



Unique Journal of Medical and Dental Sciences

Available online: www.ujconline.net

Research Article

ANTIBACTERIAL EFFICACY OF METRONIDAZOLE AND GLUTARALDEHYDE WITH HAND AND ULTRASONIC IRRIGATION-AN IN VIVO STUDY

Jain Shweta^{1*}, Thakur Sophia²

¹Department of Conservative Dentistry and Endodontics, ESIC Dental College and Hospital, Rohini, New Delhi, 110085, India

²Department of Conservative Dentistry and Endodontics, Bapuji Dental College and Hospital Davangere, Karnataka. 577004, India.

Received: 07-06-2013; Revised: 24-07-2013; Accepted: 02-08-2013

*Corresponding Author: **Dr. Shweta Jain**

BDS, MDS Lecturer, Conservative Dentistry and Endodontics, ESIC Dental College and Hospital, New Delhi, 110085, India

E-mail: drshweta6@yahoo.co.in, Contact No.: 09911791150, Mailing address: Flat No 105, Plot No 41, Sec 9, Vasudha Apartment, Rohini, New Delhi 110085

ABSTRACT

Aim: Various irrigants and irrigating methods have been used in past 50 years but very few studies have been done on use of Metronidazole and Glutaraldehyde as endodontic irrigants. Hence, an in vivo study was planned to compare antibacterial efficacy of 0.5% Metronidazole and 2% Glutaraldehyde with saline, as final irrigating solutions and of hand/rotary instrumentation with conventional hand irrigation alone versus additional 3 minutes of ultrasonic irrigation, in single rooted teeth.

Methodology: The study sample comprised of seventy-two teeth that were randomly divided into three groups (I,II,III) based on the use of Saline, 0.5% Metronidazole, and 2% Glutaraldehyde as final irrigant, respectively. Canals were sampled before (s1) and after (s2) completion of hand/rotary instrumentation with conventional hand irrigation. Subsequently, additional 3 minutes of ultrasonic irrigation with respective irrigant was done and samples (s3) were collected. Samples were analysed and data was statistically evaluated using one way ANOVA, Tukey's multiple range test and Paired 't' test.

Results: Final flush with 0.5% Metronidazole and 2% Glutaraldehyde resulted in significant reduction in colony forming units (CFUs) counts following completion of hand/rotary instrumentation ($p < 0.001$). The addition of 3 minutes of ultrasonic irrigation resulted in significant reduction in CFUs counts in each group ($p < 0.001$).

Conclusion: Metronidazole and Glutaraldehyde appeared to be superior as final endodontic irrigants with higher antibacterial efficacy compared to saline. Ultrasonic irrigation also resulted in greater reduction in microbial count when compared to conventional hand irrigation alone.

Keywords: Metronidazole, Glutaraldehyde, Endodontics, Ultrasonic irrigation, Antibacterial efficacy.

INTRODUCTION

Bacteria and their byproducts play an essential role in initiation and perpetuation of pulpal and periapical disease^{1,2}. It is well known that endodontic infections are mixed infections with complex floral interactions. Therefore, one of the major objectives of endodontic therapy is disinfection of the root canal system. Irrigating solutions when used along with mechanical instrumentation play a major role in the disinfection process³.

Apart from the ability to flush out loose debris, lubricate the dentinal walls and dissolve organic matter in the canal, one of chief attributes of an irrigant is its antimicrobial action. Studies clearly show that in comparison to saline, chemical compounds that possess antibacterial property have superior effectiveness in bacterial elimination^{4,5}. It is also shown that regardless of the type of irrigant used, the bacterial population

inside the root canal is significantly reduced by the mechanical effects of the irrigation⁶.

In instances where anaerobic bacteria are acting as sole or major pathogens, metronidazole acts specifically without disturbing the commensal aerobic flora. The resistance to it develops very rarely⁷. Hence, it has been suggested for use as an irrigating solution. Furthermore, Weimes reported that the best procedure for mechanical root canal cleaning involved the use of a fixative agent as an irrigant⁸. As glutaraldehyde fixes tissues instantaneously, its 2% solution appears to be yet another possible agent. Apart from this, ultrasonic's having proved to be a useful adjunct to endodontic debridement both in time and efficiency⁹. Perhaps a more effective technique would be the passive activation of the file, ultrasonically, inside a canal as a final step in root canal debridement and this

needs further evaluation¹⁰. Various irrigants and irrigating methods have been used in past 50 years but very few studies have been undertaken in particular reference to metronidazole and glutaraldehyde.

Hence, the present study intended to compare antibacterial efficacy of 0.5% metronidazole and 2% glutaraldehyde with saline, as final irrigating solutions and of hand/rotary instrumentation with conventional hand irrigation alone versus additional 3 minutes ultrasonic irrigation in single rooted teeth.

MATERIALS AND METHODS

Following the approval of institutional review board, the study was undertaken at the Department of Conservative Dentistry and Endodontics, Bapuji Dental College and Hospital, Karnataka. Recently traumatized teeth and those associated with calcified canals, swelling, sinus or root resorption or required retreatment were excluded.

This research was conducted in full accordance with ethical principles, including the World Medical Association 'Declaration of Helsinki (version 2008)'. Written consent was taken from all the subjects involved in the study. Seventy-two randomly selected single rooted teeth, all with pulpal necrosis and indication for endodontic treatment in systemically healthy patients of age group 20-45 years were evaluated.

Teeth were randomly divided into three groups (I,II,III) based on the use of Saline (Goa Formulation limited), 0.5% Metronidazole (Unique's Metrogyl), and 2% Glutaraldehyde (Bioclenz-G) as final irrigants, respectively.

Disinfection and isolation:

Following anesthesia and application of the rubber dam, the operative field was disinfected with 30% H₂O₂ (MERCK) and 5% povidone iodine.

The tooth was accessed with sterile # 4 round bur. 0.5% NaCl was used to flush the debris from the pulp chamber. 1ml of saline was added to each canal via sterile 2ml syringe. K flex file was pumped 5 times within 1mm of estimated working length to disperse the canal contents. Sterile paper points were used to absorb the canal contents and then transferred to a test tube containing Stuarts transport media (Hi Media Laboratories). This constituted initial bacterial sample (s1).

Canal preparation:

K flex files (Dentsply) and rotary ProTaper (Dentsply) were used for canal preparation. After achieving Glide path canal was shaped with S1 upto 3/4th of the estimated working length. #10 K flex file was used to recapitulate and established patency to full working length. SX was then used to improve straight line access. S1 and S2 was then used up to full working length. 2ml of irrigating solution (1ml 0.9% saline + 1ml of 4% NaOCl) was used alternatively after each instrumentation.

Apical preparation was done using F1, F2, F3. Final apical preparation was done with #45 hand K flex file. After completion of cleaning and shaping an additional 15ml each of saline, 0.5%Metronidazole, 2% Glutaraldehyde was used in group I,II,III respectively with the help of 5ml syringe with 26 gauge needle. A second bacterial sample (s2) was collected in the similar manner as (s1). Additional 3 minutes of ultrasonic irrigation was carried out using P MAX ultrasonic unit and

ultrasonic # 25 K- files. On activation of file it was moved passively in up and down motion upto working length, for 3 minutes using 15ml of respective irrigant of each group followed by sample collection (s3).

Temporary restorative material was placed in teeth and the patients were then scheduled to have the root canal treatment to be completed at later date.

Microbiologic preparation and evaluation

All samples were transferred to microbiology laboratory for quantification of colony forming units (CFUs). The samples were streaked on nutrient agar (Hi media Laboratories) by using cell spreaders. The plates were incubated in anaerobic chamber for 7 days at 37°C. CFUs were counted after 7 days with aid of digital colony counter.

STATISTICAL ANALYSES

Comparison of reduction in CFUs counts (s1-s2) among different groups were made using one way ANOVA followed by Tukey's multiple range test for pair wise comparison (Table I). Paired "t" test was used to compare mean CFUs counts after instrumentation and conventional hand irrigation (s2) and after ultrasonic irrigation (s3). $p < 0.001$ was considered as statistically significant (Table II).

RESULTS

Final flush with 0.5% Metronidazole and 2% Glutaraldehyde resulted in highly significant reduction in CFUs counts following completion of hand/rotary instrumentation when mean differences (s1-s2) values of CFUs were subjected to one way ANOVA ($p < 0.001$) (Table I). However there was no significant difference among Metronidazole and Glutaraldehyde when used as last irrigants, when mean difference in samples (s1-s2) values, of reduction in CFUs were subjected to Tukey's multiple range test.

There was significant reduction in CFUs count at sample (s3) after 3 minutes of ultrasonic irrigation in all the groups when compared to sample (s2) using paired "t" test ($p < 0.001$) (Table II).

DISCUSSION

In the present study, 72 single rooted teeth of individuals between age group of 20-45yrs were included. The finding of no significant differences between the two groups for tooth distribution, age, root morphology and working length suggest that the groups were of equal difficulty. The in vivo nature of this study makes isolation, access, working length determination and instrumentation more clinically relevant than in vitro studies. That is, in vitro studies can control for poor access, can determine working lengths accurately by visualizing files at the apical foramen and instrument teeth without regard to difficult access or clinical time constraints. Therefore in vivo studies may be more clinically relevant than in vitro studies².

A culture dependent approach was used in the present study for the determination of microbial count before and after the instrumentation. It is one of the most reliable methods of detecting viable bacteria particularly when samples are taken immediately after antimicrobial treatment where viability may not be ascertained by most of culture independent methods¹¹. In present study biomechanical preparation was carried out with hand/rotary instrumentation using ProTaper rotary

system (Dentsply) and K-flex files (Dentsply). The apical preparation in all the samples irrespective of the tooth type was done upto #45 K-flex file. According to published data, Khademi et al¹² conducted a study to determine minimum instrumentation size required for the effective penetration of irrigants and elimination of debris and smear layer from the apical third of the root canal. Based on the results it was shown that the minimum instrumentation size needed for penetration of irrigants to the apical third of the root canal was #30 k file. Coldero et al¹³ conducted a study to compare in vitro intracanal bacterial reduction using NiTi rotary instruments with and without apical enlargement. And it was concluded that it may not be necessary to remove dentine in the apical part of the root canal when a suitable coronal taper is achieved to allow satisfactory irrigation of the root canal system with antimicrobial agents. These studies therefore justify the apical preparation selected in the present study.

Metronidazole is bactericidal for anaerobic organisms at relatively low concentration¹⁴. The sensitive organisms possess certain biochemical pathways involving ferredoxin or flavodoxin type electron transport proteins which are capable of reducing the nitro group of metronidazole. The reduced derivative exerts a deleterious action on cell, possibly by combining with important cellular macromolecules. Reduced ferredoxin is oxidized in the presence of oxygen providing competition for electrons needed for metronidazole reduction. This serves as possible explanation of why metronidazole is so efficient at killing anaerobes but not aerobes⁷. There was found to be 88% reduction in CFUs counts after using

metronidazole as final irrigant in Group II. These results were consistent with the finding of Sanjiwan and others who concluded that 0.25% metronidazole solution is more effective than saline solution as root canal irrigant¹⁵.

Weimes et al¹⁶ reported that the best procedure for the mechanical root canal cleaning was using a fixative agent as root canal irrigant. A fixative drug can be considered to be a disinfecting drug since fixation of bacteria results in their death. In addition resulting products cannot be used as a substrate by remaining living bacteria. Diffusion of Glutaraldehyde through dental hard tissue is limited to the first +/- 200µm of dentinal tubules thus preventing chemical irritation of periodontal structures¹⁷. There was found to be 93% reduction in CFUs counts after instrumentation, when Glutaraldehyde was used as final irrigant.

In present study, 5ml syringe with 26 gauge beveled needle with 45° bend was used for conventional hand irrigation. The needle should remain loose inside the canal to allow the irrigant to reflux and causes more debris to be displaced coronally. At the same time needle is moved up and down in the canal to enhance mechanical action¹⁸. Nevertheless, the mechanical flushing action created by conventional hand held syringe needle irrigation, is relatively weak¹⁹⁻²¹. With conventional syringe needle irrigation, irrigating solution is delivered only 1mm deeper the tip of the needle, which is usually located in the coronal third of a narrow canal, or at best, the middle third of wide canals hence inaccessible canal extensions and irregularities are likely to harbor debris and bacteria^{22,23}.

Table I: Comparison of reduction in CFUs counts (s1-s2) among different groups.

Groups	Group I (Saline)	Group II (Metronidazole)	Group III (Glutaraldehyde)	F* Value	Significant Pairs**
Mean Difference (s1-s2)	55	327	279.83	106.9 P<0.001 HS [#]	I&II, I&III
SD	33.10	45.99	62.47		

*One way ANOVA, **Tukey's multiple range test, # Highly Significant

Table II: Comparison between mean CFUs counts after instrumentation and conventional hand irrigation (s2) and after ultrasonic irrigation (s3).

Sub Groups		s2	s3	Mean difference	t* Value	Significance
Group I (Saline)	Mean	246.75	130.25	116.50	21.1	P<0.001 HS [#]
	SD	19.76	17.59			
Group II (Metronidazole)	Mean	50.58	17.33	33.25	4.2	P=0.001 HS [#]
	SD	30.16	3.94			
Group III (Glutaraldehyde)	Mean	27.58	14.33	13.25	17.4	P<0.001 HS [#]
	SD	2.81	2.35			

*Paired 't' test, # Highly Significant

The passive ultrasonic irrigation system has the potential to achieve the goals, which conventional syringe irrigation failed to achieve. An ultrasonically oscillating file transmits energy, causing acoustic micro streaming, which results in mixing of the irrigant, enabling it to reach those inaccessible regions^{24,25}.

During passive ultrasonic irrigation energy is transmitted from an oscillating file or a smooth wire to the irrigant into the root canal by means of ultrasonic waves. These waves induce acoustic streaming and cavitation of the irrigants^{26,27}. Patrick concluded that 3 minutes of activation with a file had

significantly less debris remaining than 1 minutes of activation with a spreader²⁸. In the present study PMAX ultrasonic device (Satelec, Meriganc Cedex, France) with #25 ultrasonic files were used to determine the efficacy of additional 3 minutes of ultrasonic irrigation, with intermittent flush technique, at medium effective power output over the conventional hand syringe irrigation. As per manufacturer instructions, the frequency employed was approximately 30 Kilo Hertz. The present study demonstrated that there was significantly greater reduction in CFUs counts by additional 3min ultrasonic irrigation than by hand syringe irrigation. There was found to be 73% reduction with saline, 96% reduction with 0.5% metronidazole, 97% reduction with 2% glutaraldehyde, when used as final flush irrigants, when compared to be 16% reduction with saline, 88% reduction with 0.5% Metronidazole, 93% reduction with 2% Glutaraldehyde, with conventional hand syringe irrigation alone.

One reason for this may be that during ultrasonic irrigation a much higher velocity and volume of irrigant flow was created in the canal. 15ml of extra irrigant was applied during ultrasonic irrigation, apart from 37ml irrigant used during syringe irrigation. The results suggested that the flushing action of the irrigant was strongly enhanced by the combined use of ultrasound and greater volume of irrigant solutions²⁹⁻³¹. The present study however, has some limitations. It would be of interest to carry out further studies to determine the efficacy of Metronidazole and Glutaraldehyde as main endodontic irrigants. It would also be of interest to analyze the effect of ultrasonic irrigation on the antibacterial efficacy of the various irrigants used, apart from its mechanical flushing action.

CONCLUSION

Our findings suggest that 0.5% metronidazole and 2% glutaraldehyde have greater antibacterial properties compared to saline, when used as final flush irrigant. Additional ultrasonic irrigation for 3 minutes resulted in greater reduction of microbial count when compared to the conventional hand irrigation.

REFERENCES

1. Schafer E, Dent M, Bossmann K. Antimicrobial efficacy of chlorhexidine and two Ca(OH)₂ formulations against E faecalis. J Endod, 2005; 31(1): 53-6.
2. Gutarts R, Nusstein J, Reader A, Beck M. In vivo debridement efficacy of ultrasonic irrigation following Hand/Rotary instrumentation in human mandibular molars. J Endod, 2005; 31(3): 166-170.
3. Jeffrey AC. The choice of irrigant during hand irrigation and ultrasonic irrigation of root canal: a scanning electron microscope study. Australian Dental Journal, 1995; 40(2): 85-90.
4. Siqueira JF, Machado AG, Silveira RM, Lopes HP, de Uzeda M. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of Enterococcus faecalis from the root canal. Int Endod J, 1997; 30(4): 279-82.
5. Bystrom A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. Oral Surgery Oral Medicine Oral Pathology, 1983; 55(3): 307-12.
6. Siqueira JF JR, Lima KC, Magalhaes FAC, Lopes HP, de Uzeda M. Mechanical reduction of the bacterial cell number inside the root canal by three instrumentation techniques. J Endod, 1999; 25(5): 332-5.
7. Mitchell DA. Metronidazole: its use in clinical dentistry. Journal of Clinical Periodontology, 1984; 11(3): 145-158.
8. Rubio AJ, Segura JJ, Llamas R. In Vitro study of effect of Sodium Hypochlorite and Glutaraldehyde on substrate adherence capacity of macrophages. J Endod, 1997; 23(9): 562-4.
9. Weller RN, Brady JM, Bernier WE. Efficacy of ultrasonic cleaning. J Endod 1980;6(9):740-43.
10. Grundling GL, Zechin JG, Jardim WM, de Oliveira SD, de Figueiredo JAP. Effect of Ultrasonics on Enterococcus faecalis Biofilm in a Bovine Tooth Model J Endod, 2011; 37: 1128-33.
11. Siqueira JF, Rocas IN. Exploiting molecular methods to explore endodontic infections: Part I, current molecular technologies for microbiological diagnosis. J Endod, 2005; 31: 411-23.
12. Khademi A, Yazdizadeh M, Feizianfard M. Determination of the minimum instrumentation size for penetration of irrigants to the apical third of root canal systems. J Endod, 2006; 32(5): 417-20.
13. Coldero LG, Mchugh S, Mackenzie D & Saunders WP. Reduction in intracanal bacteria during root canal preparation with and without apical enlargement. Int Endod J, 2002; 35(5): 437-46.
14. Finegold. Metronidazole. Proceedings of the international Metronidazole conference, Montreal, Quebec, Canada, may 26-28, 1976, Sessions, II, IV, VI, VII.
15. Sanjiwan R, Chandra S, Jaiswal JN, Mastan . The Effect of Metronidazole on the anaerobic microorganisms of the root canal-a clinical study. Federation of Operative Dentistry, 1995; 1(2): 30-6.
16. Jain A, Chandra S, Srivastava R. Effects of various root canal irrigants on the adaptation of root canal sealer in permanent teeth- a SEM study. Endodontology, 1999; 11: 21-26.
17. Wemes, JC, Veldkamp DF, Lewis DP. Glutaraldehyde in endodontic therapy: philosophy and practice. Journal of Dentistry, 1983 ;11(1):63-70.
18. Khan FH, Rosenberg PA, Gliksberg J. An in vitro evaluation of the irrigating characteristics of ultrasonic and sonic hand pieces and irrigating needles and probes. J Endod, 1995; 21(5): 277-80.
19. Nair PN, Henry S, Cano V, Vera J . Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one visit" endodontic treatment. Oral Surgery Oral Medicine Oral Pathology, 2005; 99(2): 231-52.
20. Wu MK, Wesselink PR. A primary observation on the preparation and obturation in oval canals. Int Endod J, 2001; 34(2) :137-41.

21. Wu MK, Dummer PM, Wesselink PR. Consequences and strategies to deal with residual post treatment root canal infection. *Int Endod J* 2006; 39(5): 343-56.
22. Siqueira JF, Rocas IN. Exploiting molecular methods to explore endodontic infections: Part I, current molecular technologies for microbiological diagnosis. *J Endod*, 2005; 31: 411-23.
23. Chow TW. Mechanical effectiveness of root canal irrigation. *J Endod* 1983;9(11):475-9.
24. Al-Jadaa A, Paque F, Attin T, Zehnder M . Acoustic hypochlorite activation in simulated curved canals. *J Endod*, 2009; 35: 1408–11.
25. de Gregorio C, Estevez R, Cisneros R, Heilborn C, Cohenca N. Effect of EDTA, sonic, and ultrasonic activation on the penetration of sodium hypochlorite into simulated lateral canals: an in vitro study. *J Endod*, 2009; 35:891–5.
26. Ahmad M, Pitt Ford TR, Crum LA. Ultrasonic debridement of root canals: acoustic streaming and its possible role. *J Endod*, 1987; 13(10): 490-9.
27. Ahmad M, Pitt Ford TR, Crum LA. Ultrasonic debridement of root canals: acoustic cavitation and its relevance. *J Endod*, 1988;14(10): 486-93.
28. Munley PJ, Goodell GG .Comparison of passive ultrasonic debridement between fluted and nonfluted instruments in root canals. *J Endod*, 2007; 33(5): 578-80.
29. Goodman A, Reader A, Beck M, Melfi R, Meyers W . An in vitro comparison of the efficacy of the step back technique verses a step/ultrasonic technique in human mandibular molars. *J Endod*, 1985; 11(6): 249-56.
30. Wu MK, Wesselink PR . A primary observation on the preparation and obturation in oval canals. *Int Endod J*, 2001; 34(2):137-41.
31. Cunningham WT, Martin H, Forrest WR. Evaluation of root canal debridement by the endosonics ultrasonic synergistic system. *Oral Surgery Oral Medicine Oral Pathology*, 1982; 53(4): 401-404.

Source of support: Nil, Conflict of interest: None Declared