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EVALUATION OF ANTIBACTERIAL EFFICACY OF THREE DIFFERENT IRRIGANTS IN INFECTED ROOT CANALS: AN INVIVO STUDY

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ABSTRACT:

Aim: To compare the levels of Antibacterial efficacy of 2.5% Sodium Hypochlorite, 2% Chlorhexidine gluconate and Biopure MTAD in infected root canals under anaerobic conditions.

Materials & method: Forty Patients were selected who fulfilled the Inclusion and Exclusion Criteria. Following Rubber dam application and Local Disinfection Protocol, the Pulp chamber was accessed and two Microbial Samples were collected- one prior to (Pre-Irrigant) and one following biomechanical preparation (Post-Irrigation). Pre-Irrigant Sample was collected using a sterile absorbent paper point kept in the canal to the Estimated working length determined from the Preoperative Radiograph for 10 seconds followed by Working length determination. Grouping of Subjects was done based on the irrigating solution used during chemo mechanical preparation - Group I-2.5% Sodium Hypochlorite, Group II-2% Chlorhexidine Gluconate, Group III- Biopure MTAD and Group IV- Saline 0.9% (Control). After irrigating, Post-Irrigant Sample was collected. Immediately after Sample collection, the paper points were transferred into test tubes containing Robertson's Cooked Meat Medium as Transport Medium which was then serially diluted, inoculated onto 5% Sheep Blood Agar plates and incubated under strict anaerobic conditions at 37°C for 3-4 days. Morphologically distinct colonies were then counted using a Digital Colony Counter.

Results: Paired t test was used to compare the Statistical Significance in the percentage reduction of Colony Forming Units.

Conclusion: All the three irrigants are found to be effective in reducing the number of Microorganisms and 2.5% Sodium Hypochlorite is found to be more effective in reducing the number of Microorganisms when compared to 2% Chlorhexidine gluconate and Biopure MTAD.

Key Words: Debridement, Sodium Hypochlorite, Chlorhexidine Gluconate, Biopure MTAD, Irrigation, Paralleling Technique.

INTRODUCTION:

Endodontic therapy is essentially a debridement procedure that requires

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removal of irritants from the canal and periapical tissue if success is to be gained. Debridement procedure includes instrumentation of the canal, placement of medicaments and irrigants[1]. The need for an Irrigating solution during Biomechanical preparation is unquestionable. Compatibility in terms of Antibacterial efficacy, tissue dissolution, cleaning properties might be considered when selecting an Irrigating solution[2]. Numerous studies have shown that the bacterial flora in endodontic infections is Polymicrobial with a predominance of anaerobic species[3]. Advanced anaerobic bacteriological techniques showed a Polymicrobial environment in necrotic teeth that consisted of Obligate and Facultative Anaerobes, Microaerophilic bacteria and Yeast.

The outcome of endodontic treatment depends on successful microbial elimination from the infected root canal system[5]. Chemo-mechanical preparation plays a critical role in disinfection by reducing the bacterial populations in the root canal. In addition to the mechanical effects of instrumentation, the use of an antimicrobial substance for irrigation is indicated because it enhances bacterial elimination [6].Sodium hypochlorite (0.5% to 5.25%) is the most widely used irrigant because of its pronounced antimicrobial activity and the ability to dissolve organic matter [7].

Sodium hypochlorite 2.5% has been the irrigant of choice for Chemo mechanical procedures. Sodium hypochlorite dissolves vital and necrotic tissue, acts as an Antimicrobial and also lubricates the canal[8]. It has minimal “clinical toxicity” when kept within the confines of the canals. It is extremely toxic to the periapical tissues if it exceeds beyond the apex of the tooth [9].

2% Chlorhexidine Gluconate (CHX) is a Cationic Biguanide. It acts by adsorbing onto the cell wall of microorganisms causing cell lysis. It has got the property of Substantivity[10]. At low concentrations (0.2%), it is bacteriostatic while at higher concentrations (2%), it is bactericidal. CHX is more effective against Gram-positive organisms than Gram-negative organisms [11]. Its activity is pH dependant and is greatly reduced in the presence of organic matter.

Bio pure MTAD, which is a mixture of Tetracycline isomer (3% Doxycycline), an Acid (4.25% citric acid), and a Detergent (Tween 80) acts as an Antimicrobial indirectly by causing removal of smear layer from the instrumented root canal walls [12]. Bio pure MTAD is effective as a final rinse to remove the smear layer with minimal erosive changes on the surface dentin and is capable of disinfecting contaminated root canals [13]. It is both Antibacterial and Chelating agent.

The present Invivo study compares the Antimicrobial effectiveness of three root canal Irrigants- 2.5% Sodium hypochlorite, 2% Chlorhexidine Gluconate and Bio pure

MTAD during chemo mechanical preparation in infected root canals under Anaerobic conditions.

MATERIALS & METHOD:

The study was conducted in the Department of Conservative Dentistry & Endodontics, Government Dental College & Hospital, Hyderabad to evaluate the antibacterial efficacy of three different irrigants used during an endodontic procedure for chemo mechanical preparation, i.e. 2**.5% Sodium Hypochlorite, 2% Chlorhexidine Gluconate and Bio pure MTAD** in infected root canals under anaerobic conditions. Required protocol permissions were obtained from the Academic Committee & Ethical Committee to conduct the study.

Forty patients (Male & Female) with Periapical pathologies were identified and screened for study participation. An Inclusion and Exclusion Criteria was established for selection of the subjects. Patients between 18 and 22 years of age, Central Incisors with single roots and single patent root canals, Teeth with established periapical radiolucency ranging from 3 to 5 mm in diameter and Teeth with no history of previous Endodontic treatment were included in the study. Teeth with Fractures of root, Carious lesions, Patients with systemic debilitating disease, Patients who had received Antibiotic therapy 4 weeks prior, Teeth with periodontal pockets more than 4 mm and Women who stated to be pregnant at the time of study were excluded in the study. The Patient's medical histories were recorded and a thorough oral examination was performed. Preoperative Periapical Radiographs were obtained for every patient using Paralleling Technique prior to the procedure. Every subject was explained about the Endodontic and Experimental procedures and Informed consent was obtained from every patient prior to the procedure.

Subjects were randomly assigned to receive the irrigants-2.5% Sodium hypochlorite, 2% Chlorhexidine Gluconate, Bio pure MTAD or Saline (Control). Asepsis was followed throughout the endodontic treatment. Supra gingival and Sub gingival Scaling were performed to remove calculus surrounding the tooth. The tooth to be treated was isolated using Rubber Dam application (Single Tooth Isolation). The operative field including the tooth, clamp and surroundings were cleaned with 3% hydrogen peroxide. All surfaces were then disinfected by vigorous swabbing with 2.5% NaOCl followed by swabbing with 5% sodium Thiosulphate to inactivate the halogens.

The pulp chamber was then accessed with a sterile diamond round bur and diamond Tapered Fissure bur. Pulp from the root canal was extirpated using a Barbed Broach. The canal orifice was enlarged & lingual shoulder removed using Gates Glidden drills up to #2 with a Micro motor Hand piece. Two microbial samples were taken- one

before biomechanical preparation (Pre-irrigation) & one following/after biomechanical preparation (Post-irrigation) using sterile absorbent paper points.

For collection of Pre-Irrigation Sample, a dry sterile absorbent paper point of size #20 was kept in the canal to the estimated working length determined from the Preoperative Radiograph for 1 minute. Later the paper point was taken out and transferred into a test tube containing 5 ml Robertson's Cooked Meat Medium as the transport medium. Working length was established using Root ZX Apex Locator to 0.5 mm short of the apex using #20 K-file. A Step Back technique was used for preparation of the root canal using frequent irrigation with the experimental irrigants. Step Back Preparation was done in 1 mm increments using stainless steel K-Files. The apex was enlarged to a diameter three times that of the diameter of initial apical file. All the changes in instrument sizes were associated with irrigation following a standardized protocol using the experimental solutions. Irrigation was passive (without binding to walls) using a syringe with needle of 24 gauge with a 30° bend in the needle. Irrigation was done with 5ml of 0.9% Saline following Working Length Determination in all the teeth over a period of 5 minutes.

Grouping of Subjects was done based upon the Irrigant used for Chemomechanical Preparation as shown in **Table 1**.

Irrigation procedure was standardised and was done in all the groups by flooding the canals with 10ml of respective irrigating solution for 5 Minutes followed by Biomechanical preparation with #25 K-File. Changes in instrument sizes #30 and #35 were accompanied by Irrigation with 5ml of 2.5% Sodium Hypochlorite for 5 minutes. This was followed by a Final rinse of 5ml of 2.5% Sodium Hypochlorite for 5 minutes.

After irrigating the root canals with the respective irrigants, all the canals were finally flushed with 5ml of 0.9% Saline for 5 minutes and a dry sterile absorbent paper point of size #35 was kept in the canal to the full Working Length for 1 minute. Later the paper point was taken out and transferred into a test tube containing 5 ml Robertson's Cooked Meat Medium as the transport medium. All the canals were finally irrigated after the procedure with 0.9% Saline and closed dressing with Zinc Oxide Eugenol Cement was done.

The test tubes containing the samples were sealed, stored in an Incubator at 37°C and then transferred to the Microbiology Laboratory (Vista Labs) within three hours of time. The transport medium was then serially diluted (1:10 serial dilutions) and inoculation was done at room temperature onto 5% sheep blood agar plates under complete aseptic and anaerobic conditions. The agar plates were then incubated in an anaerobic jar under anaerobic conditions at 37°C for 3-

4 days. Morphologically distinct colonies were then counted using a Digital Colony Counter.

RESULTS:

The number of Colony Forming Units (CFUs) and Percentage Reduction of Microorganisms were determined from all the Pre-irrigation and Post-irrigation Samples. Mean, Median, Standard Deviation, t-value and p- value were calculated for each group (**Table 2**). The tabulated observations were then statistically analyzed using Paired t-test and Significance was established at $p<0.05$. **Paired t test** was used to compare the Statistical Significance in the percentage reduction of Colony Forming Units. The percentage reduction of Microorganisms is represented graphically as shown in **Graph 1**.

DISCUSSION:

Successful endodontic treatment requires a combination of factors such as accurate diagnosis, thorough cleaning and shaping of the pulp space, a predictable disinfection protocol achieved with the help of various irrigating solutions. This is followed by Three Dimensional Obturation of the pulp space and adequate Final restoration.

Mechanical preparation of the root canal achieved with either Hand or Rotary Nickel-Titanium instruments helps in removal of vital and necrotic remnants of pulp tissue, Microorganisms and Microbial toxins to a certain extent. However, with the use of recent imaging techniques, it is certain that some part of the pulp space remains uninstrumented. These areas might harbor necrotic tissues, microorganisms and their byproducts resulting in persistent periradicular inflammation. Therefore, Irrigation is an essential part of debridement because it allows for cleaning beyond what might be achieved by root canal instrumentation alone [14].

Bacteria in the Apical 5 mm of Infected Root Canals were evaluated by J. Craig Baumgartner and William A. Falkler Jr [15] in 1991. Of the 50 bacterial isolates, 34 (68%) were strict anaerobes and demonstrated the presence of predominately Anaerobic Bacteria in the apical 5 mm of infected root canals in teeth with carious pulpal exposures and periapical lesions. The choice of Irrigant and irrigation protocol should be aimed at removal of all forms of Microorganisms from the root canal.

Elimination of bacteria from the root canal system is paramount to the healing of periapical lesion, therefore being the focus of endodontic treatment. Persistence of Bacteria in the root canal system has a negative impact on the outcome of endodontic treatment. Different Irrigants are currently used to flush out Debris, dissolve Smear Layer, Lubricate Dentin walls, destroy Microbes and remove Tissue remnants (Nair et al 1990)[16]. Harrison et al[17] in 1984 proposed that an Irrigant should also possess Anti bacterial Activity since

Residual Bacteria may be one of the causes of Post treatment disease.

Sodium hypochlorite (NaOCl) is the most frequently and dominantly used Irrigant for Root canal irrigation. Two unique properties of NaOCl include Antimicrobial activity and Organic tissue dissolution[18]. NaOCl acts as an organic and fat solvent, degrading fatty acids and transforming them into fatty acid salts (soap) and glycerol (alcohol) which reduces the Surface tension of the remaining solution. NaOCl neutralizes amino acids forming water and salt. Hypochlorous acid, a substance present in sodium hypochlorite solution, when in contact with organic tissue acts as a solvent and releases chlorine, which combines with protein amino groups to form chloramines. The chloramination reaction between chlorine and the amino group (NH) forms chloramines that interfere with cell metabolism. Chlorine has an Antimicrobial action, inhibiting bacterial enzymes. Efficacy of Sodium hypochlorite can be increased by increasing Temperature of the solution or by Ultrasonic activation [19].

Chlorhexidine Gluconate is a synthetic cationic Bis-guanide. Its efficacy is because of the interaction of the positive charge of the molecule and the negatively charged Phosphate groups on microbial cell walls (Gomes et al. 2003)[12], thereby altering the cell's osmotic equilibrium. This increases the permeability of the cell wall, which allows the Chlorhexidine molecule to penetrate into the bacteria. At low concentration (0.2%), it is Bacteriostatic as Low molecular weight substances, specifically Potassium and Phosphorous will leak out of the cell. On the other hand, at higher concentration (2%), CHX is bactericidal as precipitation of the cytoplasmic contents occurs, which results in cell death (Gomes et al. 2003) [20].

Torabinejad et al [20] have shown that Bio pure MTAD [Mixture of Tetracycline isomer(3% Doxycycline),an Acid (4.25% citric acid) & a Detergent(Tween 80)] is able to safely remove the smear layer and that it is effective against Enterococcus faecalis, and it can also eliminate bacteria in human root canals that had been infected by whole saliva. Their results showed that Bio pure MTAD is effective as a final rinse to remove the smear layer with minimal erosive changes on the surface dentin.

Moller [21] established the Culturing method for Endodontic microbiological analysis used consistently in many of the studies. This method relies on root canal sample acquisition with paper points, cultivation of recovered bacteria on culture media and enumeration by Colony Forming Units.

False positive results can occur because of contamination during sampling, from salivary leakage or from improper handling of the specimens in the clinic or laboratory. To minimize these False Positive results, the

operative field was isolated using Rubber Dam and the tooth and Rubber Dam being disinfected using 3% Hydrogen peroxide and 2.5% Sodium Hypochlorite. As suggested by Moller, the halogens were later neutralized by swabbing with 5% Sodium Thiosulphate solution.

In the present study, Irrigation with 2.5% Sodium Hypochlorite (Group I) showed the Maximum Reduction in number of Colony Forming Units compared to 2% Chlorhexidine gluconate (Group III), Bio pure MTAD (Group II) and 0.9% Saline (Group IV). These results are in accordance with the studies carried out by Vianna et al [22] who showed in vivo that 2.5% NaOCl in comparison to 2% Chlorhexidine Gluconate has not only a higher capacity to kill microorganisms but is also more able to remove necrotic pulp cells from the root canal. Sandeep Singh et al [23] have also concluded in vivo that the use of 2.5% Sodium Hypochlorite resulted in significantly greater reduction of anaerobic organisms in infected root canals when compared to Bio pure MTAD.

Irrigation with Bio pure MTAD (Group II) showed a weaker reduction in number of Colony Forming Units compared to 2.5% Sodium Hypochlorite (Group I) and 2% Chlorhexidine gluconate (Group III) but very significant when compared to 0.9% Saline (Group IV). The inferior result outcome of Bio pure MTAD may be attributed to its ineffectiveness in the presence of organic matter or Bio film. According to a Study conducted by Giardino et al [24], Bio pure MTAD shows a better action than Sodium Hypochlorite but the goal could be achieved only after 30 to 60 minutes of Irrigation which is too long for a clinical use of this Irrigation Regime. Beltz RE, Torabinejad M [25] has shown that Bio pure MTAD has only 45% solubilising effect on organic tissue whereas Sodium Hypochlorite has more than 90% effectiveness in dissolving the organic tissue which may be responsible for inferior results of Bio pure MTAD.

Irrigation with 2% Chlorhexidine Gluconate (Group III) showed better reduction in number of Colony Forming Units compared to Bio pure MTAD (Group II) and 0.9% Saline (Group IV) but the efficiency was less when compared with 2.5% Sodium Hypochlorite (Group I). The inferior results of Antimicrobial efficacy when compared to 2.5% Sodium Hypochlorite may be because Chlorhexidine is unable to dissolve necrotic tissue remnants and is less effective against Gram negative bacteria compared to Gram positive bacteria which are predominant in anaerobic infections. This effect is especially pronounced in periapical infections where Gram negative anaerobes are predominant. Further, the activity of 2% Chlorhexidine Gluconate is also pH dependant.

Irrigation with 0.9% Saline (Group IV) showed the least Reduction in the number of Colony Forming Units compared to 2.5% Sodium Hypochlorite (Group I), Bio pure MTAD (Group II) and

0.9% 2% Chlorhexidine gluconate (Group III) probably because of lack of Antimicrobial activity, inability to remove smear layer or inability to dissolve the organic debris and necrotic tissue. However, the little reduction in the number of Microorganisms following Irrigation might be attributed to the removal of Microorganisms along with the dentin shavings along the dentin walls during Biomechanical preparation or Cleaning & Shaping of the canal. However, the Microorganisms left in the canal may proliferate rapidly increasing their number between appointments when treated without an effective Antimicrobial agent resulting in treatment failure.

CONCLUSION:

Within the limitations of study, it can be finally concluded that all the three irrigants-2.5% Sodium Hypochlorite, 2% Chlorhexidine Gluconate and Bio pure MTAD are found to be effective Antibacterial agents during Chemo mechanical Preparation. However, 2.5% Sodium Hypochlorite is found to be a more potential Antibacterial Agent followed by 2% Chlorhexidine Gluconate and Bio pure MTAD.

REFERENCES:

1. Weine FS. Endodontic therapy, Sixth Edition, Mosby 2004:4
2. Cruz-Filho AM, Soousa-Neto MD, Saquy PS, Pecora JD. Evaluation of the effect of EDTAC, CDTA and EGTA on radicular dentin micro hardness Journal of Endodontics 2001:27: 183-184
3. Lana MA, Ribeiro-Sobrinho AP, Stehling R, et al. Microorganisms isolated from root canals presenting necrotic pulp and their drug susceptibility in vitro. Oral Microbiology& Immunology, Vol.16,2001
4. Siqueira JF Jr, Rocas IN Clinical implications and microbiology of bacterial persistence after treatment procedures Journal of Endodontics, Vol.34, 2008
5. Brito PR, Souza LC, Machado de Oliveira JC et al Comparison of the effectiveness of three irrigation techniques in reducing intracanal Enterococcus faecalis populations: An in vitro study. Journal of Endodontics,Vol.35, 2009
6. Grossman LI, Meiman BW Dissolution of pulp tissue by chemical agents. Journal of American Dental Association 1941
7. Harrison JW, Hand RE. The effect of dilution and organic matter on the anti-bacterial property of 5.25% sodium hypochlorite. Journal of Endodontics Vol.7, 1981
8. Harrison JW, Svec TA, Baumgartner JC. Analysis of clinical toxicity of endodontic irrigants. Journal of Endodontics Vol.4,1978

9. Kuruvilla JR, Kamath MP. Antimicrobial activity of 2.5% sodium hypochlorite and 0.2% chlorhexidine gluconate separately and combined, as endodontic irrigants. Journal of Endodontics Vol.24,1998
10. Zehnder M. Root canal irrigants. Journal of Endodontics Vol.32, 2006
11. Torabinejad M, Khademi AA, Babagoli J, et al A new solution for the removal of the smear layer Journal of Endodontics Vol.29, 2003
12. Torabinejad M, Shabahang S, Aprecio R, Kettering JD. The antimicrobial effect of MTAD: An in vitro investigation. Journal of Endodontics Vol.29, 2003
13. E. L. Pashley, N. L. Birdsong, K. Bowman, BS, and D. H. Pashley Cytotoxic Effects of NaOCI on Vital Tissue Journal of Endodontics Vol. 11, No. 12,
December 1985
14. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965
15. Zehnder M. Root canal irrigants. Journal of Endodontics Vol.32, 2006
16. Harrison JW, Hand RE. The effect of dilution and organic matter on the anti bacterial property of 5.25% sodium hypochlorite. Journal of Endodontics, No.7, 1981
17. Dakin HD. On the use of certain antiseptic substances in treatment of wounds. Brazilian Medical Journal 1915; 2:318–20.
18. Paragliola R, Franco V, Fabiani C. Final Rinse Optimization: Influence of Different Agitation Protocols. Journal of Endodontics, No.36, 2010
19. Greenstein G, Berman C, Jaffin R. Chlorhexidine: An adjunct to periodontal therapy. Journal of Periodontology, 57, 1986
20. Torabinejad M, Khademi AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K, et al. A new solution for the removal of smear layer. Journal of Endodontics 2003;29:170–5
21. Moller JR. Microbiological examination of root canals and periapical tissues of human teeth-methodological studies 1966
22. Lisane Paquette, Milos Legner, Edward D. Fillery and Shimon Friedman Antibacterial efficacy of chlorhexidine gluconate intracanal medication in vivo Journal of Endodontics Vol.33, No. 7, July 2007
23. Sandeep singh, Sushma Krishna,Shashi rashmi acharya,P.Sugandhi rao, Vasudev Ballal Comparison of antimicrobial efficacy of Bio pure MTAD and 2.5% sodium hypochlorite irrigation in infected root canals following single visit endodontics - An in vivo study Endodontology
24. M. R. Leonardo, M. Tanomaru Filho, L. A. B. Silva, P. Nelson Filho, K. C. Bonifacio In Vivo Antimicrobial Activity of 2% Chlorhexidine Used as a Root Canal Irrigating Solution Journal of Endodontics Vol. 25, No. 3, March 1999

25. Beltz RE, Torabinejad M, Pouresmail M Quantitative analysis of the solubilizing action of MTAD, sodium hypochlorite, and EDTA on bovine pulp and dentin. Journal of Endodontics Vol.29, No.5, May 2003

TABLES & GRAPH:

TABLE 1: GROUPING OF SUBJECTS AS PER THE IRRIGATING SOLUTION USED

GROUP	NUMBER OF SUBJECTS	IRRIGANT USED
I	n=10	2.5% Sodium Hypochlorite
II	n=10	2% Chlorhexidine gluconate
III	n=10	Biopure MTAD
IV	n=10	Saline 0.9% (Control)

TABLE 2: STATISTICAL ANALYSIS USING PAIRED t TEST

GROUP	MEAN (%)	SD (%)	DIFFERENCE (%)	t value	p value
GROUP I	99.141	1.060	16.045±3.758	10.285	0.000 Significant
GROUP II	83.096	4.818			
GROUP I	99.141	1.060	3.623±3.624	2.386	0.028 Significant
GROUP III	95.518	4.684			
GROUP I	99.141	1.060	67.011±5.405	32.344	0.000 Significant
GROUP IV	32.130	6.465			
GROUP II	83.096	4.818	12.422±0.134	-5.846	0.000 Significant
GROUP III	95.518	4.684			
GROUP II	83.096	4.818	50.966±1.647	19.989	0.000 Significant
GROUP IV	32.130	6.465			
GROUP III	95.518	4.684	63.388±1.781	25.107	0.000 Significant
GROUP IV	32.130	6.465			

GRAPH 1 : COMPARISON OF REDUCTION OF COLONY FORMING UNITS FOLLOWING IRRIGATION WITH RESPECTIVE IRRIGATING SOLUTIONS