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COMPARISON OF ANTIBACTERIAL EFFICACY OF CHLORHEXIDINE AND SODIUM HYPOCHLORITE AT TWO DIFFERENT CONCENTRATIONS AGAINST E.FAECALIS: AN IN -VITRO STUDY

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ABSTACT

Introduction: The purpose of this In -Vitro study was. **Aim:** To compare the antimicrobial efficacy of two different root canal irrigants two different root canal irrigants at two different concentrations (Chlorhexidine- 0.2% and 2% and NaOCl- 0.5% and 2%) against E.Fecalis in primary tooth- In-Vitro study. **Materials and Methods:** Sixty extracted human mandibular second primary molars were divided into four equal groups. Group-A-chlorhexidine- 2%, Group-B- Chlorhexidine-0.2%, Group-C- NaOCl-2.5% and Group-D- NaOCl-0.5%. Colony forming units were counted in order to check for the antibacterial efficacy of these root canal irrigants, against E.faecalis. **Results:** Before treatment and after treatment of every single sample were compared and between the groups. Colony forming units were counted and tabulated. Chi-square test and SPSS software were used for the statistical analysis of the results. Paired T-test with SPSS 15.0 version for windows, Kruskal-Wallis, Non-Parametric test was selected, as the mean value is less than two times of standard deviation. Post Hoc Test with Turkey HSD was calculated at $p<0.05$ for multiple comparison between the groups. Chlorhexidine-2% is very good antimicrobial irrigating solution against E.Faecalis amongst the tested irrigants as the colony forming units were reduced to the maximum when compared with other groups. **Conclusions:** Treatment protocols using irrigation with either NaOCl or CHX succeeded in significantly reducing the number of bacterial taxa and their levels in infected root canals, with significant difference between their concentrations. Chlorhexidine-2% and Sodium hypochlorite-2.5% have a good potential to keep a low E.Faecalis colony forming units count compare to the Lower concentration. **Clinical Significance:** Feasibility of Root canal irrigants, antibacterial efficacy at lower concentration in primary tooth were tested. Also, antibacterial reduction at different concentration of sodium hypochlorite and chlorhexidine will guide us in selection, for safe and effective concentration.

KEYWORDS: Antibacterial efficacy, Chlorhexidine, E.faecalis, Sodium Hypochlorite.

INTRODUCTION

Root canal treatment may be defined as the complete removal of the irreversibly damaged pulp followed by thorough cleaning, shaping and obturation of root canal system, so that the tooth may remain as a functional unit within the dental arch.[1] Access cavity preparation, biomechanical preparation and obturation of root canal constitutes the endodontic triad. Of these three steps biomechanical preparation plays a major role in determining the success of treatment.[2]

The morphology of the root canals in primary teeth makes endodontic treatment difficult and often impractical. If the canal cannot be cleansed of necrotic material, sterilized, and adequately filled, endodontic therapy is likely to fail.[3]

Though the chemo mechanical preparation and use of antimicrobials are effective in reducing the bacterial load, some bacteria can still persist.[3] Enterococcus Faecalis is one among the facultative organism which is persistently found in root canal failures[4] and is resistant to various intracanal medicaments.[5] The microorganism, found in the root canals of deciduous teeth are similar to those in the root canals of permanent teeth.[6,7]

Due to the mechanical complexities of many root canals, even after mechanical procedures, organic residues and bacteria loaded deep in the dentinal tubules cannot be reached. Therefore, various irrigating solutions have been used during and immediately after the root canal preparation to remove debris and necrotic pulp tissue and

to eliminate microorganism that cannot be reached by mechanical instrumentation.[6]

It is highly desirable that the chemical agents selected as endodontic irrigants possess various major properties such as gross debridement, sterilization or at least disinfection, tissue or debris solvent, non-toxicity to periapical tissue, low surface tension, lubricant, removal of smear layer and antimicrobial activity.[8] Additionally, they should also have low viscosity, for a better penetration depth, long duration of action and easily available.

Numerous solutions have been recommended as root canal irrigants such as distilled water, saline, hydrogen peroxide, sodium hypochlorite, calcium hydroxide, iodine potassium iodide, povidone iodine, chlorhexidine gluconate, MTAD and citric acid.

The most popular endodontic irrigant is sodium hypochlorite, it dissolves necrotic pulp tissue and denatures collagen. Although it is an effective antimicrobial agent and an excellent organic solvent it is known to be highly irritant to the periapical tissues at high concentrations.[9]

Even though, it has been widely used in endodontics as an irrigant, there is no consensus regarding the ideal concentration to be used. The risk benefit ratio should be considered during the choice of the irrigant. Data concerning the anti-microbial effectiveness of different sodium hypochlorite concentrations have also revealed conflicting results. Some clinical studies have not found any clinical significant difference in anti-bacterial effect between 0.5% and 5%. In contrast other studies have reported that the antibacterial effectiveness of sodium hypochlorite is significantly reduced after dilution.

Chlorhexidine in the chemical form is a cationic bis-biguanide that is usually marked as a gluconate salt. The most common preparation is with the digluconate salt because of its stability and high-water solubility. It has that in cell lysis property at higher concentration. Chlorhexidine contains 1, 6-bis-p-chlorophenyl biguanidohexane and been used for endodontic disinfection.

Chlorhexidine has been recommended as a root canal irrigant because of its broad-spectrum antimicrobial action, substantivity, and low grade of toxicity,[10] and it is active against gram-positive and gram-negative bacteria, spores, viruses and yeast. However, the inability of chlorhexidine to dissolve pulp has a problem. At low concentrations, chlorhexidine is only bacteriostatic, and at high concentration, bactericidal effect. Many of the In-vitro studies have demonstrated the antibacterial effect, depends on concentration of the solution used and, alternating between specific types of intracanal solution or using them in combination has been shown to improve the potential for cleaning.[11]

But at higher concentration during irrigation, accidental extrusion into periapical region may affect the permanent tooth bud formation. So, whether at lower concentration, is it feasible to eliminate microorganism in primary tooth and also to compare the antibacterial reduction at two different concentration using sodium hypochlorite (0.5% and 2.5%) and chlorhexidine (0.2% and 2%) against E.faecalis, the current In-Vitro study was conducted.

MATERIALS AND METHODS

The present study was conducted in the Department of Pedodontics and Preventive Dentistry, Faculty of Dental Sciences, Sri Ramachandra University, Chennai. Ethical committee clearance was obtained and sixty extracted human mandibular second primary molars were collected and stored in formalin, and any attached soft tissue and calculus were removed with an ultrasonic scaler. The storage and handling of the teeth were performed as per Occupational Safety and Health Administration guidelines and regulations. Collected second primary molars were extracted due to non-restorability, parents request as they were not willing for pulp therapy.

Inclusion Criteria involves, Mandibular second primary molars teeth with intact distal root. Minimum 2/3rd of distal root length. Tooth extracted without any crown and root fracture.

Exclusion criteria – extracted tooth with fractured crown and root. Tooth with resorbed roots. The selected teeth were then divided into four equal groups -15 in each group, according to the root canal irrigants used. Group-A(15): chlorhexidine-2%, Group-B(15): chlorhexidine-0.2%, Group-C(15): sodiumhypochlorite-2.5%, Group-D(15): sodiumhypochlorite-0.5%.

Completed 60 tooth samples were packed into autoclave packs and sealed. Dry heat sterilization was done, and the sterile tooth samples were transferred to Department of Microbiology, Sri Ramachandra University, Chennai. Five tooth samples were selected randomly from sterilized 60 tooth samples and immersed into broth solution and placed in an incubator for twenty-four hours. It was done to provide a suitable environment for any viable organisms to grow. toIt was done to Absence of turbidity confirms the proper sterilization of tooth samples. Preparation of chlorhexidine 0.2% was done by diluting the concentrated 2% in isopropyl alcohol (Analytical Reagent) and 0.5% and 2.5% Sodium hypochlorite by diluting the concentrated 5.25% in water for injection.

Standard access cavities were made for all teeth. The distal root canal for all teeth was negotiated by number 10 handheld stainless-steel file. Distal root of primary mandibular second molar were preferred, since they generally have a curved, large, single root canal with a uniform canal outline and relatively less intracanal ramifications compared to the mesial roots. The conventional method of canal debridement was done

used with Stainless steel 'H-File' instrument with a push-pull along with and irrigation done with saline .irrigation and the Root canals were dried using with paper points. and apex was sealed with root canal sealer (APF). Due to the resorption nature of root apex, apical seal is done using root canal sealer (APF) to contain the broth solution.

Microbiological Process of Tooth Samples was done by immersing selective tooth samples in BHI broth and stored in incubator. Absence of turbidity proved the proper sterilization. BHI broths were prepared and contaminated with Enterococcus Faecalis, which was procured from Department of microbiology. And stored in an incubator prior to the inoculation procedure. Sterile McCartney bottles were packed with sterile gauze in an aseptic chamber and the tooth samples were mounted on top of it and wrapped around with aluminium foil. Contaminated broth was dropped into all 60 the tooth samples at the same time using Insulin syringe in an aseptic condition. These bottles were closed with the respective Aluminium foils of the same bottles and kept into the incubator at 2^{o} for a period of one week. For a total period of seven days, all the sixty tooth samples were kept in the incubator to maintain the vitality of the organism. During every alternate day the tooth samples were taken out and contaminated broth solution were added, to provide better nourishment. Totally three times of broth replacement was done during the whole procedure. That is on Day-1; initially organism inoculation was done, followed by that, Day-3, Day-5 and Day-7.

After one week in incubator, tooth samples of all four groups were placed in aseptic chamber and paper points were introduced into the root canal and the sample was collected to an ependorff tubes containing clear BHI broth, to check for the viability of organism. Viability check was done in all the sixty samples and organism count was also done in colony forming units. Prepared root canal irrigants were loaded into the 5ml syringe and Canal Clean 30-gauge needle with close-end tip and side-port opening. A contact period of 5mins was given after irrigating with all the four different irrigants for four different groups. After 5 mins of contact time, canal dried and Gates gliden drill was introduced into the distal canal till apical third and the dentin chips were collected and introduced into Eppendorf tubes containing BHI broth.

These tubes were centrifuged to detach the bacterial cells from the dentin chips. Then 0.1ml of this solution was transferred into 0.9ml of broth in another tube to get master dilution. This will be counted as 10^{1}. Then later 10^{2}, 10^{3}, 10^{4}, dilution was made, using micropipette. During each sample dilution pipette tips were changed to avoid cross contamination and to get an accurate measurement. Samples from 10^{4} dilution were taken and streaks were made on Trypticase Soya Agar plates. Agar plates were incubated for 48 hours and colony forming units were counted and expressed in 10^{2} and 10^{4}.Before treatment and after treatment of every single sample were compared and between the groups. Colony forming units were counted and tabulated. Chi-square test and SPSS software were used for the statistical analysis of the results.

STATISTICAL ANALYSIS

The following analysis was employed to statistically evaluate the results:
One-Way ANOVA test
Kruskal – Wallis test
Turkey-HSD (Honesty Significant Difference)
Data were analyzed by using paired T-test with SPSS 15.0 version for windows.
Kruskal-Wallis, Non-Parametric test was selected, as the mean value is less than two times of standard deviation.
Post Hoc Test with Turkey HSD was calculated at $p<0.05$ for multiple comparison between the groups.

RESULT

A total number of 60 mandibular second primary molars were used for the study. Colony forming units were counted to check for the antibacterial. Contaminated samples were assessed before irrigation and after irrigation, at 5minute contact time. Microorganism samples were cultured on agar plates and colony forming units were calculated and the results were tabulated for further statistical analysis.

Table-I: Colony Forming Units Counts, Before And After Irrigation.
GROUP-A - (CHLORHEXIDINE - 2%)

Sample	Pre-irrigation Cfu / 0.01ml	Post-irrigation Cfu / 0.01ml
1	53	0
2	97	12
3	59	17
4	34	0
5	84	20
6	60	0
7	79	8
8	120	14
9	44	0
10	107	10

11	98	16
12	66	11
13	44	5
14	41	4
15	15	0

INFERENCE FROM TABLE-I

Inference from Table-1, shows the total colony count recorded before irrigation for all the 15 samples to be 1001. And a significant reduction of colony forming units count is seen after irrigation that is 117.

TABLE-II: Colony Forming Units Counts, Before And After Irrigation.
GROUP-B - (CHLORHEXIDINE - 0.2%)

Sample	Pre-irrigation Cfu / 0.01ml	Post-irrigation Cfu / 0.01ml
1	29	12
2	101	24
3	24	19
4	54	11
5	65	20
6	48	16
7	59	8
8	89	18
9	76	22
10	39	17
11	143	13
12	68	19
13	71	21
14	26	10
15	104	24

INFERENCE FROM TABLE-II

Inference from Table-II shows colony forming units count before and after irrigation to be 996 and 254 per 0.01ml, when counted for all the 15 samples, showing similar result of colony forming unit reduction is seen.

Table-III: Colony Forming Units Counts, Before And After Irrigation.
GROUP-C - (SODIUM HYPOCHLORITE - 0.5%)

Sample	Pre-irrigation Cfu / 0.01ml	Post-irrigation Cfu / 0.01ml
1	104	12
2	45	17
3	50	8
4	48	9
5	70	11
6	65	13
7	79	4
8	96	11
9	65	16
10	43	5
11	85	15
12	53	3
13	115	13
14	44	6
15	67	16

INFERENCE FROM TABLE-III

From the inference, of the Table-III maximum colony counting units, counts before treatment (1029) was recorded. And also, when compared with after treatment, colony forming units count, (159) significant difference is noted.

Table-IV: Colony Forming Units Counts, Before And After Irrigation.
GROUP – D (SODIUM HYPOCHLORITE – 2.5%)

Sample	Pre-irrigation Cfu / 0.01ml	Post-irrigation Cfu / 0.01ml
1	85	18
2	50	16
3	40	14
4	56	37
5	112	23
6	85	17
7	44	15
8	65	11
9	40	19
10	110	33
11	35	16
12	48	22
13	76	16
14	44	23
15	78	20

INFERENCE FROM TABLE-IV

Inference of Table-IV gives us the similar result, of difference in colony forming units counts before irrigation (968) and after irrigation (300). And also, least number of colony forming units count before treatment is seen when compared with other groups.

Table-V: Descriptive Statistics
(BEFORE TREATMENT)

Groups	N	Range	Mean	Std. Error	95% confidence interval for Mean	
					Upper bound	Lower bound
A	15	15 to 120	66.73	7.727	50.16 to 83.31	
B	15	24 to 143	66.40	8.517	48.13 to 84.67	
C	15	43 to 115	68.60	5.934	55.87 to 81.33	
D	15	35 to 112	64.53	6.527	50.53 to 78.53	
TOTAL	60	15 to 143	66.57	3.535	59.49 to 73.64	

INFERENCE FROM TABLE-V

Inference of Table-V list outs the mean value and standard error of all the four groups before treatment. Group-A shows a mean value of 66.73 with a standard error 7.727, whereas in Group-B 66.40, the mean value and standard error 8.517, followed by Group-C which recorded to be 68.60 with a standard error 5.934 and Group-D with a mean value of 64.53, standard error 6.527.

Table-Vi: Descriptive Statistics
(AFTER TREATMENT)

Groups	N	Range	Mean	Std. Error	95% confidence interval for Mean	
					Lower bound	Upper bound
A	15	0 to 20	7.80	1.824	3.89 to 11.71	
B	15	8 to 24	16.93	1.318	14.11 to 19.76	
C	15	3 to 17	10.60	1.186	8.06 to 13.14	
D	15	11 to 37	20.00	1.805	16.13 to 23.87	
TOTAL	60	0 to 37	13.83	.989	11.85 to 15.81	

INFERENCE FROM TABLE-VI

From the inference of Table-VI, the mean value and standard error of all the four groups after treatment were evaluated. Group-A with a mean value of 7.80 and standard error 1.824, where the maximum difference of mean value is noted when compared with before treatment. Followed by Group-B, mean and standard error of 16.93 and 1.318, Group-C, mean value 10.60 with a standard error 1.186, whereas Group-D, showing 20.00 and 1.805, with least difference of mean values when compared with before treatment.

GRAPH-I: COMPARISON OF COLONY FORMING UNITS – MEAN VALUE.

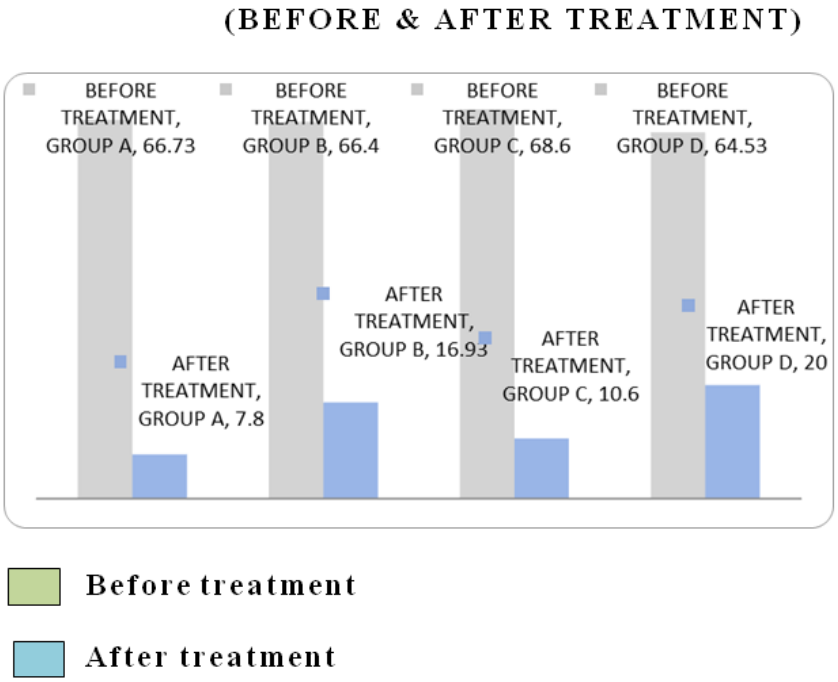

Table-Vii: Inferential Statistics (Oneway Anova).

	Sum of squares	df	Mean square	F	Sig.
(Before Treatment)					
Between groups	124.867	3	41.622	.053	.984
Within groups	44115.867	56	787.783		
Total	44240.733	59			
(After Treatment)					
Between groups	1417.400	3	472.467	12.951	.000
Within groups	2042.933	56	36.481		
Total	3460.333	59			

INFERENCE FROM TABLE-VII

From the inference of Table-VIII, significance of organism growth before treatment and after treatment between all single groups were evaluated based on the obtained F-value. From the results of F-value (.053) before treatment a non-significant P-value (.984) has been achieved. Whereas after treatment, the recorded F-value is 12.951 showed a statistically significant P-value of (.000).

Table- Viii: Post Hoc Test For Multiple Comparison. (Before Treatment)

Before treatment		Mean difference	Std. Error	Sig,	95% confidence interval		Kruskal-Wallis Test P-value
					Upper bound	Lower bound	
Group-A	Group-B	.333	10.249	1.000	-26.80 to 27.47		
	Group-C	-1.867	10.249	.998	-29.00 to 25.27		
	Group-D	2.200	10.249	.996	-24.94 to 29.34		
`Group-B	Group-A	-.333	10.249	1.000	-27.47 to 26.80		
	Group-C	-2.200	10.249	.996	-29.34 to 24.94		
	Group-D	1.867	10.249	.998	-25.27 to 29.00		
Group-C	Group-A	1.867	10.249	.998	-25.27 to 29.00		
	Group-B	2.200	10.249	.996	-24.94 to 29.34		
	Group-D	4.067	10.249	.979	-23.07 to 31.20		
`Group-D	Group-A	-2.200	10.249	.996	-29.34 to 24.94		
	Group-B	-1.867	10.249	.998	-29.00 to 25.27		.945
	Group-C	-4.067	10.249	.979	-31.20 to 23.07		

INFERENCE FROM TABLE-VIII

Inference from Table-VIII, evaluated multiple comparison between groups based on mean value and results found to be not significant. And Kruskal-Wallis test, (a non-parametric test) based on the colony forming units counts evaluated the same results. So, when compared between groups there was no significant P-value.

Table- Ix: Post Hoc Test For Multiple Comparison (After Treatment)

After treatment		Mean difference	Std. Error	Sig,	95% confidence interval		Kruskal-Wallis Test P-value
					Upper bound	Lower bound	
Group-A	Group-B	-9.133	2.205	.001	-14.97 to -3.29		
	Group-C	-2.800	2.205	.586	-8.64 to 3.04		
	Group-D	-12.200	2.205	.000	-18.04 to -6.36		
Group-B	Group-A	9.133	2.205	.001	3.29 to 14.97		
	Group-C	6.333	2.205	.029	.49 to 12.17		
	Group-D	-3.067	2.205	.511	-8.91 to 2.77		
Group-C	Group-A	2.800	2.205	.586	-3.04 to 8.64		
	Group-B	-6.333	2.205	.029	-12.17 to -.49		
	Group-D	-9.400	2.205	.000	-15.24 to -3.56		
Group-D	Group-A	12.200	2.205	.000	6.36 to 18.04		
	Group-B	3.067	2.205	.511	-2.77 to 8.91		.000
	Group-C	9.400	2.205	.000	3.56 to 15.24		

INFERENCE FROM TABLE-IX

Inference from Table-IX, which used Post Hoc test, revealed a statistically significant value between each group. When Group-A was compared with Group-B, C and D, (Group-B and D,) showed a significant value of (.001 and .000). Similarly Group-B was statistically significant with (Group-A and C), (.001 and .029), when compared with Group-A, C and D. Followed by Group-C, significant with Group-B and D, (.029 and .000). Group-D is significant with Group-A (.000) and Group-C (.000), when compared with Group-A,B and C. And also, when a non-parametric test used (Kruskal-Wallis test) similar results were been achieved, with a P-value .000.

Table – X: Student T-Test For Paired Samples Of Individual Groups.

Groups	Before			After				
	Mean	N	Std. Error	Mean	N	Std. Error	t.value	Sig.
A	66.73	15	7.727	7.80	15	1.824	8.983	.000
B	66.40	15	8.517	16.93	15	1.318	6.072	.000
C	68.60	15	5.934	10.60	15	1.186	10.191	.000
D	64.53	15	6.527	20.00	15	1.805	7.194	.000

INFERENCE FROM TABLE-X

Inference from Table-X, using a Student-t-test for paired samples resulted in a significant value before and after treatment of all four groups individually. A mean value for Group-A, before and after treatment (66.73 and 7.80) showed a significance (.000) and similar results were noted in all the other groups. Group-B with mean (before treatment-66.40 and after treatment-16.93), followed by Group-C before and after treatment to be (68.60 and 10.60) and Group-D, before treatment-64.53 and after treatment-20.00, with significant P-value .000 for all the groups.

Table-Xi: Wilcoxon Signed Rank Test.

Groups	Colony forming units Counts		Wilcoxon test	
	Before	After	Z – value	P - value
A	1001	117	-3.408	.001
B	996	254	-3.408	.001
C	1029	159	-3.408	.001
D	968	300	-3.410	.001

INFERENCE FROM TABLE-XI

Inference from Table-XI shows a significant P-value when a non-parametric test (Wilcoxon Signed rank test), was used based on colony forming units counts every group individually. Group-A showing significant P-value of .001 with colony forming units counts before and after treatment to be 1001 and 117, Group-B significant value of .001, with a (before-996 and after treatment-254)

colony forming units. Followed by Group-C with before-1029 and after 159 colony forming units and statistically significant P-value of .001, and also in Group-D with a significant value of .001, from before and after treatment (968 and 300), colony forming units counts.

The present study was conducted in the Department of Pedodontics and Preventive Dentistry, Faculty of Dental Sciences, Sri Ramachandra University, Chennai, in March 2010 to May 2010. Ethical committee clearance was obtained and sixty extracted human mandibular second primary molars were collected and stored in formalin, and any attached soft tissue and calculus were removed with an ultrasonic scaler. The storage and handling of the teeth were performed as per Occupational Safety and Health Administration guidelines and regulations. Collected second primary molars were extracted due to non-restorability, parents request as they were not willing for pulp therapy.

After one week in incubator, tooth samples of all four groups were placed in aseptic chamber and in all 60 samples, paper points were introduced and the liquid broth sample were collected and those paper points were immersed into an Eppendorf tube containing clear BHI broth, and paper point were discarded. This was done to check for the viability of organism, by calculating in colony forming units. Prepared root canal irrigants were loaded into the 5ml syringe of Canal Clean 30-gauge needle with close-end tip and side-port. Followed by Root canal irrigation was done, allowing a contact period of 5mins for all four different irrigants and different groups. After which canal were dried and Gates gliden drill was introduced into the distal canal till apical third and the dentin chips were collected and introduced into Eppendorf tubes containing BHI broth. Paper points were used as a tool to collect liquid and dentinal chips samples, as it can observe maximum samples from the canal.

These tubes were centrifuged to detach the bacterial cells from the dentin chips. Then 0.1ml of this solution was transferred into 0.9ml of broth in another tube to get master dilution. This will be counted as 10^1. Then later 10^2, 10^3, 10^4, dilution was made, using micropipette. During each sample dilution pipette tips were changed to avoid cross contamination and to get an accurate measurement. Samples from 10^4 dilution were taken and streaks were made on Trypticase Soya Agar plates. Agar plates were incubated for 48 hours and colony forming units were counted and expressed in 10^2 and 10^4. Before treatment and after treatment of every single sample were compared and between the groups. Colony forming units were counted and tabulated. Chi-square test and SPSS software were used for the statistical analysis of the results.

DISCUSSION

The treatment of primary teeth that have been pulpally exposed by the caries process, accidentally during cavity preparation or fracture of a tooth because of trauma, that has long presented a challenge in treatment.[12] Endodontic treatments of primary teeth may be complex according to the type of lesion affecting it. The anatomical features of primary tooth, that influence pulp treatment includes thin dentin on the floor of the pulp chamber, curvature of the primary tooth root canal system, ovular-shaped canals, impossibility to establish the exact location of the apical foramen and possible damage to the permanent tooth bud during pulp debridement.[7]

Use of a chemical, based on antiseptic and antibiotic properties of endodontic irrigants and medications becomes mandatory. Disinfection of root canal is major determinant in the healing of periapical tissues.[13] E.faecalis is the most commonly implicated microorganism in asymptomatic persistent infections. It is a facultative anaerobe present in small proportion of the flora of untreated canal as a part of polymicrobial flora. In post-treatment apical periodontitis, the prevalence ranges from 24% to 77%.[6,7] It can enter the root canal system during treatment, between appointments, or even after the treatment has been completed.[7]

EDTA has little antibacterial activity but is important in its ability to remove the inorganic portion of the smear layer thus allowing other irrigants access to the dentinal tubules.[14,15] Enterococcus Faecalis have been commonly recovered from root canals where the endodontic treatment has failed. Molander et al examined the microbiological status of root filled teeth with periradicular lesions and found enterococci in 32% of the investigated teeth. Under similar conditions, Moller reported 29% and Sundqvist et al reported 38% occurrence. Enterococci usually can survive even under unusual environmental stresses and may be extremely resistant to medications used during the endodontic therapy.

Thus towards finding an effective irrigant and their effective concentration to eradicate E.faecalis have been done in the present study.

Matthias zehander have mentioned that, 3% to full strength NaOCl, if used in adequate amounts and exchanged regularly, has the capability to destroy E.faecalis in the root canal system.[16] Chlorhexidine in a 2% gel or liquid concentration is effective at reducing or completely eliminating E.faecalis from the root canal space and dentinal tubules.[17]

The present study is done to compare the antibacterial efficacy of two commonly used root canal irrigants: chlorhexidine and sodium hypochlorite, against Enterococcus Faecalis. and to evaluate the effects of the two-root canal irrigants at two different concentrations (Chlorhexidine- 0.2% and 2% and sodium hypochlorite- 0.5% and 2%).

Sixty extracted mandibular second primary molar were used in the present study, as it provides conditions close to clinical situation. Primary first molar has connections involving furcation and horizontal anastomoses[18] therefore the mandibular second primary molars were chosen for the study to avoid obtaining a high variance in the canal systems.

Distal roots were preferred in this study, since they generally have a curved, large, single root canal with a uniform canal outline and relatively less intracanal ramifications compared to the mesial roots.

Contradicting the microorganism, of choice selected for testing antibacterial efficacy, Sundquist et al have stated that most of the In-vitro studies have used extracted bovine or human teeth mono-infected with E.faecalis, a gram positive facultative species associated with failed root canal treatment. However in primary endodontic infections, usually poly-microbial gram negative anaerobes were in predominant and he concluded that E.faecalis is rarely encountered in primary endodontic infection.

In contrast, Ramachandran nair, have stated that E.faecalis property of inherent antibacterial resistance, ability to adapt to harsh environmental changes and its growth in root canal walls as biofilm make it responsible for endodontic failure. And this statement gives us an substantiating support in selection of E.faecalis, even though they were not predominantly seen in primary endodontic infection.

Sterilized samples were contaminated using Insulin syringe in an aseptic condition. Contamination by injection of whole broth and incubation in Brain Heart Infusion broth for 24 hours was carried out in this study. The same methodology has been used by shahrokh et al in his previous study. But in his study the incubation period was 48 hours. *It is to avoid cross contamination of samples that a reduced incubation period was chosen for this study.*

During every alternate day the tooth samples were taken out and contaminated broth solution were added, to provide better nourishment. Like In the study done by, Jose F. Siqueira, where contamination of samples during every alternate day provided better nourishment and also helped to maintain the viability of organisms.

In a previous study, Rachel et al, aseptically cultured the samples and incubated for a period of 4 days, prior to the test irrigants.[19] Literature also, supports incubation for a longer duration of better organism growth and penetration into dentinal tubules.

Paper points have been widely used for sampling, but it is acknowledged that bacteria located in the other regions of the entire root canal system can pass unnoticed by this identification method. Thus, Bacterial sampling with paper points only detects the microorganisms that are present in the main root canal, whereas the bacteria located in the dentinal tubules are inaccessible.[20] It is not in the purview of this study to locate microorganisms or to assess their penetrating depth into the dentinal tubules. *Only the presence or absence of these organisms was needs to be evaluated, hence the paper point method of sampling was used.*

*Irrigation of the contaminated samples using closed end tip, side-port opened 30-gauge needle was used in current study.*In agreement with the use of 30-gauge Christos et al, have explained the average duration of an irrigant between successive instruments with a 30-G needle is 17-23seconds.[21], even a z velocity of 0.001m/s could ideally provide some irrigant replacement. Velocities in the order of 0.01 m/s could provide some effect, but velocities higher than 0.1 m/s should be considered clinically significant for adequate irrigant replacement. Nevertheless, the higher the velocity of the irrigant, the replacement is faster and more adequate.[21]

It has been suggested that the side-vented needle is more efficient than the bevelled and notched ones in the removal of bioluminescent bacteria.[22] The reported superior performance of the side-vented needle has been attributed to turbulence. Vinoth kumar et al reported that the unidirectional performance of the side-vented and double side-vented needle showing a better irrigation and clearance of smear layer.

The efficacy of Chlorhexidine at 2% which is bactericidal has been proven in other studies, also at 0.2% it has been shown to exhibit bacteriostatic properties. This study looks at comparing the efficacy of these two concentrations.

The 2% concentration of chlorhexidine is available over the counter whereas the 0.2% had to be prepared. This was done by diluting the concentrated 2% solution in isopropyl alcohol (Analytical Reagent), for this study.

Chlorhexidine is a potent antiseptic, which is widely used for chemical plaque control in the oral cavity. Addy stated that aqueous solutions of 0.1 to 0.2% are recommended for that purpose, while 2% is the concentration that is used for root canal irrigation. This literature supported the use of chlorhexidine as a potent root canal irrigant. It was to check its efficacy at a lesser concentration of about (0.2%).

In previously done clinical trials the efficacy of sodium hypochlorite over chlorhexidine in obtaining a negative culture has been noted. Hence sodium hypochlorite was taken as the second choice of irrigant. There has been much controversy over the concentration of hypochlorite solutions to be used in endodontics.[16]

It was to address this controversy that a lower and higher concentration of Sodium hypochlorite was tested. A

concentration of 2.5% and 0.5% were evaluated.

Use of aqueous sodium hypochlorite in endodontics as the main irrigant has been in place from as early as 1920. Furthermore, Frais S et al stated that sodium hypochlorite solutions are cheap, easily available, and have a good shelf life.

Sim T. P et al said that the added advantage of using 0.5% solution was that they do not decrease the elastic modulus and flexural strength of human dentin compared to 5.25% solution. And, at higher concentrations Hulssman M, reported occurrence of severe irritations to periapical tissue.

As Bonnie retamozo, explained, that the paper points only detect the microorganisms that are present in the main root canal, whereas the bacteria located in the dentinal tubules are inaccessible, and thus dentin was drilled till the apical third, to collect the dentin chips.

A culture-dependent approach was used in the present study as it is one of the most reliable methods of detecting viable bacteria, especially when samples are taken immediately after antibacterial treatment where viability may not be ascertained by most other methods.[23]

All root canals included in this investigation harboured bacteria before treatment, and their viability was confirmed. Irrespective of the irrigant used, substantial bacterial reduction was observed after chemo-mechanical preparation, which also parallels other findings from the literature.[24,22]

Before treatment and after treatment of every single sample were compared and between the groups. Colony forming units were counted and tabulated.

Chi-square test and SPSS software were used for the statistical analysis of the results.

In the present study when we compared the antibacterial efficacy of all the four groups, statistical difference was seen between all groups except between Group A – Group C and Group B – Group D.

Group A (2% chlorhexidine) was shown to have the highest efficacy against E.feacalis.

2% chlorhexidine has been proven to be a more potent antimicrobial when used for root canal irrigation when compared to sodium hypochlorite. Gomes et al and Vianna et al after comparison of sodium hypochlorite and chlorhexidine in different concentrations claimed that chlorhexidine 2% was an ideal irrigant as it showed maximum antibacterial efficacy in the shortest duration.[25]

Gomes et al evaluated the efficacy of different concentrations of chlorhexidine solutions and concluded that the efficacy of chlorhexidine increases with higher concentration.[26] This also holds true for this study where 2% chlorhexidine showed higher efficacy than 0.2% chlorhexidine.

Sequera et al noted that chlorhexidine at concentrations as low as 0.12% also showed comparable effects in eliminating bacteria and concluded that the concentration of the irrigant is not the only factor that affects the efficacy of chlorhexidine as an irrigant.[27] Chlorhexidine has also been proven to be more effective than other commonly used irrigants. Basson and Tait compared chlorhexidine (2%), calcium hydroxide and Iodine Potassium Iodide as irrigants. The chlorhexidine group was the only irrigant that eliminated microorganisms from all samples. Also, its efficacy was noted till 60 days where no culture growth was reported.[28]

Group C-(sodium hypochloride-2.5%) was shown to be the second best efficient irrigant with antibacterial properties among the tested irrigants.

Berber et al found that 5.25% sodium hypochlorite was the most efficient antimicrobial irrigant followed by 2.5% of the same solution. This agreed to our study as the efficacy increased with increase in concentration.[29]

On the basis of this study, it appears that high concentration and long exposure to Chlorhexidine and NaOCl are needed for elimination of E.faecalis contaminated teeth.

Further studies on efficacy of these agents on other microorganisms present in the root canal and other factors influencing the efficacy of an irrigating solution like, contact time, irrigation technique used, need to be further evaluated.

CONCLUSION

The present, In-vitro study was conducted to evaluate the antibacterial efficacy of chlorhexidine 2% and 0.2% and sodium hypochlorite 2.5% and 0.5%, against Enterococcus faecalis, in 5-minute contact time.

Extracted human mandibular second primary molars were used in this study and the 60 samples were divided into four groups depending on the irrigants used. They were assessed using Trypicase soya agar culture plates, based on the colony forming units before and after irrigation the values were analysed statistically. Of the treatment steps involved with infection control, the chemomechanical preparation assumes a pivotal role in root canal disinfection, because instruments and irrigants together act primarily on the main canal, which is the most voluminous area of the system and consequently harbours, the largest number of bacterial cells. As the morphology of the root canals in primary teeth makes endodontic treatment difficult and often impractical in case if the canal cannot be cleansed of necrotic material,

sterilized, and adequately filled, endodontic therapy is likely to fail. Root canal irrigation becomes mandatory to perform a successful root canal treatment.

The following conclusions are made based on the findings of the present study.

Chlorhexidine-2% is a very good showed better antimicrobial property irrigating solution against E.Faecalis amongst the tested irrigants as the colony forming units were reduced to the maximum when compared with other groups of irrigations. Both Chlorhexidine-2% and Sodium hypochlorite-2.5% have a good potential to keep a low E.Faecalis colony forming units count.

Lower concentration of chlorhexidine (0.2%) and sodium hypochlorite (0.5%) have also shown promising antimicrobial activity, although not comparable to higher concentration.

In the present study, the tested irrigants have shown promising results. Their efficacy was checked based on the concentration of root canal irrigants and the organisms were counted by colony forming units. The main limitation of the present study is that, being an In-Vitro study the efficacy of irrigants against E.Faecalis was tested. Apart from E.faecalis the root canal harbours other organisms too. Hence further In-Vivo studies need to be carried out to evaluate the efficacy of irrigants to bring it close to clinical situations.

Another limitation is the duration of the study. Longer time of incubation of the organism would give us a clear picture of the depth of penetration of the organism into the dentinal tubules. Further studies must be carried out to check for the efficacy of the irrigant based on their concentration, contact time, method of irrigation, and other factors promoting their efficacy.

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