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In vivo comparative evaluation of microbial reduction after biomechanical preparation of human root canals containing necrotic pulp tissue when using three endodontic irrigants by culture test

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Abstract

Background: Chemo-mechanical debridement is the most essential element in the root canal therapy (RCT). The use of precise irrigant in an accurate way is the key of success to limit recurrent poly-microbial endodontic infection. The present in vivo study aimed to compare effectiveness of three irrigants (1% povidone iodine, 0.2 % ciprofloxacin, 0.5% metronidazole) in microbial reduction during the RCT.

Materials and methodology: RCT was performed in 60 maxillary anterior teeth which were divided into four subgroups, 15 in each. Normal saline was used in the control group while in experimental groups 1% povidone iodine, 0.2 % ciprofloxacin, 0.5% metronidazole were used. Total 15 ml of each irrigant was used in 3 visits (5ml/ visit). Sample collection was done four times with the help of sterile paper points- preoperative, immediate after biomechanical preparation, at 48 hours and at 72 hours. Microbial reduction was evaluated in both aerobic and anerobic cultures.

Result: All three irrigants were effective in microbial reduction during RCT. Ciprofloxacin was more effective on aerobic microorganisms while metronidazole was more effective on anerobic microorganisms.

Conclusion: 0.2 % ciprofloxacin and 0.5 % metronidazole are very effective root canal irrigants.

Keywords: Endodontic infection, Biomechanical preparation, Irrigants

Introduction

The chief motive of treating an endodontically compromised tooth is to maintain the functionally and aesthetically pleasing occlusion along with the healthier periodontium for longer period of time as they play most substantial role in preserving healthier oral cavity.[1] Root canal therapy (RCT) consists of three main steps biomechanical preparation, irrigation and disinfection, and obturation, collectively termed as 'ENDODONTIC TRIAD".[2] Cleaning and shaping of the endodontium is extreme challenging as the internal morphology of root very intricate with the presence of anastomoses, cul-de-sacs, and deltas which leads to hoard of pathogens and their toxic products, ultimately leading to recurrence of endodontic infections.[3-4] Thus microbial riddance from the root canal system is decisive factor in the management of endodontically compromised tooth as residual bacteria may provide a substrate for the re-growth of microbial colonies.[5] For efficient debridement use of antimicrobial medications is necessary along with the mechanical instrumentation. Thus use of antimicrobial chemical agents is a vital part of RCT in acquiring better success results.[2]

Researchers have found oral cavity harbours sundry microorganisms beyond 700 bacterial species along with viruses, fungi and protozoa.[5-7] The primary endodontic infection is of mixed type where both aerobic and anaerobic bacteria are available in abundance although gram-negative anaerobic rods dominate.[3,8] Therefore broad-spectrum antibacterial action is a supreme property to be an irrigant along with the qualities of being systemically non-toxic, non-caustic to human body, disestablishment necrotic pulp tissue remnants, avert founding of smear layer.[2] There are several studies on the effectiveness of antimicrobial irrigants focused on E feacalis which is a facultative anaerobic bacteria and reported to be the prime suspect of seconday infection.[3,9-12] The presence of other microbes is often been neglected even after knowing as primary endodontic infection is mixed in nature.[3] Therefore this study was aimed to evaluate the effectiveness of different irrigants on the microbial reduction (including both aerobic and anaerobic bacteria) during the root canal therapy in order to find an ideal irrigant.

Material and methodology

The present study was conducted after getting approval from the ethical review board. A total number of 60 anterior permanent teeth of 45 patients, aged 10-15 years were selected from the outpatient department in the department of pedodontics and preventive dentistry, K.D. Dental College and hospital, Mathura (U.P.), India, who required endodontic treatment. Patient inclusion criteria involved teeth, with negative response to electric pulp testing, history of night pain, tender on percussion, swelling or sinus formation. Exclusion criteria involved teeth with, any sign of internal or external root resorption, calcification or atypical anatomy, extensive caries where isolation cannot be achieved or where temporary seal is difficult to maintain, not having adequate bone support and patient under antimicrobial agent within the last one month. Study was well explained to the patients and signatures were taken on an informed consent. All the teeth were randomly divided in to four groups, 15 in each. Out of which one was control group where normal saline was used as root canal irrigant while other three groups were experimental groups, had 3 different irrigation solutions- 1% povidone iodine, 0.2 % ciprofloxacin, 0.5% metronidazole.

Sample Collection

Sample collection was done four times in 3 visits- preoperatively (immediate to access opening, prior to the use of irrigant), just after the biomechanical preparation, at 48 hours (2^{nd} visit) and at 72 hours (3^{rd} visit). Each time one by one 2 paper points were kept for about 1 minute to soak the contents of the root canal. One paper point was used to incubate aerobic bacteria while other was used to culture anaerobic bacteria. If the root canal was dry, a small amount of sterile saline solution was introduced in the canal. For the aerobic culture, paper point was inserted into the tube containing peptone water in front of a spirit lamp. While for anaerobic organisms the cap of the culture tube containing fluid thioglycollate was loosened without removal. Paper point inserted deep into the medium. The cap was tightened as soon as possible.

PROCEDURE- Before starting the endodontic procedure, full mouth scaling was done. After achieving local anaesthesia rubber dam isolation was performed. An aseptic technique was used for instrumentation during access to sample taking. An access opening was made into the pulp chamber with a sterile no. 4 round bur at high speed with water spray. The walls of the access cavity were modified with a sterile round or tapered fissure bur at low speed as per the need. Canal length was determined by a radiograph after placing a No.l5 file into the canal. The file was then manipulated for 30-60 seconds to contact all canal walls to suspend as many bacteria as possible. The file was then withdrawn and two sterile paper points (one after another) was then picked up with a sterile tweezer and was inserted to reach the apical portion into the canal for sample collection. The tube was labelled to indicate the patient's name, the date and the tooth cultured. The walls of the canals were prepared biomechanically with the help of files and the debris was flushed out at regular interval with 5 ml of test irrigant at each appointments. The irrigant was introduced slowly into the canal by means of 27 gauze Luerlock syringe. After biomechanical preparation with a test irrigant the second sample is taken in another two paper point for both aerobic as well as for anaerobic bacteria on the same appointment day as we have taken before. The canals were then washed with 5 ml of distilled water after biomechanical preparation and irrigation. It was then dried with the help of sterile paper points. The access opening was then sealed with zinc oxide eugenol cement prior to which a sterile cotton piece was introduced into the pulp chamber without any intracanal medicaments and the patient was recalled after 48 hours and then 72 hours. In the subsequent appointment, same procedure was followed to obtain the bacterial samples.

For Anaerobic bacteria, the inoculated paper point in thioglycollate media was incubated at 37°C for 24-48hours and then inoculated on Kanamycin blood agar (selective blood agar) 100 µg/ml. 100 µg, disc of metronidazole was placed on first series of streaks. This was incubated in anaerobic McIntosh and Fildes jar at 37^{o}C for 48-72hours. After 72 hours plates were taken out from the jar and colonies were picked up and subcultured again anaerobically on selective blood agar. Isolate was identified with the help of colony morphology, Gram staining, motility and biochemical reaction.

For aerobic bacteria, the inoculated paper point in the peptone water was transported to microbiology laboratory. It was then inoculated on solid agar (like blood Agar, Macconkey Agar) for 48 hours. Isolate was identified with the help of colony morphology, Gram staining, motility and biochemical reaction. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Results

Pie diagram 1 and 2 are showing prevalence percentage of different aerobic and anaerobic bacteria isolated from the root canal system prior to treatment respectively.

The table 1 showed the aerobic culture test evaluation in four samples for all used irrigants at different intervals. Results suggested all three test irrigants were effective in the microbial reduction. At 72 hours infected tooth were 7, 3 and 5 in the povidone iodine, ciprofloxacin and metronidazole groups respectively, suggesting maximum effectiveness of ciprofloxacin group followed by metronidazole group. On intergroup comparison statistically significant differences were seen among all 4 groups at 72 hours with the p value of 0.014.

The table 2 showed the anaerobic culture test evaluation in four samples for all used irrigants at different intervals. We found that there was no significant bacterial reduction observed even after 48 hours but at 72 hours there were significant differences seen among all four groups with the p value of 0.022 where metronidazole group had minimum number of infected root canals i.e. one followed by ciprofloxacin group, here it was five.

The table 3 showed combined (both aerobic and anaerobic) culture test evaluation in four samples for all used test irrigants at different intervals. There were significant differences seen among all four groups at 48 as well as at 72 hours with the p values of 0.047 and 0.001. The metronidazole group had Minimum infected tooth that was six, followed by the ciprofloxacin group, where the infected tooth samples were eight.

Discussion

Existence of residual microbes within the root canal system is the core cause of failure of root canal therapy.[11] This can be limited by enhancing the microbial reduction with the use of chemical agents during the cleaning and shaping phase of root canal therapy that is biomechanical preparation.[13] Povidone iodine, commonly known as betadine is a combination of aqueous iodine and polyvinyl pyrrolidone. Torneck in 1976, was the first one to use it as endodontic irrigant. It has antimicrobial action on a wide array of microbes including gram positive and negative organisms as well as fungi and viruses. Povidone iodine has few other advantageous properties like better permeability with in the dentinal tunules without stating dentine, very mild toxicity, non-allergenicity.[2,11] That is why we decided to took it for the evaluation. In the present study povidone iodine has shown its effectiveness in both aerobic and anaerobic cultures specifically at 48 and 72 hours intervals. This may be due to its vapour forming ability, low surface tension and the above mentioned properties. The results were similar to the studies of Shahani MN and Reddy S2 and Baker NE et al.[10] Baker NE et al reported the effectiveness of povidone iodine at 24 hours which was statistically not significant. Buck et al[14] reported that the efficacy of povidone iodine is reliant on bacteria present with in the canals.

Ciprofloxacin, an outstanding antibacterial agent has not gained much lime light as an irrigant despite of showing admirable pharmacokinetics and having very mild adverse effects. It has mild toxicity, stumpy allergenicity yet high intrinsic solubility. This is a second-generation fluoroquinolone, immediate hypersensitivity reactions in the human body are very rare with this drug which includes urticaria, itching, erythema, skin rash, and shock.[9,15-16] In the present study we have used 0.2 % ciprofloxacin to avoid even those mild side effects. Result showed 0.2 % ciprofloxacin has reflected superior effectiveness against aerobic microbes at different time intervals compared to 0.5 % metronidazole solution. The results were in concordance with the studies of Kaushik SN et al[9] and Karatas E et al[16] but both of them have used ciprofloxacin in combination to other drugs.

Metronidazole (5-nitromidazole) is a wide spectrum antibacterial drug, effective against the bacteria containing electron transport elements, like ferrodoxin that bequeaths electrons to metronidazole to reduce its nitro-group resulting in highly efficient radicals via inhibition of DNA synthesis of bacteria. In the present study 0.5 % metronidazole solution used as irrigant has shown outstanding results in limiting the endodontic microbial infection, specifically highly effective against anaerobes.[17-18] A few researchers have reported that the work field of metronidazole is limited to strict anaerobes but not efficiently effective against facultative anaerobes like E. faecalis, which is the main culprit in secondary endodontic infection thus we recommend use of metronidazole along with other irrigants. The results of the present study were similar to the studies of Dubey S[17], Gaetti-Jardim Júnior et al [19] and Effat Khodeinae et al. [20] and Jain S and Thakur S[21]

Conclusion

As we know endodontic infection is polymicrobial in nature where chemical debridement plays a substantial role so the selection of correct irrigant for sufficient time period is of utmost important.

1% Povidone iodine, 0.2% ciprofloxacin and 0.5% metronidazole irrigation solutions are highly effective in microbial reduction during the root canal therapy.

0.2% Ciprofloxacin is has shown a great effectiveness against aerobic pathogens.

0.5% Metronidazole is the most promising irrigant for limiting the anaerobic as well as mixed polymicrobial endodontic infections.

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Legend Figure and Table

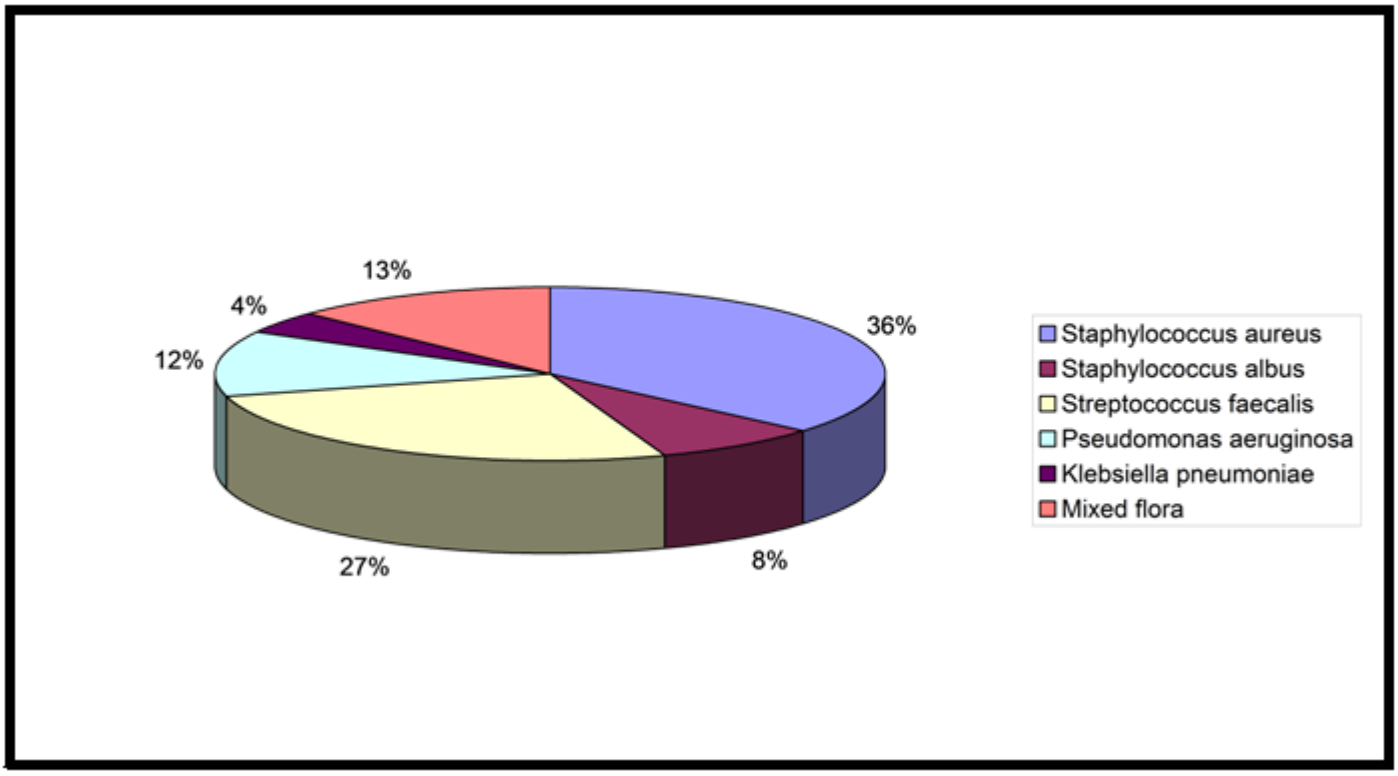

Figure 1: showing prevalence percentage of isolated aerobic bacteria inside the root canal

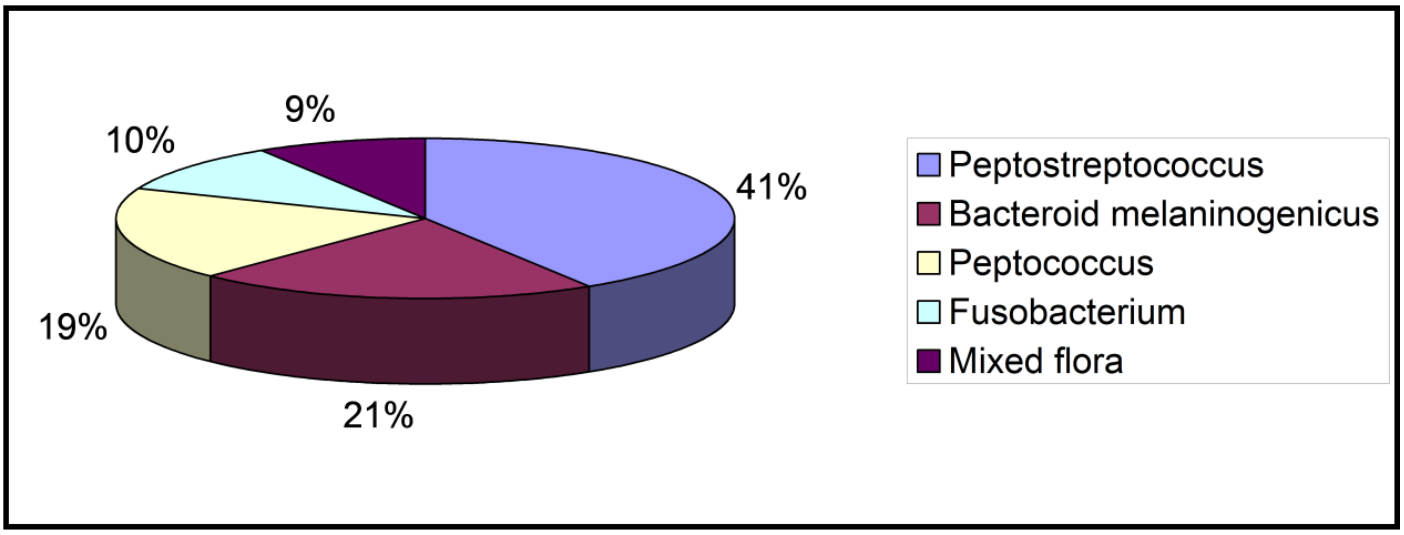

Figure 2: showing prevalence percentage of isolated anaerobic bacteria inside the root canal

	Pre	Post	48 hrs	72 hrs
Normal saline (0.9%)	15	15	13	11
Povidone iodine (1%)	15	14	10	7
Ciprofloxacin (0.2%)	15	12	8	3
Metronidazole (0.5%)	15	12	10	5
χ^2	0	4.367	3.928	10.629
"p"	1	0.225	0.269	0.014

Table 1: Showing Aerobic Culture Test Evaluation in four samples for Different Irrigant Used.

	Pre	Post	48 hrs	72 hrs
Normal saline (0.9%)	15	15	12	9
Povidone iodine (1%)	15	13	10	6
Ciprofloxacin (0.2%)	15	13	8	5
Metronidazole (0.5%)	15	13	6	1
χ^2	0	2.222	5.556	9.597
"p"	1	0.528	0.135	0.022

Table 2: Showing Anaerobic Culture Test Evaluation in four samples for Different Irrigant Used.

	Pre	Post	48 hrs	72 hrs
Normal saline (0.9%)	30	30	25	20
Povidone iodine (1%)	30	27	20	13
Ciprofloxacin (0.2%)	30	25	16	8
Metronidazole (0.5%)	30	25	16	6
χ^2	0	5.780	7.937	17.626
"p"	1	0.123	0.047	0.001

Table 3: Showing Culture Test Evaluation in four samples for Different Irrigant Used (Both Aerobic + Anaerobic combined)