

IN VITRO EVALUATION OF INTRACANAL BACTERIAL REDUCTION USING TWO DIFFERENT INSTRUMENTATION TECHNIQUES AND IRRIGATION REGIMENS

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ABSTRACT

BACKGROUND

Enterococcus faecalis, a facultative anaerobic Gram-positive coccus is involved in the endodontic failures. The bacterial elimination from the infected root canal is often achieved by mechanical cleaning and shaping along with irrigants. This study compares the intracanal bacterial reduction using two instrumentation techniques and irrigation regimens.

METHODS

50 extracted human mandibular bicuspid teeth with single canal were decoronated at cemento-enamel junction and pulpectomy done. Working length determined and apical foramen sealed with acrylic resin and specimens autoclaved at 121^o centigrade for 20 minutes. Samples were divided in to six groups. Group I - Hand instrumentation with 0.9% saline irrigant; Group II - Hand instrumentation with 5% sodium hypochlorite as irrigant; Group III - Rotary instrumentation with 0.9% saline irrigant; Group IV - Rotary instrumentation with 5% sodium hypochlorite as irrigant; Group V - Control-Only saline irrigation; Group VI - Samples taken immediately after sterilization. Sterilized teeth infected with *E. faecalis* and incubated for one day at 37^o centigrade. Samples were collected from the canals before and after instrumentation and irrigation. The colony forming units were then counted and transformed to log numbers and analysed statistically.

RESULTS

The reduction in number of colony-forming units was statistically significant. Statistical analysis reveals bacterial reduction in the following order GIV>GIII>GII>GI>GV.

CONCLUSION

Bacterial reduction is higher with greater taper (0.06 mm/mm) instrumentation and it is enhanced with the use of 5% sodium hypochlorite compared to 0.9% saline solution.

KEYWORDS

Hand Files 0.02%, Rotary Files 0.06%, 0.9% Saline, 5% Sodium Hypochlorite, *E. faecalis*, Colony Forming Units.

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INTRODUCTION: Periradicular lesions develop mainly due to pulpal infection and elimination of bacteria, their substrate and their by-products from the pulp canal should be the primary intent of the root canal therapy.

In root canal disinfection, chemo-mechanical preparation is considered as an essential step.¹ A region of canal 0.25 mm in diameter and 1 mm long can contain approximately 80,000 Streptococci. This is quite sufficient to

produce an inflammatory reaction.² The back and forth flow of the irrigant solution and the mechanical action of instrument helps in removal of irritants in the root canal and subsequently bacterial cells are removed by antibacterial irrigants.¹

During endodontic treatment of infected root canals, bacterial organisms are reduced by the mechanical instrumentation. Although Nickel-Titanium instruments, considered less efficient in cutting yet most commonly used, owing to its superior flexibility, great resistance to torsional fracture and modulus of elasticity.¹

0.5% to 0.6% sodium hypochlorite solution was used as antiseptic in open and infected wounds by Dakin. Coolidge introduced the usage of Sodium hypochlorite as a root canal irrigant in 1919³ which was followed by Walker who recommended that double strength chlorinated soda (5% Sodium hypochlorite) solution as another canal irrigant.

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However, in endodontic practice, Sodium hypochlorite has been found to be the most effective irrigant till date.³

AIMS AND OBJECTIVES: This study was performed to compare intracanal bacterial reduction provided by hand and rotary instrumentation, using two different irrigants-0.9% saline and 5% Sodium hypochlorite as canal irrigant. In this study, *Enterococcus faecalis* (ATCC29212) is used because of its supposed involvement in endodontic failure.

METHODOLOGY: The study included 50 extracted human mandibular bicuspid teeth with single canal. After pulpectomy, the teeth were decoronated at the level of cemento-enamel junction. The working length was established using 15 size K-file and irrigated with tap water. Followed by this the apical foramen was sealed using self-cure acrylic resin in order to prevent bacterial leakage. For the ease of handling and identity the teeth were mounted vertically in plaster blocks. Individual specimens were wrapped using Kraft paper. Followed by this, the prepared teeth were autoclaved at 121° centigrade for 20 minutes. Bacterial suspension prepared in tubes which is equivalent to McFarland's 0.5 standard is prepared by adding 1% solution of anhydrous BaCl₂ and a cold 1% v/v solution of pure H₂SO₄.⁴

Sterilized teeth were opened under laminar airflow. The prepared canals were completely filled with suspension of *Enterococcus faecalis* using Sterile No. 15 files, again wrapped with the same Kraft paper and incubated for 1 day at 37° C.

The study materials were divided into six groups based on instrumentation and irrigation techniques.

Group I: Hand instrumentation done using Ni-Ti files with 0.9% saline as irrigant.

Group II: Hand instrumentation done using Ni-Ti files with 5% Sodium hypochlorite as irrigant.

Group III: Rotary instrumentation done using 0.9% saline as irrigant.

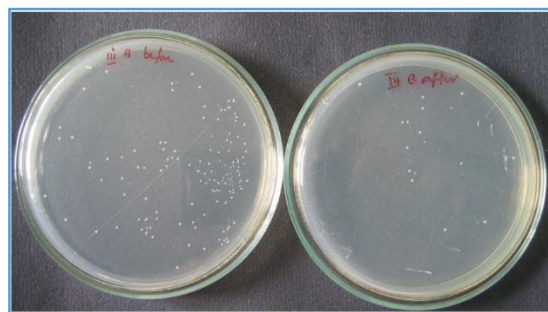
Group IV: Rotary instrumentation done using 5% Sodium hypochlorite as irrigant.

Group V: Control-Only saline irrigation done.

Group VI: Sample taken immediately after sterilization.

The samples were collected from the canals for nutrient agar culture using paper points before instrumentation. The step-back technique using Ni-Ti flex files was performed in Group I and Group II. In group III and Group IV, root canals were prepared with rotary files in a crown down manner as recommended by the manufacturer. In Group V, no instrumentation done and canals were irrigated with 0.9% Saline. In Group VI, samples were taken immediately after sterilization. Before instrumentation canals were filled with saline. In Group I, II, III, IV the pulp canals were irrigated using 0.5 ml of the test solutions after the usage of each file. A final irrigation using 0.5 ml of saline solution was performed. However, each root canal was always irrigated with a total volume of 3 ml solution. After instrumentation, the samples were taken from the root canals for culture using No. 15 paper points. The colony forming units were

then counted and transformed to log numbers and analysed statistically.



RESULTS: The quantities of bacterial colony forming units before and after instrumentation are presented in table.

Group I	Before	After
1.	200	75
2.	90	52
3.	57	22
4.	200	50
5.	195	69
6.	88	34
7.	76	19
8.	200	120
9.	160	56
10.	250	88

Table 1A

Group II	Before	After
1.	80	45
2.	110	25
3.	180	17
4.	250	20
5.	73	23
6.	56	13
7.	60	40
8.	43	NIL
9.	118	28
10.	107	35

Table 1B

Group III	Before	After
1.	95	NIL
2.	38	3
3.	15	NIL
4.	120	3
5.	80	2
6.	112	13
7.	55	6
8.	89	11
9.	120	3
10.	40	NIL

Table 1C

Group IV	Before	After
1.	90	NIL
2.	80	13
3.	72	NIL
4.	250	NIL
5.	25	NIL
6.	160	NIL
7.	54	2
8.	81	NIL
9.	60	NIL
10.	130	NIL

Table 1D

Group V	Before	After
1.	150	105
2.	105	60
3.	34	23
4.	70	36
5.	45	32
6.	102	61
7.	18	13
8.	43	27
9.	120	75
10.	120	96

Table 1E

Samples taken immediately after sterilization	NIL
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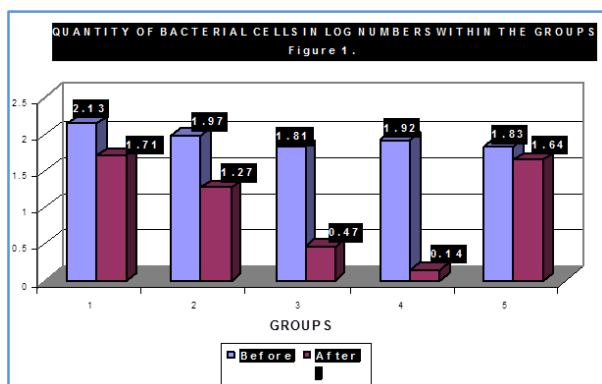
Table 1F

1. Paired "t" test was used to determine whether the difference between the mean log₁₀ values. The level of significance was set at 0.05 for all analyses.

Groups	Initial (log numbers)	Final (log numbers)	Reduction % (Mean of log numbers)
I	2.13	1.71	20.05
II	1.97	1.27	35.85
III	1.81	0.47	74.27
IV	1.92	0.14	92.65
V	1.83	1.64	10.24

Table 2

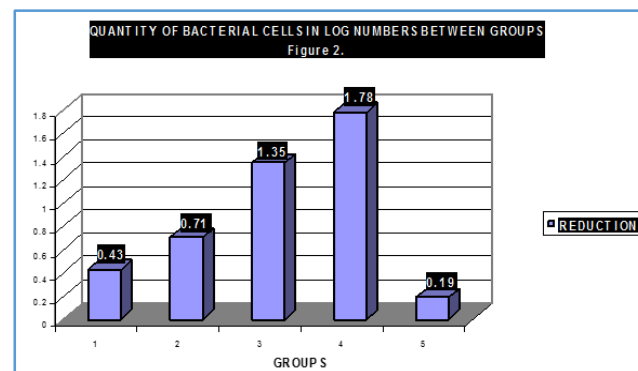
Reduction percentage of bacterial cells in mean of log numbers from highest to lowest is as follows GIV>GIII>GII>GI>GV (figure 1).



Unpaired "t" test were used to determine whether the difference between the mean log₁₀ values between the groups was 0 (figure 2).

Groups	Mean	Standard deviation
I	0.43	0.13
II	0.71	0.44
III	1.35	0.37
IV	1.78	0.47
V	0.19	0.06

Table 3



All the values obtained were analysed statistically, a statistically significant difference detected between GI and GIII, GII and GIV, GI and GV, GI and GIV, GII and GIII, GII and GV, GIII and GIV, GIII and GV, GIV and GV (P<0.05).

No statistically significant difference was detected between GI and GII. (P>0.05).

GI	GII	GIII	GIV	GV
NS				

Table 4

S- Significant (P<0.05).

NS- Not Significant (P>0.05).

DISCUSSION: The major aetiology of Pulpal and Periapical pathology are microorganisms and their end products. Bystrom and Sundqvist⁵ stated that "mechanical instrumentation reduced bacteria from human root canals by only 50%". Enterococcus faecalis, a Gram-positive facultative anaerobe is a commensal of the oral cavity and gastrointestinal tract.¹

The use of *Enterococcus faecalis* can be justified owing to its repeated resistance to chemo-mechanical procedures. It has been found to play a role in endodontic failures. Also it is relatively easy to culture and manipulate the organism. During endodontic treatment³, bacterial reduction or elimination can be achieved by chemo-mechanical preparation. The mechanical action of instruments, flow and back flow of the irrigant solution helps in removing the pulp canal irritants.

Regardless of the irrigant used, the bacterial population inside the root canal can significantly be reduced by the mechanical effect of irrigation. However, studies have revealed that chemical compounds that possess anti-bacterial effects show clearly superior effectiveness in bacterial elimination when compared with the routine saline solution.

Studies have established Sodium hypochlorite destroying vegetates, spore forming bacteria, protozoa, fungi and viruses, thereby it acts as a broad spectrum antimicrobial.⁶

Sodium hypochlorite acts by oxidation that destroys the activity of amino acids such as cysteine, methionine and sulfhydryl groups. Sodium hypochlorite can also have deleterious effect on bacterial DNA by forming chlorinated derivatives of the nucleotide bases. In addition, Sodium hypochlorite has also been reported to induce bacterial membrane disruption. It is a well-known fact that an increase in NaOCl concentration in a solution brings a corresponding increase in antibacterial activity as long as the temperature, pH and organic content are being held constant.⁶

In this study, two different instrumentation techniques and irrigation materials were used and their antimicrobial efficiency evaluated. The results clearly reveal that the amount of bacterial reduction is more in Group IV (rotary instrumentation with NaOCl).

In Group IV, crown down preparation done using Ni-Ti instruments and canal size enlarged to 0.06/30. The following factors play an important role in maximizing bacterial reduction. The cross sectional geometrics of rotary instruments have three radial lands separated by three 'U' shaped configuration aids in effective debridement of the canal.⁷ Kavanaugh and Lumley et al found that the use of 0.06 taper improved canal shape.⁸ Research evaluating canal cleanliness had clearly shown that preparations need to taper at least 0.08 mm/mm-0.10 mm/mm to ensure that a sufficient volume of irrigant can efficiently circulate into canal anatomy.⁹ The use of orifice shapers 0.07 and 0.06 mm/mm facilitate shaping by removing the restrictive dentin,¹⁰ and by pre-flaring the coronal two third of the canal.¹¹

Removal of restrictive dentin and pre-flaring the coronal two third of the canal allows the irrigation needle to be placed deep in the canal and thereby have more flushing action, and it also allows effective volume of irrigant to work deeper. In this group Sodium hypochlorite is used as an irrigant, apart from flushing action, sodium hypochlorite has antimicrobial action and tissue dissolving property. Dr. Elio Berutti and his team in 1990s demonstrated that Sodium

hypochlorite cleans a root canal system after shaping, and it can penetrate deep into the dentinal tubules when used at the correct temperature and concentration at an appropriate amount of time.¹²

In Group III (rotary instrumentation with Saline), bacterial reduction is mainly achieved by instrumentation and mechanical action of irrigant. In Group II (Hand instrumentation with NaOCl), canals were treated by only hand instrumentation with a taper of 0.02 mm/mm, which is two times lesser taper than rotary instruments. Therefore, instrumentation by hand files results in poor canal shape, working time for the penetration and circulation of irrigant is reduced, which results in lesser bacterial reduction. Group I (Hand instrumentation with Saline), received 0.9% Saline solution as irrigant has no antibacterial effect on *Enterococcus faecalis*.⁶ Group V (only saline irrigation), bacterial reduction is lesser than other groups. This group received no instrumentation at all and also canals irrigated with 0.9% saline only. Bacterial reduction in the group is mainly due the mechanical action of irrigants.

CONCLUSION: The results of this study clearly indicates that the quantity of bacterial reduction is higher when the root canals are instrumented with greater taper (0.06 mm/mm) rotary files compared to conventional ISO (0.02 mm/mm) taper Ni-Ti Flex files.

In addition, the bacterial reduction is enhanced with irrigation of 5% Sodium hypochlorite as compared to a 0.9% saline solution.

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