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Antimicrobial Efficacy of Intracanal Medicaments on an E Faecalis-An In Vitro Study

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Abstract: *Disinfection of root canal plays a major role on long term success of endodontic treatment .Reduction of endodontic microbiota has been achieved by series of antimicrobial stratigies that include preparation ,irrigation,intracanal medicaments and root canal filling materials.it was suggested that when antibiotics were applied locally into infected root canals instead of systematic administration ,it wouldreduce the risk of adverse sysytemic effects.*

Due to polymicrobial nature of infected root canal multiple antibiotic formulation might be recquiredRecently triple antibiotic paste containing ciprofloxacin,metronidazole and minocycline has been introduced for lesion sterilization and repair.Hence,The study concluded that triple antibiotic paste has the greatest effectivenessagainst E. faecalis to completely eradicate bacteria.

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I. Introduction

Disinfection of root canal plays a major role on the long term success of endodontic treatment. Reduction of endodontic microbiota has been achieved by a series of antimicrobial strategies that include root canal preparation, irrigating solutions, intracanal medicaments, and root canal filling materials.[1-4]

The outcome of endodontic treatment is unsuccessful, especially in cases where bacteria remain in the root canal systems that results in inflammation of pulpal or periradicular tissue.[5,6]Therefore such microorganism must beeliminated in order to achieve successful treatment outcomes.

Role of intracanal medicaments in root canal treatment is to eliminate any remaining bacteria after canal instrumentation,reduce inflammation of periapical tissues and pulp remnants and it also act as a barrier against leakage from the temporary filling and also to preventspost-operative pain.

Antibiotics can be used as an adjunct to endodontic treatment in a number of ways – locally (i.e., intracanal), systemically and prophylactically. In endodontics,antibiotics are prescribed to reduces the chances of secondary infection in the pulp space and periapical area.

It was suggested that when the antibiotics were applied locally into the infected root canals instead of administering systematically, it would reduce the risk of adverse systemic effects [7]

Due to the polymicrobial nature of infected root canal, multiple antibiotic formulation might be required to disinfect the root canal.Nonspecific antibiotic suppresses most of the microbial flora and allow residual virulent micro-organisms to repopulate the root canal. Therefore it is essential to use combination of antibiotics to act against all endodontic pathogens and to prevent resistance.

Recently triple antibiotic paste containing ciprofloxacin, metronidazole and minocycline has been introduced for lesion sterilization and repair.Metronidazole is a nitroimidazole compound that exhibits broad spectrum of activity against protozoa and anaerobic bacteria. Metronidazole is selectively toxic to anaerobic microorganisms.

Tetracycline, which includes doxycycline and minocycline are primarily bacteriostatic. Minocycline exhibit broad spectrum of activity against gram positive and gram negative microorganisms.

Ciprofloxacin is a synthetic floroquinolone with rapid bactericidal action.

Linezolid is a member of oxazolidinone class of medications. Linezolid is active against most gram positive bacteria that cause disease,including*streptococci,vancomycin resistant staphylococcusaureus.*

Most researcher have found mixed result with intracanal medicaments regarding disinfection of root canal contaminated by *E.faecalis.*

II. Materials And Method

This prospective comparative study was carried out in department of conservative dentistry and endodontics at sardarpatel post graduate institute of dental and medical sciences lucknowuttar Pradesh in collaboration with Sanjay Gandhi post graduate institute of medical sciences Lucknow from November 2017 to November 2018.

Study Design: Prospective Comparative Study
Sample Size: 20 agar plates
Distribution of Samples:-
Samples were divided into three experimental groups and one negative control group.
Group 1:- Triple antibiotic paste
a. Ciprofloxacin 500mg.
b. Metronidazole 200mg.
c. Minocycline 100mg.
Group II:- Linezolid (600mg)
Group III:- Linezolid (600mg) with calcium hydroxide.
Group IV:- Calcium hydroxide.(control group)

Preparation of Triple Antibiotic Paste(Group I)
Triple antibiotic paste was prepared by crushing of antibiotic i.e. Tab ciprofloxacin (Ciplox 500 mg,Cipla, India),Tab metronidazole (metrogyl 200mg), Cap minocycline (Cyomin 100mg. The powder thus obtained were weighed separately and mixed in a 1:1:1 proportion respectively with the weighing machine to obtain triple antibiotic mixture. A total of 100 mg of triple antibiotic mixture was dispensed and mixed with one drop of normal saline to get a thick paste like consistency.
Preparation of Linezolid paste(Group II)
Linezolid paste was prepared by removing the coating of tablet and crushing linezolid (tab linox 600 mg) with the help of mortar and pestle. The powder obtained was dispensed and mixed with one drop of normal saline to get paste like consistency.
Preparation of Linezolid with Calcium Hydroxide paste (Group III)
Linezolid and calcium hydroxide paste was prepared by crushing of Tab Linezolid(linox tab 600mg) tablets by using a mortar and pestle. The calcium hydroxide powder was taken. The linezolid powder and calcium hydroxide powder obtained were weighed separately and mixed in a 1:3 proportion respectively to obtained the mixture. A total 100 mg of linezolid + calcium hydroxide powder was dispensed and mixed with one drop of normal saline to get a thick paste like consistency.
Preparation of calcium hydroxide paste(Group IV)
Calcium hydroxide powder(safe plus-calcium hydroxide powder) was taken and was weighed separately and mixed with one drop saline to get thick paste like consistency**.**

III. Experimental Procedure

Pure strain of *Enterococcus faecalis* (ATCC 29212) was obtained in single use disposable vial from American type culture collection(Manassss,VA) by Himedia Laboratory(Mumbai,India) which was subcultured in tryptonsoya broth agar medium by overnight culturing in an incubator at 37^{o}C, after the bacterial growth was confirmed using microscope.

The surface of twenty freshly prepared Miller-Hinton agar plates were inoculated with 0.2ml Tryptone Soya broth culture of *E. faecalis* with the help of platinum loop. Four wells of 4mm depth and 10 mm diameter were punched in each of the agar plates. The four freshly mixed medicaments were placed in the wells of each agar plate. Positive control plates were streaked with bacteria. The plates were then incubated at 37^{0}C for 24hrs, 48hrs and 72hrs.

The diameter of the zone of inhibition around each well was then measured in millimeters using antibiotic zone scale at an interval of 24hrs, 48hrs and 72 hrs. The test were repeated thrice for all the tested intracanal medicaments against *E. faecalis* and the raw data collected and the statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software and Kolmogorov-Smirnov test, Kruskal Wallis test non parameteric ANOVA and Mann -Whitney U test.

IV. Results

The Mann–Whitney U test was used for intragroup analyses. Results after the placement of medicaments were presented graphically (Box and Whisker plot). The Kruskal– Wallis test was used for the intergroup comparative analysis of data. The reduction in the number of E.faecalis after the treatment protocol for 24 hr,48 hr and 72 hr was highly significant for all groups ($P < 0.001$)given in table2,3,4.Triple antibiotic paste was significantly superior over the linezolid with calcium hydroxide paste ,linezolid paste and calcium hydroxide paste in reducing E. faecalisat 24 hr,48 hr and 72 hr.

Table 1: Intergroup comparison of Zones of Inhibition at 24 hours

Group	N	Min.	Max.	Mean	SD	Median
Group I (TAP)	20	26	30	28.35	1.39	28.00
Group II (Linezol)	20	17	19	17.85	0.81	18.00
Group III (Linezol with $Ca(OH)_2$)	20	20	23	20.90	1.02	21.00
Group IV ($Ca(OH)_2$)	20	11	16	13.40	1.67	13.00
Total	80	11	30	20.13	5.62	19.50

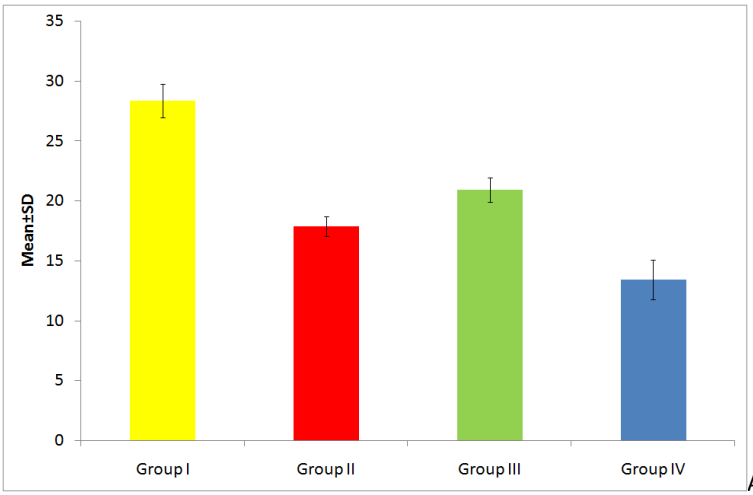

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Table 2: Between Group differences in Zone of Inhibition values at 24 h (Mann-Whitney U test)

	Mean	**S.E.**	**Z**	**P**
Group I Vs Group II	10.50	0.40	5.489	<0.001
Group I Vs Group III	7.45	0.40	5.502	<0.001
Group I Vs Group IV	14.96	0.40	5.475	<0.001
Group II Vs Group III	3.05	0.40	5.506	<0.001
Group II Vs Group IV	4.45	0.40	5.479	<0.001
Group III Vs. Group IV	7.50	0.40	5.491	<0.001

Between group difference was found to be maximum between Group I Vs. Group IV (14.96±0.40) followed by Group I vs. Group II (10.50±0.40) while minimum difference was found between Group II & Group III (3.05±0.40) followed by Group II & Group IV (4.45±0.40). Between group differences were found to be statistically significant for all the combinations. Hence, order of zone of Inhibition at 24 h was: **Group I > Group III > Group II > Group IV**

Table 3: Between Group differences in Zone of Inhibition values at 48 h (Mann-Whitney U test)

	Mean	**S.E.**	**Z**	**P**
Group I Vs Group II	10.55	0.40	5.492	<0.001
Group I Vs Group III	7.35	0.40	5.477	<0.001
Group I Vs Group IV	14.80	0.40	5.439	<0.001
Group II Vs Group III	3.20	0.40	5.453	<0.001
Group II Vs Group IV	4.25	0.40	5.477	<0.001
Group III Vs. Group IV	7.45	0.40	5.476	<0.001

Between group difference was found to be maximum between Group I Vs. Group IV (14.80±0.40) followed by Group I vs. Group II (10.55±0.40) while minimum difference was found between Group II & Group III (3.20±0.40) followed by Group II & Group IV (4.25±0.40). Between group differences were found to be statistically significant for all the combinations. Hence, order of zone of Inhibition at 48 h was:

Group I > Group III > Group II > Group IV

Table 4: Between Group differences in Zone of Inhibition values at 72 h (Mann-Whitney U test)

	Mean	S.E.	Z	P
Group I Vs Group II	10.35	0.41	5.464	<0.001
Group I Vs Group III	7.40	0.41	5.474	<0.001
Group I Vs Group IV	14.75	0.41	5.438	<0.001
Group II Vs Group III	2.95	0.41	5.052	<0.001
Group II Vs Group IV	4.40	0.41	5.448	<0.001
Group III Vs. Group IV	7.35	0.41	5.471	<0.001

Between group difference was found to be maximum between Group I Vs. Group IV (14.75±0.41) followed by Group I vs. Group II (10.35±0.41) while minimum difference was found between Group II & Group III (2.95±0.41) followed by Group II & Group IV (4.40±0.41). Between group differences were found to be statistically significant for all the combinations. Hence, order of zone of Inhibition at 72 h was:

Group I > Group III > Group II > Group IV

V. Discussion

Bacteria in the root canal system initiate and maintain pulpal and periradicular lesions**(Moller et al. 1981*)***. Chemomechanical cleaning and shaping of the root canal greatly reduce the number of bacteria, but it has been shown that it is impossible to obtain complete disinfection in all cases(**BPFA Gomes et al., 2002*)*[8] therefore concern exists about the fate and subsequent activity of the remaining microorganism in the canal.

Amongst four different groups in agar diffusion test the mean zone of inhibition was observed at the interval of 24 hr,48 hr and 72 hr with the help of antibiotic zone scale.

The result showed the mean zone of inhibition after 24 hr was maximum for triple antibiotic paste (28.35±1.39) and minimum for calcium hydroxide paste (13.40±1.67).

The result suggests that calcium hydroxide paste alone was least effective against the *E.faecalis*compared to other groups.

The mean zone of inhibition of calcium hydroxide paste(Group IV) was (13.40±1.67) after 24 hours of incubation and after 48 hrs of incubation the value ranged from (14.40±1.50) which increased to 14.45±1.47 after 72 hours. When Calcium Hydroxide is placed in agar, its high pH starts to precipitate it, preventing its diffusion[9]. Moreover, the release of Ca and OH ions decrease the pH of the media, enhancing growth of the organisms being tested.[47]These factors may have been responsible for its lack of effectiveness against *E.faecalis* in blood agar. Moreover, the proton pump of *E.faecalis* carries protons to the interior of the cell, acidifying its cytoplasm in situations of increased alkalinity when subjected to Calcium Hydroxide.[10] All these factors might have contributed to the pH decline of Calcium Hydroxide.

Linezolid paste(Group II) also showed good results as compared to calcium hydroxide paste against *E.faecalis* with the mean zone of inhibition being 17.85±0.81 after 24 hours,18.65±0.99 after 48 hrand18.85±1.18 after 72 hours.

Linezolid with calcium hydroxide paste (Group III) showed the better results in comparison to calcium hydroxide paste(Group 1V) and Linezolid paste(Group II) with a mean values of 20.90±1.02 after 24 hr,21.85±1.50 after 48 hrand 21.80±1.47 after 72 hours, respectively.

Among all the antibiotics Groups the Triple antibiotic paste(Group I) showed the best results at the interval of 24,48, and 72 hrs. The mean zone of inhibition of Triple antibiotic paste was 28.35±1.39 after 24 hours of incubation, which increased to29.20±1.47 after 48 hours and the value was constant at 72 hrs.

Thus,the reason behind the Triple antibiotic paste to be most effective medicament in our study can be,its biocompatibility and it also has an antimicrobial effect against the microorganisms in which minocycline inhibits collagenases and matrix metalloproteinases which is non cytotoxicand has an antibacterial effect against gram positive and gram negative bacteria in addition metronidazole is active against protozoa and anaerobic bacteria while ciprofloxacin can generate fibroblast and also effective against gram negative bacteria.

Hence,in the present study intra group analysis indicated that all the medicaments succeeded in promoting reduction in bacterial growth which was calculated by measuring zone of inhibition.Group I was significantly more effective at killing *E.faecalis* followed by Group III as compared to other groups. But there was least significant difference between Group II and Group III at interval of 24,48 and 72 hr.

Thus from the literature, it is clear that triple antibiotic paste is effective in disinfection of root canal and successful healing of large periradicular lesionsand also the result of the present study concluded that thetriple antibiotic paste completely inhibited the microbial growth at the time interval of 24,48 and 72 hr against the tested microorganism i.e*E.faecalis*among the different compared groups.

Within the confines of design and materials of this in vitro study, triple antibiotic paste seems to be a promising intracanal medicaments owing to its greater antimicrobial efficacy. Further studies are necessary to substantiate the result of the present study.

I.CONCLUSION

The medicament that provided the best result in our study was triple antibiotic paste.It was followed by linezolid and linezolid with calcium hydroxide. The least value was shown by calcium hydroxide.

The study concluded that there is no ideal antibiotic paste as all of them failed to completely eradicate bacteria.

Group I > Group III > Group II > Group IV

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