Evaluation of the antimicrobial efficacy of 5%Doxycycline, 5%Tetracycline, 5%Metronidazole and 2.5%Sodium hypochlorite used alone or in combinations with 2.5%Sodium hypochlorite against Enterococcus faecalis.An *Invitro* Study

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Abstract:

Aim:*The study investigated and compared the antimicrobial efficacy of 5%Doxycycline,5%Tetracycline,5%Metronidazole and 2.5%NaOCl used alone or in combinations against E.faecalis.*

Material & Methods: *The testorganismE.faecalis was streaked on agar plates.A total of 140 wells, 20 for each group were made in 50 plates.5gms of each powder was mixed with 100ml of ionized water.This 50ìl of the test irrigant solution were pipetted into each well and placed in a CO_2incubator at 37°C for 48 hours. The seven groups were are as follows:Group A:2.5%NaOCl,Group B: 5%Tetracycline, Group C: 5%Metronidazole, Group D: 5%Doxycycline, Group E: 5%Doxycycline+2.5%NaOCl, Group F: 5%Tetracycline+2.5%NaOCl, Group G: 5%Metronidazole+2.5%NaOCl. The zones of inhibition were measured using a mm scale.Agar diffusion test was done to evaluate the antimicrobial efficacy of the various intracanal irrigantsand the results was statistically analyzed using ANOVA and Post hoc Tukey's test.*

Results:*GroupEshowed highly significant differences amongst all the groups and GroupCand GroupAshowed non-significant differences amongst all.*

Conclusion:*Combination of 5%Doxycycline and 2.5%NaOCl is more effective irrigant amongst all the experimental groups againstE.faecalis while Metronidazole and NaOClshows least antimicrobial effect against E.faecalis.*

Keywords: *Agar diffusion test, Metronidazole,Tetracycline, Doxycycline.*

Introduction

Complete debridement of the contaminated root canal system is the paramount as well as a precondition for successful pulp therapy.Root canal morphology is complex and it contains numerous ramifications and anatomical irregularities. Microorganisms and their byproducts play a crucial role in contaminating the root canal system.[1]The microorganisms in root canals not only invade the anatomic irregularities of the root canal system but they are also present in the dentinal tubules which causes persistent endodontic disease after root canal therapy leading to the failure of the treatment.

Current techniques of root canal debridement may leave some areas of the root canal system completely untouched by the instruments and so precise instrumentation is necessary inorder to gain the outcome of successful treatment.[2] Biomechanical preparation and chemical preparation are used concomitantly in order to cleanse the root canal system and also been shown that this instrumentation without irrigation reduces but does not predictably eliminate bacteria in the canal. Thus, a root canal irrigant is needed inorder to aid in the debridement of the root canals, should consist the ability to dissolve pulp remnants, should remove the smear layer, and be anti-microbial in nature. Scientific evidence of this irrigant relating to its desirable properties reveals its efficiency and so it can be said that sodium hypochlorite is currently the irrigant of choice and is preferred by most clinicians. Various concentrations of sodium hypochlorite (NaOCl) have been used as root canal irrigants according to the use since many decades.[3]

Sundqvist *et al*.recovered numerous species of anaerobic bacteria from failed root canal cases, of which E.faecalis was found to be the most prevalent bacteria in leading the persistent infections.[4]Enterococci are less dependent on their virulent factors and their emergence as pathogens may be related to their resistance to several antimicrobial agents.[5]

Doxycycline belongs to a broad spectrum antibiotic group,an effective antimicrobial agent and is a hydroxy derivative of tetracycline. It is the most potent anti-collagenase antibiotic amongst all the commercially available tetracyclines.[6]Being bacteriostatic in nature,it carries the advantage of not releasing the antigenic

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byproducts such as endotoxins in absence of the bacterial lysis.[7]

There are less reports comparing the antimicrobial efficacy of Doxycycline, Tetracycline and Metronidazole and Sodium hypochlorite.Hence the present study was undertaken to evaluate and compare the antimicrobial efficacy of 5%Doxycycline, 5%Tetracycline, 5%Metronidazole and 2.5 %Sodium hypochlorite when used alone and in combinations against *E.faecalis*.[7]

Aim

The purpose of this study was to determine and compare the antimicrobial efficacyof 5% Doxycycline,5%Tetracycline, 5%Metronidazoleand 2.5%Sodium hypochlorite used alone or in combinations against Enterococcus faecalis.

Material & Methods

In the present study, the standard strain of Enterococcus faecalis was used as a test strain.Four morphologically similar colonies were picked up from an agar medium with a wire loop and the growth was transferred to a test tube containing 4ml of sterile peptone water.

The antimicrobial activity was carried on sheep blood agar (HiMediaPvt.Ltd, Mumbai, India) as per standard protocol of inoculum preparation. After adjusting the inoculums to 0.5 McFarland tube the lawn culture was done on blood agar plates.A total of 140 wells, 20 for each group were made of diameter 6mm and depth 4mm equidistantfrom each other in 50 plates.5gms of antimicrobial powder (each of Tetracycline, Metronidazole, Doxycycline) was mixed with 100ml of ionized water.This 50ìl of the test irrigant solution were pipetted into each well and were placed in a CO_2incubator at 37° C for 48 hours.

The seven groups in the present study are as follows:

Group A:2.5% Sodium hypochlorite

Group B: 5% Tetracycline(Research-LAB fine CHEM Industries)

Group C: 5% Metronidazole(Research-LAB fine CHEM Industries)

Group D: 5% Doxycycline(Research-LAB fine CHEM Industries)

Group E: 5% Doxycycline+2.5% Sodium Hypochlorite

Group F: 5% Teracycline+2.5% Sodium Hypochlorite

Group G: 5% Metronidazole+2.5% Sodium Hypochlorite

The agar plates were then carefully placed in a CO_2incubator (NuAire Inc, Plymouth, MN)at 37° C for 48 hours.The zones of inhibition were measured using a millimeter scale, passing through its diameter to the point of complete inhibition of growth on either side and the average was calculated**. (Fig1,2 and 3)**ANOVA and Post hoc Tukey's test was applied to evaluate the mean and the intergroup comparison between the various groups($p<0.05$).

Fig No 1: Zone of inhibition(mm) of Tetracycline &Doxycycline

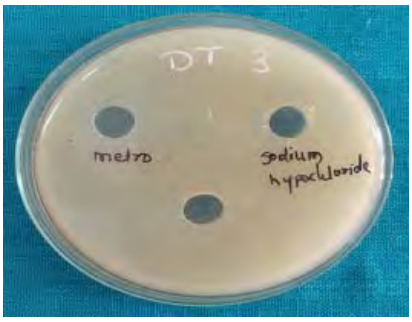


Fig No 2:Zone of inhibition(mm) Metronidazole &Sodium Hypochlorite

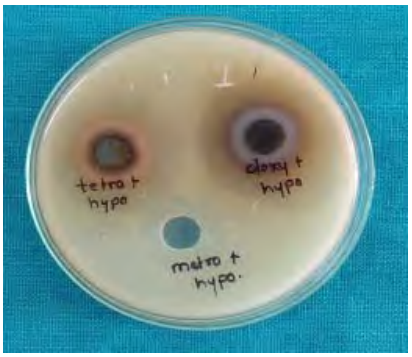


Fig No 3: Zone of inhibition(mm) Tetracycline+ NaOCl, Doxycycline+ NaOCl, Metronidazole+ NaOCl

Statistical Analysis

The data was coded and entered into Microsoft Excel spreadsheet. Analysis was done using SPSS version 20 (IBM SPSS Statistics Inc., Chicago, Illinois, USA) Windows software program. Descriptive statistics included computation of means and standard deviations. The analysis of variance (ANOVA) [for quantitative data within three groups] with post hoc Tukey's test (to make more intra-groups comparison) was used for quantitative data comparison of all clinical indicators. Level of significance was set at $P<0.05$.

Results

Group A(NaOCl) showed statistical differences with all other groups but non significant differences with Group G(Metro+NaOCl) when p value was set at$P<0.05$, showing better results with Group E(Doxy+Hypo) depending upon the mean difference(-20)

Group B(Tetra) when compared, showed highly significant differences amongst all the groups with better results showing with GroupE(Doxy+Hypo) depending upon the mean difference.(-7)

Group C(Metro)showed highly significant differences amongst all the groups with better results of Group E(Doxy+Hypo) considering the mean difference(-31)

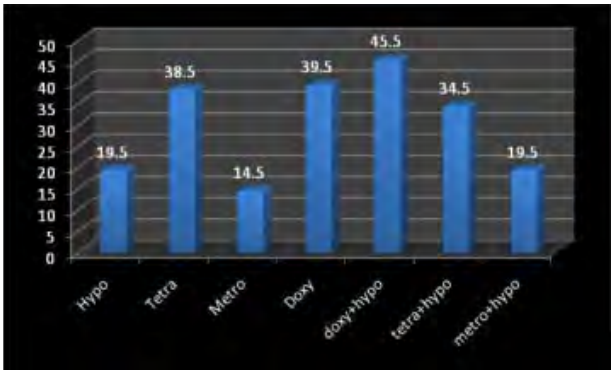
Group D(Doxy) when compared with other groups showed highly significant differences.When mean differences were checked(-6), Group E(Doxy+Hypo)exhibited higher significance.

Group E(Doxy+Hypo),when compared showed statistically significant differences amongst all other groups when p value was set at$P<0.05$

Table No 1: Intra-group comparison among all the groups.

		Mean differences	P value
A (Hypo)	tetra	-19.0	0.000 (S)
	metro	5.0	0.000 (S)
	doxy	-20.0	0.000 (S)
	doxy+hypo	-26.0	0.000 (S)
	tetra+hypo	-15.0	0.000 (S)
	metro+hypo	0.00	1.000(NS)
B (Tetra)	metro	24.0	0.000 (S)
	doxy	-1.0	0.000 (S)
	doxy+hypo	-7.00	0.000 (S)
	tetra+hypo	4.0	0.000 (S)
C (Metro)	doxy	-25.0	0.000 (S)
	doxy+hypo	-31.0	0.000 (S)
	tetra+hypo	-20.0	0.000 (S)
	metro+hypo	-5.00	0.000 (S)
D (Doxy)	doxy+hypo	-6.0	0.000 (S)
	tetra+hypo	5.0	0.000 (S)
	metro+hypo	20.0	0.000 (S)
E (Doxy+Hypo)	tetra+hypo	11.0	0.000 (S)
	metro+hypo	26.0	0.000 (S)
F (Tetra+Hypo)	metro+hypo	15.0	0.000 (S)
G (Metro+Hypo)	tetra+hypo	-15.0	0.000 (S)

P< 0.05 (S-statistically significant; NS-not significant)



Graph No 1: Mean zones of inhibition(mm)

Discussion

The ultimate goal of endodontic treatment is either to prevent the development of apical periodontitis or to create adequate conditions for periapical healing in presence of disease.Thus, the rationale of endodontic treatment is to remove microorganisms from infecting or re-infecting the root canal system.[8]Although obligate anaerobic bacteria dominate the root canalmicrobiota,there are some facultative strains such as *E.faecalis* which have been involved in causing persistent infections, which influences the prognosis of the involved tooth.

In order to successfully eliminate this species from the root canal system, a thorough debridement of the root canal system is most important.Not that only the debridement procedures like instrumentation of the root canals are necessary, but irrigation with proper irrigating agent has been the norm. In spite of these various bactericidal irrigants available, residual microorganisms such as *E.faecalis* still survive and creates a dilemma which increases the rate of persistent infections of the root canals which leads to root canal failures.

Therefore, this study was confined to elucidating the antimicrobial property of various test irrigants when used alone or in combinations and comparing them with each other when tested against *E.faecalis.*

E.faecalis is the most commonly isolated microorganism from the root canals of failed endodontic cases. It consists of various virulence factors which enables it to survive and persist as a pathogen in the root canals,which can also survive in the root canal as a single organism or as a major component of the oral flora. Considering the nature of the organism and its capability to populate and survive in unsuccessful cases of therapy and cause persistent infections, this organism holds the importance and is an imperative choice for several antimicrobial studies.

The agar diffusion method was used in the study for antimicrobial activity assessment because it is said to be the most accepted method or technique to test the antimicrobial activity of various dental materials and irrigants as it allows the direct comparison of the materials against the organisms, indicating thepotential of the test materials to eliminate microorganisms in the local microenvironment of the root canal system.[9] But there are also some disadvantages over this test that its results depends upon the material's ability to diffuse into the agar medium.

Sodium hypochlorite is a most popular irrigating solution, a broad spectrum antimicrobial activity which enhances the potential of the solution to inhibit the bacterial growth in the root canals. The hypochloric acid of NaOCl disrupts several vital functions in the microbial cell resulting in tissue death, provides as a good tissue dissolving capacity, has high pH, offers ease in the instrumentation as it acts a lubricant and can flush loose debris out from root canals. It carries a primary antibacterial property due to its tissue dissolving capacity and its proteolytic enzymes,Consideringall the functions of the NaOCl, it was the irrigant of choice in this study.

The results of the present study showed that, Group E (5%Doxy +NaOCl) had the greatest zones of inhibition(mm) followed by Group D(5% Doxycycline) and Group B(5% Tetra), this might be due to the synergistic antimicrobial action of doxycycline and NaOCli.e.anti-collagenase action, inhibition of protein synthesis by passive diffusion through the bacterial cell wall, adsorption of the drug on the bacterial cell wall, and ability of NaOCl to denature proteins which affects the bactericidal property on the agar.[10]

The results also determines that Doxycycline and Tetracycline were effective alone against *E. faecalis*, but Doxycycline promoted better action when combined with NaOCl. Metronidazole alone and in combination exhibited least antimicrobial efficacy against *E.faecalis* when compared to other groups in the study.

The reason behind it might be that Doxycycline, is a broad spectrum antibiotic and hydroxy derivative of tetracycline and has shown a great potential for expanded use in the treatment of bacterial infections in different parts of the body. It is an effective against*E.faecalis*.[11] and also Tetracyclines are a group of broad-spectrum antibiotics which are effective against a wide range of microorganisms. They not only offer anti-microbial property, but also has anti-inflammatory action and serves as an appropriate irrigant in concerns of root canal debridement.They are bacteriostatic, which provides a more advantageous use since there is no bacterial lysis and consequently no release of antigenic by-products such as endotoxins.[7] It is said that the substantivity of tetracyclines lasts for more than 12 days when compared to other irrigating solutions.[12]

The advantage of the Doxycycline over others is that its prolonged use of the drug does not facilitate the bacterial mutation to generate the tetracycline resistant microorganism.The bacteriostatic nature of Doxycycline inhibits the bacterial protein synthesis by binding to and interfering with ribosomes. Intracellularly, it inhibits the binding of aminoacyl-tRNA to the acceptor site on the mRNA-ribosome complex preventing the addition of aminoacids to the growing peptide and also binds to the 30S subunit of the bacterial ribosome.[13] Tetracyclines also have many unique properties such as the inhibition of mammalian collagenases which helps in preventing the tissue breakdown, and the inhibition of clastic cells which results in anti-resorptive activity.[14]

There are some likely to be similar studies evaluated which can support the results of this study. In one study, Ramta Bansal RB*et al*.evaluated that MTAD exhibited best antibacterial efficiency against *E.faecalis*than others.[15] andY.Abdul Rahman G*et al*evaluated that Biopure MTAD had the strongest antibacterial effect against E.faecalis than 5.25% NaOCl and distilled water.[16]also F.G.Pappen*et al*in his antimicrobial study evaluated that Tetraclean was more effective than MTAD against*E.faecalis*.[17]

Metronidazole in this study has not been much effective against *E.faecalis*,as it is active against strict anaerobes, but is ineffective against facultative anaerobes.

Thus, the present study determines the better antimicrobial efficacy of combination of Doxycycline and NaOCl when used against *E.faecalis*.

Conclusion

Within the limitations of the study,

The combination of 5%Doxycycline and 2.5% Sodium Hypochlorite is a most effective irrigant amongst all the experimental groups against *E.faecalis* followed by 5% Doxycyclineand 5% Tetracycline while Metronidazoleshowed least antimicrobial efficacy followed by Sodium Hypochloriteagainst *E.faecalis*.

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