COMPARATIVE EVALUATION OF DIFFERENT IRRIGATION METHODS IN REMOVAL OF TRIPLE ANTIBIOTIC PASTE- AN IN-VITRO STUDY.

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Article History: Received: 12.12.2022 **Revised:** 29.01.2023 **Accepted:** 15.03.2023

Abstract

Introduction: The aim of this study was to to evaluate and compare the efficacy of manual dynamic activation, passive ultrasonic irrigation and laser in removal of triple antibiotic paste (TAP) from root canal walls.
Methods: Thirty-six extracted human mandibular premolars were prepared using ProTaper rotary files up to size F3. The roots were divided longitudinally and a standardized groove was prepared in the apical part of one segment. The root halves were assembled after inserting TAP into the grooves. According to the irrigation protocol used, the specimens were randomly divided into three experimental groups: manual dynamic activation, passive ultrasonic irrigation (PUI) and laser activated irrigation using with 5.25% NaOCl and 17% EDTA. The root segments were separated, and a four-grade grading system was used to determine the remaining TAP under a stereomicroscope at 10x magnification. The data were evaluated statistically using Student t test and ANOVA test with a 95% confidence level (P = 0.05).
Results: There were significant differences amongst the groups (P < 0.001). Passive ultrasonic irrigation (PUI) and laser assisted irrigation removed significantly more TAP than manual dynamic activation. However, no significant difference was seen among the passive ultrasonic irrigation and laser assisted irrigation.
Conclusions:PUI was most successful at removing TAP from root canal artificial grooves. However, TAP could not be totally eliminated from root canals.

Keywords: Triple antibiotic paste, ultrasonic activation, diode laser, manual dynamic activation, Protaper.

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DOI: 10.31838/ecb/2023.12.s2.062

1. Introduction

The complete eradication of microorganisms from the root canal system is the primary objective of endodontic treatment. The root canal system has various complexities that includes the presence of apical deltas, fins, anastomoses and isthumus.[1] The routine chemo-mechanical methods cannot eliminate the microbes from these complexities.[2] However, combination of mechanical instrumentation, various irrigation solutions and intracanal medicaments can result in reduction of bacterial count in infected root canal.

Prognosis of endodontic treatment can be improved with the help of intracanal medicaments. They have an intended pharmacological action. The triple antibiotic paste was introduced by Hoshino et al in the year 1998.The mixture that he proposed contained ciprofloxacin, metronidazole, and minocycline each at a combination of 25µg per ml of paste and it was able to disinfect the root dentine in-vitro.[3]Triple antibiotic paste was developed to offer a combination that might be effective against all microorganisms seen in root canal pathology. The introduction of triple antibiotic paste aimed to provide a combination that might be efficient against every microorganism in the root canal pathology. 3-MIX MP is a commercial version of this combination.

Despite being proven to have a significant advantage in the root canal system's disinfection, there are a few drawbacks that make the total removal of TAP necessary. Discolouration of the crown is the major drawback of TAP. The use of minocycline in the formulation resulted in this discoloration.[4]Another disadvantage of using TAP is that, it was found to be detrimental to the apical papilla stem cells, when it is used in the regeneration procedure for the complete disinfection of the root canal.[5] Additionally, it has been demonstrated that using TAP for more than a month significantly reduced the micro-hardness of dentin.[6] TAP can also affect the sealer setting and sealer penetration which can further lead to reduction in bond strength.[7]

The removal of triple antibiotic paste from the apical third poses a significant challenge to the clinicians as the remnants of this medicaments can be found in apical ramifications. Hence, several irrigation techniques have been used to eliminate it from these ramifications.

TAP has been eliminated using a variety of irrigation techniques, however limited information is available on this issue.The aim of this study was to to evaluate and compare the efficacy of various irrigation protocols on the removal of TAP from an artificial groove created in a root canal. The null hypothesis was that there is no difference amongst the various irrigation protocols in the removal of TAP.

2. Materials and Methods

Specimen preparation

This study was approved by the Ethical Committee/ Institutional Review Board of Bharati Vidyapeeth Deemed to be University Dental College and Hospital, Pune. Identity of all patients whose teeth were used for study were kept confidential. Thirty-six single-rooted, non-carious mandibular premolars with similar sizes and completed apices were selected. The selected teeth were ultrasonically cleaned to remove any soft tissue or calculus present on the root surface. Buccolingual and mesiodistal radiographs were taken from the specimens to evaluate their anatomy. The teeth were verified radiographically as having a single root canal. The teeth were then stored in 4 °C distilled water until used. Specimens were decoronated with a diamond disc under water coolant to obtain a standardized root length of 13 mm. Then, the working length (WL) was established 1 mm short of the root length. The root canals were prepared with ProTaper rotary files) up to a F3 master apical file size.Irrigation was done with 2ml of 5.25% sodium hypochlorite solution between each instrument and a final flush was done with 2ml of 17% EDTA solution. After instrumentation, the specimens were fixed in clear acrylic resin (DPI) with a mould made of silicone material (3M). After removal from the impression material, all the roots were grooved longitudinally on the buccal and lingual surfaces with a diamond disc under copious water irrigation, avoiding penetration into the root canal. The roots were then split into two halves. A longitudinal groove of approximately 4mm long,0.2mm wide and 0.5mm deep was then cut in the root canal wall of one half of each tooth at 2-5mm from the apex using a small cylindrical bur. The purpose of this was to simulate an un-instrumented canal extension in the apical region.

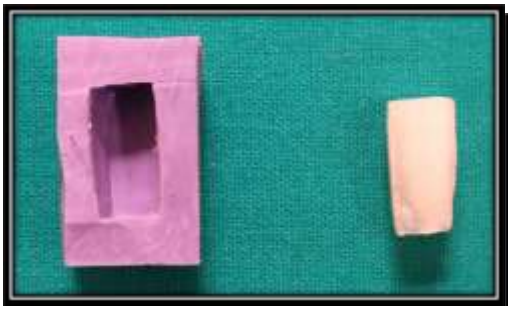


Fig 1: Specimens mounted in clear acrylic using putty mould

Fig 2: Longitudinal grooves made for splitting using diamond disc.

Fig 3: Longitudinally split specimens

Fig 4: Standardized longitudinal Groove in apical third

A tooth brush was used to remove debris from the root halves and grooves. A final flush was applied using 5ml of 17% EDTA and 5ml of 5.25% NaOCl each for one minute. The root canal was dried with paper points.

Preparation of triple antibiotic paste

Triple antibiotic paste was prepared in the ratio of 1:1:1 by using commercially available tablets metronidazole (Metrogyl 400,Jb Chemicals and Pharmaceuticals Ltd), ciprofloxacin (Ciprodac 500,Cipla Ltd.) and minocycline (Divonine 100,Cipla Ltd.). The enteric coating of the tablets was removed and pulverised into a powder using motor and pestle. The powder was then transferred to a dapen dish and mixed with propylene glycol in the ratio 3:1 to obtain a paste form.

Fig 5: Filling the longitudinal groove with Triple Antibiotic paste

Fig 6: Specimen reassembled using sticky wax

The grooves were filled with TAP, the root halves were reassembled using sticky wax. Access to the root canals was temporarily sealed with a cotton pellet and Cavit (Espe, Seefeld, Germany), and the specimens were then kept at 37 ℃ with 100% humidity for 1 week. The specimens were divided randomly into three groups(n = 12) and irrigated as follows:

Group 1 (n=12) -Manual dynamic activation.
5 ml of 5.25% NaOCl was delivered to the root canal with the side-vented needle and agitated with a ProTaper gutta-percha cone (size F3) (Dentsply Maillefer, Switzerland) approximately 1mm short of working length and moved for 1 minute in a corono-apical direction using back and forth strokes of approximately 3 mm with a frequency of 100 movements per minute.

Group 2 (n=12)-Passive ultrasonic irrigation
Specimens were subjected to passive ultrasonic irrigation using Ultra X activator (Orikam,India) at high frequency of 45 kHz. The ultrasonic tip was placed into the root canal 1mm short of the working length along with 5.25% NaOCl. It was activated in the root canal without touching any walls. The tip was activated for 20 secs and was used in pumping motion. When the irrigating solution declined it was replaced by fresh irrigating solution. Three applications with total agitation time of 60s were performed with a total of 5ml of 5.25% NaOCl.

Group 3 (n=12)-Laser activated irrigation.
Specimens were subjected to laser activated irrigation where NaOCl was activated by laser irradiation (Diode laser; Novolase Gold) using endodontic fibre with a diameter of 200 µm and 15mm length. 1 ml of 5.25% NaOCl was delivered to the canal and the optical fiber tip was placed in the canal about 5 mm short of the apex and activated for 20 secs. when the irrigating solution declined it was replaced by fresh irrigating solution. Three applications with total agitation time of 60 secs were performed with a total of 5ml of 5.25% NaOCl. The specimens were flushed with normal saline. This was followed by final flushing with 5ml of 17% EDTA for 60 secs. Following each irrigation procedure, root canals were given a final flush with distilled water and dried with paper points. Finally, the roots were dissembled to evaluate the removal of TAP.

Evaluation of the remaining TAPin the canal
All the specimens were analysed for the remaining TAP using stereomicroscope at 10x magnification. Digital images of the root halves were obtained by digital camera connected to the stereomicroscope. The images were then transferred to the computer. The images were evaluated with Image Analysis System (Chroma Systems Pvt. Ltd., India) for TAP remaining in the canal.
The remaining TAP was measured in mm^2 and was recorded as a percentage of overall surface area of the root canal space. A scoring system as proposed by Aksel et al[8] was used to score the obtained percentage of remaining TAP in the canals:

- Score 1 – less than 25% of the root canal filled with TAP,
- Score 2 – 25-50% of the root canal filled with TAP,
- Score 3 – 50-75% of the root canal filled with TAP,
- Score 4 – 75-100% of the root canal filled with TAP.

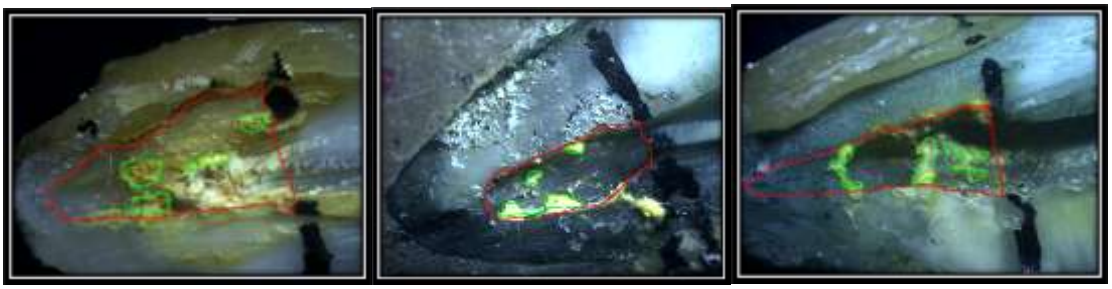


Fig 7: Stereomicroscopic Images of group 1, group 2 and group 3

Statistical Analysis

Student t tests (two tailed, unpaired) was used to find the significance of study parameters on continuous scale between two groups. Analysis of variance (ANOVA) was used to find the significance of study parameters between the groups (Inter group analysis). Further post hoc analysis was carried out if the values of ANOVA test were significant.Level of significance was fixed atp=0.05 and any value less than or equal to 0.05 was statistically significant. The Statistical software IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses of the data and Microsoft word and Excel were used to generate graphs, tables etc.

3. Results

The unpaired t test, revealed no significant difference in the values of residual TAP for both the Groups. Group 2 (Ultrasonic irrigation,12.35) showed lower residual TAP value than Group 3 (Laser Activated Irrigation,12.55) **[p - value > 0.001]**. It is observed that Group 1 shows the maximum amount of TAP after irrigation protocols (26.66) whereas Group 2 shows minimum amount of TAP after the irrigation protocol (12.35).

Table 1: Comparison of the remaining triple antibiotic paste in terms of {Mean (SD)} among all the 3 groups using ANOVA test.

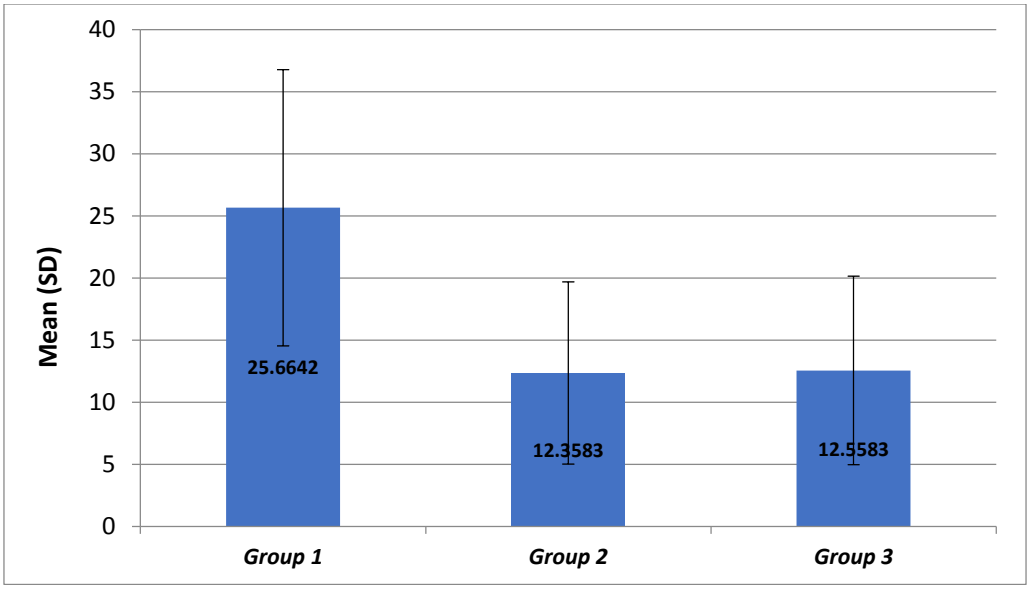
Group	N	Mean	Std. Deviation	F value	P value
Group 1	12	25.6642	11.12923	**8.893**	**<0.001****
Group 2	12	12.3583	7.33937		
Group 3	12	12.5583	7.59228		
Total	36	16.8603	10.66965		

($p < 0.05$ - Significant*, $p < 0.001$ - Highly significant**)

Table 2: Represents Tukey's post hoc analysis

	Group 1	Group 2	Group 3
Group 1	**-**	**0.002***	**0.003***
Group 2	**0.002***	**-**	**0.998**
Group 3	**0.003***	**0.998**	**-**

Graph 1: Comparison of the remaining triple antibiotic paste in terms of {Mean (SD)} among all the 3 groups using ANOVA test



ANOVA test and Tukey's post hoc analysis shows that there was statistically significant difference amongst all the groups except between Group 2 & Group 3 (**p = 0.99**). Highly significant difference (p < 0.001) observed between: Group 1 and Group 2 alsoGroup 1 and Group 3.

4. Discussion

The success of root canal therapy depends on the complete elimination of these diverse varieties of pathologic micro-organisms. Mechanical instrumentation of the root canal is considered as a primary way to eradicate these micro-organisms but the complex anatomy of the root canal system poses a significant challenge in achieving the same. Antibiotics are now effectively used for the targeted, localised antibacterial action in recent years. Due to the presence of various species of bacteria in the root canal system, a single empirical antibiotic does not seem to provide sufficient disinfection of the root canal system. Triple Antibiotic paste was introduced by Hoshino et al in the year 1996 as an intracanalmedicament. TAP is a combination of ciprofloxacin, metronidazole and minocycline in the ratio of 1:1:1. Many researches were conducted to evaluate the antimicrobial efficacy of TAP. In a study conducted by Mozayeni et al in the year 2014, they compared the antimicrobial efficacy of four different intracanal medicaments against Enterococcus feacalis and showed that TAP was more effective than calcium hydroxide, chlorhexidine gel and nanosilver[9] Despite the various advantages of TAP in endodontics, some disadvantages have been reported. When TAP is present in the root canal for a longer period, it could cause discolouration of the root canal dentin. This discolouration is due to the presence of minocycline in its composition.[10] Yilmaz et al in 2016 concluded that TAP caused reduction in the microhardness of the dentin when used for more than a month.[11] Otkay et al in 2018 proposed that TAP interfered with the sealer penetration causing a weaker interface between the sealer and the root dentin.[7]Thus, these studies advocate the removal of TAP from the root canal system completely. In general, previous studies have demonstrated that several irrigating solutions and irrigation techniques including activation with ultrasonic energy were effective in removing intracanal medicaments (van der Sluis et al. 2007, Tasdemir et al. 2011)[12]. The aim of this study was to determine the efficacy of various irrigation protocols in removing TAP from an artificial groove. The results of this study showed that none of the irrigation technique could completely remove the triple antibiotic paste from the standardized groove made in apical third of root canal. This is in accordance with many previous studies.[13,14] The amount of TAP remaining after manual dynamic irrigation (Group 1) was 25.66%, while that with PUI (Group 2) and LAI (Group 3), was 12.35% and 12.55% respectively. Further using tukey's post hoc analysis significant difference was found between Group I and Group II (p value: 0.002) and Group 1 and Group 3 (p value:0.003). No significant difference was found between Group 2 and Group 3 (p value: 0.99). Therefore, the null hypothesis that there is no difference amongst the various irrigation protocols in removing TAP can be rejected. The irrigants chosen in the present study were 5.25% NaOCI (sodium hypochlorite) and 17% EDTA (ethylene diamine tetraacetic acid) which are the most common irrigants used in clinical practice. NaOCl has been systematically used as an endodontic irrigant for the chemo mechanical preparation of root canals because of its excellent antimicrobial

action, capacity of dissolving organic tissue remnants.Since EDTA acts by dissolving the inorganic component of smear layer several authors have recommended its use in combination with NaOCl (0.5-5.25%) in order to remove organic remnants. Manual dynamic activation is a technique that helps in the distribution of the irrigant and its exchange within the root canal. It also enhances the effectiveness of the irrigant solution used. In this process, the irrigant present in the canal reaches the root apex and disrupts the vapor lock.[15] It creates an increased intracanal pressure change within the canal during the forward and backward movement of the gutta-percha cone which creates turbulences that enhance the diffusion of shear stresses.[16] However, the results of this study indicate that the apical penetration of the irrigant in MDA group was inferior to PUI group and LAI group. This could be because the frequency of push–pull motion of the gutta-percha point at 100 strokes per minute generates currents at a lower frequency (3.3 Hz)[17] as compared with higher frequency of 40 to 45 kHz generated due to secondary acoustic streaming during PUI.[18]Manual dynamic irrigation has been shown to be significantly more effective than auto dynamic and conventional irrigation.[17] It is also simple and less expensive, but has the disadvantage of being more laborious and time consuming.[19] Irrigation techniques which cause activation of irrigant would be more effective in removing organic and inorganic debris, hence PUI and LAI have been included in this study. As mentioned earlier, the results indicate a significant difference (p value: 0.002) between Group 1 (Manual dynamic activation) and Group 2 (Passive Ultrasonic Irrigation) and similarly between Group 1 and Group 3 where laser activated irrigation was used (p value:0.003).This agrees with studies of Antony et al. and Mansi et al. who reported that the use of ultrasonic was more efficient than conventional irrigation and sonic irrigation.[20,21] Several other authors have also reported that ultrasonic irrigation has the potential to remove dentin debris and organic tissue from inaccessible root canal areas and planktonic bacteria.[22] Passive ultrasonic irrigation (PUI) is based on the transmission of energy from an ultrasonically oscillating instrument (frequency 25-30 kHz) to the irrigant inside the root canal. The ultrasonic tips operate in a transverse vibration, setting up a characteristic pattern of nodes and anti-nodes along their length, resulting in acoustic microstreaming and cavitation of the irrigant in the root canal.[12] The resultant high velocity of irrigant flow generated by PUI flushes out triple antibiotic paste from root canals. The efficiency is also improved with fresh irrigant replacement. [23,24]

According to Srirekha et al. (2017)[25] another explanation for the increased effectiveness of PUI could be the biomechanical preparation prior to the PUI, as done in this study. As the apical root canal is widened, the file can move freely without dampening of its vibrations, this would enhance the flow of irrigant and there is also better exchange of irrigant between the coronal and apical part. Therefore, enhancement of the flushing action is seen even when the ultrasonically activated tip does not reach the full working length.[26]In this study Ultra X- Cordless Ultrasonic Activation Device (Orikam, India) was used for PUI. It works at 45 kHz ultrasonic frequency. Diode lasers that have recently been introduced into clinical practice for soft-tissue surgery.Diodes of 810-980 nm are used in the disinfection of periodontal pockets and root canals. The effect by which diode kill the microorganisms are the photo thermal effect (laser energy converted to thermal energy). Laser activated irrigation (LAI) regime was evaluated in this study, using diode laser (Novolase Gold,India) at wavelength of 980 nm. The endodontic fiber with a diameter of 200 µm and 15mm length was placed in the canal about 5 mm short of the apex and activated for 20 secs. when the irrigating solution declined it was replaced by fresh irrigating solution. Three applications with total agitation time of 60 secs was followed. The use of lasers can cause some side effects in the root canal, such as carbonization and cracks, collateral damage, apical extrusion of the solution, and temperature increases. To avoid this the fiber tip was placed 5mm short of the working length and activated for 20 sec and during laser irradiation, the hydration of root canal was maintained by continuously replenishing the irrigant. The results of this study showed that the LAI was superior to manual dynamic activation (p value:0.003). No statistically significant difference was seen in PUI and LAI in removal of triple antibiotic paste from the root canals (p value:0.99). The probable cause for this could be that the cavitations developed after several seconds of laser operation. This clearly differs from the action of erbium lasers, where the bubble forms instantly (only a few microseconds after onset of the pulse) at the fiber tip and is invisible to the human eye because it exists for only a few hundred microseconds.[27] In this study, the diode laser was significantly more effective at removing debris from the groove than manual dynamic activation but less effective than PUI. The bubble formation for the diode laser was limited to the area just around the fiber tip and was considered more to be the result of streaming than of generation of vapor bubbles (cavitation).[28] Another reason for better performance of PUI can be the physical properties of NaOCl. It is a salt water suspension. Bubbles formed in salt water tend to be more numerous in

number and are smaller in size. These bubbles thus formed are less prone to coalesce.[28] Because of this, the acoustic microstreaming will be different and could perhaps be more powerful.

5. Conclusion

Within the limitations of the study,none of the irrigation technique could remove triple antibiotic paste completely from the artificial standardized groove in the apical third of the roots.Among the irrigation techniques used Passive ultrasonic irrigation was found to be the most effective in removing triple antibiotic paste from root canals closely followed by Laser activated irrigation with Diode laser.

Limitations

Clinical scenarios may be quite different as the biological root canal system and the variations in the canal morphology seen in-vivo could affect the efficacy of different irrigation systems. Also, the irrigation techniques were compared in straight root canals in the present study and further studies should be conducted to evaluate the effectiveness of the techniques in curved root canals.

Acknowledgement

The author wishes to express her gratitude to the colleagues and faculty of The Department of Conservative Dentistry and Endodontics, BVDU Dental College and Hospital, Pune for the constant help, support and valuable suggestions provided in making the manuscript.

Conflict of Interest

The authors report no conflict of interest.

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