84	0.001
		E.faecalisPost irrigation	10	0.28	0.27	
	Pair2	E.coliPreIrrigation	10	0.50	0.21	0.02
		E.coliPost-irrigation	10	0.11	0.07	
GroupC	Pair1	E.faecalisPre-Irrigation	10	4.07	1.60	0.001
		E.faecalisPost-irrigation	10	3.18	1.22	
	Pair2	E.coliPre-Irrigation	10	0.63	0.28	0.01
		E.coliPost-irrigation	10	0.52	0.27	

P<0.05:Statistically Significant

Table 1 shows Pre and post irrigation comparison within group using paired t test, whose results indicate that there was a significant reduction in post irrigation values in all the three groups.

Table 2: Comparison Of Mean Percentage Reduction Among Three Different Groups Using Post HocTest.

Dependent variable	GroupName(I)	GroupName(J)	pvalue
E.faecalis % age reduction	GroupA	GroupB	0.76
		GroupC	<0.01
	GroupB	GroupA	0.76
		GroupC	<0.01
	GroupC	GroupA	<0.01
		GroupB	<0.01
E.coli % age reduction	GroupA	GroupB	0.81
		GroupC	<0.01
	GroupB	GroupA	0.81
		GroupC	<0.01
	GroupC	GroupA	<0.01
		GroupB	<0.01

P<0.05: StatisticallySignificant

Table 2 indicates pairwise comparison between three groups using Post Hoc analysis with respect to percentage reduction in CFU post irrigation. Results indicate that Group C showed highly significant difference from group A and Group B indicating that Group A and Group B were effective against both E.faecalis and E.coli. Results also show that there was no significant difference in efficacy of Group A and Group B.

DISCUSSION:

The main cause of endodontic failure is the persisting infection in the root canal system. We are constantly searching for new antimicrobial agents that can provide us with a cleaner root canal and thereby improve the success rate of endodontically treated teeth.

Of all the currently used irrigants, sodium hypochlorite appears to be the most ideal, as it covers more of the requirements for endodontic irrigant than any other compound. Various investigations have shown that NaOCl might irritate the periodontal and periapical tissues (Spangberg et al 1973)[6]. The biocompatibility problems associated with the use of sodium hypochlorite have led to the use of substances with known antimicrobial properties.

Ozone has been discussed as a possible agent when treating root canals, but the antimicrobial effects on the oral biofilm are questioned. Most studies[7,8] on this subject are in vitro and they show various results. In this study we used bacterial sampling to indicate the presence of infection in the canal. All the teeth treated with ozonated water showed a positive reduction in bacterial growth. This study showed that there was no significant difference in reduction of infection if ozonated water or 3% sodium hypochlorite was used during irrigation. The difference between the groups is small to show a significant difference. Nagayoshi et al (2004)[9] also showed that ozonated water had nearly the same antimicrobial activity as 2.5% NaOCl during irrigation, especially when combined with sonication, and showed a low level of toxicity against cultured cells.

Teeth treated without ozone i.e., with 3% sodium hypochlorite which showed equally good results. In fact some samples of this group have shown 100% reduction in bacterial count. However, Hypochlorite is acutely operator sensitive, requiring careful application during root canal cleaning to prevent inoculation through the apex into bone or soft tissue, which can cause alarming and destructive oedema, pain and tissue damage, whereas ozone has therapeutic effect on oral tissues. Nagayoshi et al[9] in 2004 along with antimicrobial activity also compared the cytotoxicity against L-929 mouse fibroblasts between ozonated water and NaOCl. They found that the metabolic activity of fibroblasts was high when the cells were treated with ozonated water, whereas that of fibroblasts significantly decreased when the cells were treated with 2.5% NaOCl.

CONCLUSION:

Keeping in mind the limitations ozonated water has proved to be an alternative antimicrobial agent. Probably increasing the ozone concentration will prove to be a better root canal irrigant without any significant side effects.

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