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Original Research Article

Efficacy of different irrigation techniques in removal of N-acetyl cysteine as an intracanal medicament- An *in-vitro* study

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ABSTRACT

Context: Intra canal medicament must be completely removed from the pulp space for better penetration of sealers. Various methods of irrigant delivery are used for removal of medicament from root canals. Removal of N-acetyl cysteine when used as an intracanal medicament hasn't been studied yet.
Aim: To compare efficacy of different irrigation techniques, ultrasonic, sonic, navi tips, side vented needles in removal of N-acetyl cysteine as an intracanal medicament- an *in-vitro* study
Materials and Methods: Fifty-two maxillary anterior teeth were decoronated and standardized to a root length of 13 mm. Root canals were prepared with Protaper Universal rotary system. N-acetyl cysteine paste was placed in root canals with lentulospiral and kept in an incubator for 7 days. Then four groups (n=13) were established according to the removal technique- Group 1- Passive ultrasonic irrigation, Group 2- Sonic irrigation, Group 3- Manual irrigation with NaviTip, Group 4- Manual irrigation with side vented needles. Each tip was placed 2 mm short of working length and irrigation was done using 5.25% sodium hypochlorite. Final irrigation was done, then roots were split buccolingually and evaluated for residual medicament under stereomicroscope at 24x magnification.
Statistical analysis used: Done using Kruskal-Wallis and Mann-Whitney tests ($P \leq 0.05$).
Results : In coronal and middle third, there was significant difference in removal of medicament by Group 1- Passive ultrasonic irrigation compared to other techniques, whereas, in apical third Group 2- Sonic irrigation showed better result. Group 3-Manual irrigation with NaviTip and Group 4- Manual irrigation with side- vented needles was significantly less effective than other techniques.
Conclusion: Use of irrigants with activation devices like ultrasonic and sonic revealed improved removal of N- acetyl cysteine intracanal medicament.

1. Introduction

The primary etiological factors in teeth with pulp and periapical disorders are microorganisms, so a complete disinfection of root canal is needed to ensure success of endodontic therapy. A combination of instrumentation, irrigation and intra canal medication is needed for success/ long term prognosis of the tooth. In multivisit endodontics, intracanal medicaments are a prerequisite to eliminate bacteria.[1]

There are various intracanal medicaments utilized to completely eradicate microorganisms, to reduce flare up and post-operative pain. Most commonly used medications are calcium hydroxide, chlorhexidine, TAP, DAP etc.

Amon the array of intracanal medicaments, NAC is a naturally occurring compound, a plant antioxidant found

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in Allium plant. It is also available in synthetic forms like NAC in powder form and NAC supplements used as an antidote for acetaminophen overdose and to prevent hepatic injury. N-acetyl cysteine (NAC) are synthetic form of amino acid cysteine, a semi essential amino acid which gets metabolised to glutathione which is mucolytic, antioxidant, potential cytoprotective, cancer preventive and anti-inflammatory.

N-acetyl cysteine (NAC) as an intracanal medicament has been recently researched. Its proven to suppress the growth of Enterococcus faecalis and destroy its biofilm.[2] It acts against all endodontic pathogens.[3] It also provides immense protection of apical stem cells for regenerative endodontic procedures (REP's) for modern endodontics and gives an added advantage of being used as intracanal medicament.[4] Hence, NAC has antibacterial, antibiofilm, anti- inflammatory properties to be used as a medicament.[5]In this study, NAC in powder form was preferred and propylene glycol was used as vehicleto prepare the medicament.

Following the placement of medicament, before obturation, the medicament must be completely removed from the pulp space for better penetration of sealers into the dentinal tubules.[6] Various methods of irrigant delivery are used to facilitate the removal of medicament from root canals. Different methods recommended to increase effectiveness of irrigation are manual irrigation using K file or master apical file in combination with NaOCl and EDTA,[7] passive ultrasonic irrigation, sonic irrigation with EndoActivator or Vibringe, canal brushes, XP Endo Finisher, laser, etc.[8,9]

Different techniques of irrigation for removal of calcium hydroxide and Triple antibiotic paste (TAP) as intracanal medicaments have been studied extensively. Sonic and ultrasonic irrigation is more effective as compared to irrigation in combination with rotary system.[10]

Considering the limited literature available on removal of N-acetyl cysteine as a intracanal medicament, in this study we aim to evaluate and compare the efficacy of different irrigation techniques in removal of N-acetyl cysteine using ultrasonic irrigation, sonic irrigation, manual irrigation with NaviTip and manual side vented needle irrigation.

2. Materials and Methods

Following the approval from ethics committee, fifty two permanent maxillary anterior teeth, extracted for periodontal reasons, were chosen and maintained conferring to OSHA guidelines. Teeth were checked for caries, attrition, abrasion, or other enamel defects, which, if present, were excluded. The teeth were cleaned of calculus and debris with ultrasonic scaler and immersed in 0.1% thymol until further use. All teeth were then evaluated using radiographs for inclusion and exclusion criteria. Decoronation was done with a diamond disc to a standardized length of 13 mm. A 10K file was inserted until it was visible at apical foramen and then working length was established by subtracting 1mm from the recorded root length. The roots were instrumented with Protaper Universal rotary files upto F4 master apical file, 2 ml of 5.25% NaOCl was used as an irrigant after change of instruments. Final irrigation was done by 5 ml of 17% EDTA for 2 min followed by 0.9% saline, root canals were then dried using paper points. The apex of all teeth were sealed with modelling wax.

The medicament was prepared by mixing N-acetyl cysteine with propylene glycol in the ratio 1:1 and root canals were filled with N-acetyl cysteine using a lentulospiral no. 35. Then the orifice was sealed with a cotton pellet and temporary filling material after which radiographs were taken to confirm the complete placement of medicament. The specimens were incubated at 37^0 Celsius under 100% humidity for 7 days.

After 7 days, fifty two teeth were randomly divided into four groups (n=13) based on the irrigating technique used.

Group 1- Passive ultrasonic irrigation - Ultrasonic file ISO 10 coupled to an adapter of P5 Newtron, Satelec XS handpiece (Acteon) was inserted 2 mm short of working length in the centre of the canal at 6 power W.

Group 2- Sonic activated irrigation- EndoActivator with blue 35/.04 tip was placed 2 mm short of working length that activated the irrigant at low frequency of 2-3 kHz.

Group 3- Manual irrigation with NaviTip- NaviTip (Ultradent) 29 gauge attached to the normal syringe was used in an up and down motion.

Group 4- Manual irrigation with side vented needle- side vented needle (29 gauge) attached to the normal syringe was used.

The volume of irrigant and time of irrigation were 12 ml and 2 min respectively for 4 cycles (5.25% NaOCl were used).

Final irrigation was done using 17% EDTA followed by 5 ml 0.9% saline then canals were dried with paper points. On all roots, grooves were made on the buccal and lingual surfaces with a double diamond disc and then were split into two halves along their long axis buccolingually with a chisel. All sectioned halves of each group were evaluated under stereomicroscope for residual medicament. Images of the coronal (12 mm from the apex), middle (8 mm from the apex) and apical (4 mm from the apex) thirds were acquired using a digital camera mounted on the stereomicroscope at 24x magnification and transferred to the computer. The residual medicament was evaluated independently in a blinding manner by two evaluators on a 4 grade scale.[10]

Score 0- Less than 25% of the root canal filled with NAC

Score 1- 25-50% of the root canal filled with NAC

Score 2- 50-75% of the root canal filled with NAC

Score 3- More than 75% of the root canal filled with NAC

2.1. Statistical analysis

All statistical tests were performed at a significance level of 5% ($p \leq 0.05$). Statistical analysis was done using Kruskal Wallis test & post hoc analysis using Mann Whitney U test. Statistical analysis was performed with SPSS software, version 21; SPSS Inc., (Chicago, IL, USA).

3. Results

As depicted in Table 1, Figure 1 Results of the intragroup comparison showed that maximum removal in coronal and middle third of the canal was observed in Group I Passive Ultrasonic irrigation and least in apical third.

In Group II Sonic irrigation, maximum removal was seen in apical third followed by coronal and middle third of the canal.

In Group III manual irrigation with Navitip irrigation and Group IV Side vented needles, maximum removal was seen in coronal followed by middle third and least by apical third of the canal.

As depicted in Table 1, Figure 1 Results of the intergroup comparison showed that maximum removal was observed in Group I Passive ultrasonic irrigation in coronal and middle third of the canal. Whereas in apical third, maximum removal was observed in Group II Sonic irrigation.

These results were statistically significant when compared to other groups.

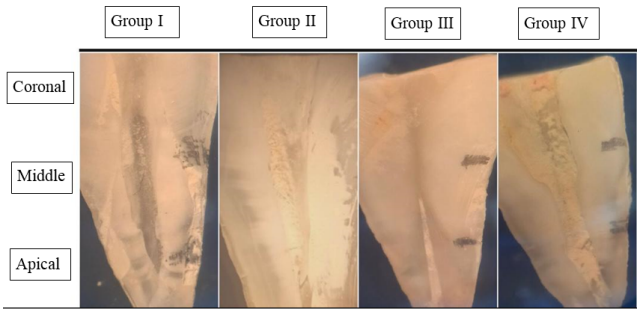

Figure 1:

Horizontal 'p' values are showing significance between the different irrigating systems and Vertical 'p' values are showing significance within groups between the coronal, middle and apical third.

4. Discussion

The use of intracanal medicaments to disrupt biofilms and eliminate residual bacteria responsible for infection within the root canals have been advocated to increase the outcome of root canal treatment. Chlorhexidine and calcium hydroxide have been widely indicated as intracanal medicaments pertaining to their excellent antimicrobial properties.[11] However, chlorhexidine is inactivated by physiological salts and has a limited ability to penetrate into deep layer of biofilms. Calcium hydroxide has low diffusibility and its antimicrobial efficacy is compromised by the buffering effect of dentin and resistance of Enterococcus faecalis to the hydroxylion.[12]

N-acetyl cysteine (NAC), a potent thiol- containing antioxidant, serves as a precursor of glutathione synthesis.[13] NAC has exhibited antibacterial activity against different medically important bacteria such as Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae.[14] The antibacterial activity is expected to be achieved by inhibition of cysteine utilization in bacteria or by reaction of its thiol group (–SH) with bacterial cell proteins, inducing irreversible damage to the essential proteins. NAC, depending on bacterial strains reduces production of extracellular polymeric substance, thereby disrupting mature biofilms and reducing bacterial adhesion to surfaces. Its applications in the medical field include its use in treatment of patients with obstructive pulmonary disease, owing to significant reduction in bacterial infections, as it breaks disulfide bonds in the mucus and inhibits bacterial biofilm formation.[15]

In recent studies, anti-bacterial effects of NAC have been demonstrated against oral pathogens found in endodontic infections such as Prevotella intermedia and E. faecalis.[2] NAC has proven to completely disrupt the mature multi species endodontic biofilms comprising of A. naeslundii, S. mutans, L. salivarius and E. faecalis, via affecting extracellular polymeric substance produced by bacterial cells. It exhibited biofilm removal from dentin surfaces and has been proposed as a replacement for ibuprofen for postendodontic pain. These characteristics demonstrate the potential of NAC to be used as alternative intracanal medicament.[16]

Removal of these intracanal medicaments before obturation is as essential as placing them to ensure quality obturation. Residual medicaments have known to prevent the sealer penetration in dentinal tubules and leave voids between the filling material and dentin surface, hence facilitating bacterial proliferation.[17] Various techniques of medicament removal have been evaluated for removal of calcium hydroxide and triple antibiotic paste from root canals. There is limited literature in removal of N-acetyl cysteine medicament from root canals. In our study, we have compared four different techniques of irrigation for removal of N-acetyl cysteine medicament to evaluate their efficacy.

Our results showed that Passive ultrasonic irrigation (PUI) showed better removal efficacy in coronal and middle third. PUI is a widely established irrigation technique for cleaning the root canals.[18] The efficacy of PUI can be owed to its large scale acoustic micro- streaming and cavitation. Streaming motion is when each impulse interacts with the water molecules, it creates shock waves leading to powerful streaming motion that can penetrate in the region of resident irrigant such as apical portion and facilitate

Table 1: Determination of less tha 25% of the root canal filled with remaining N acetyl cysteine at all the three levels

Group	Passive ultrasonic irrigation (n = 13	Sonic irrigation (n = 13	Navitip irrigation (n = 13	Side vented needle irrigation (n = 13	P-value
Coronal third	$100^{b\alpha}$	$84.6^{c\alpha}$	$69.2^{d\alpha}$	$46.2^{a\alpha}$	0.013*
Middle third	$100^{b\alpha}$	$84.6^{c\alpha}$	$53.8^{a\alpha}$	$38.5^{a\alpha}$	0.002*
Apical third	$50.9^{b\beta}$	$70.2^{c\alpha}$	$15.4^{a\beta}$	$7.7^{a\beta}$	<0.001**
P-value	<0.001**	0.491	<0.001**	<0.001**	
Overall	83.63	79.80	46.13	30.80	

All values are expressed as a percentage. The statistical test used for intergroup comparison and intragroup comparison: Kruskal Wallis test, *P ≤ 0.05, significant, ** P ≤ 0.001, Highly statistically significant; 95 % CI

three-dimensional movement of the irrigating solution thus playing a chief part in debriding the apical anatomy.[19–21] This non cutting threads and blunt working end removes the smear layer and also prevents perforation to the canal walls. It also creates undesirable dampening effect of amplitude of its characteristics nodes and antinodes pattern. This also might be the reason for lesser effectiveness in the apical third of the canal.[22] The streaming pattern also leads to maximum contact of solution with the walls of canals for removal of medicaments in a large prepared canal space. The result of this is in accordance with the previous studies done by Arslan et al 2014, Akman et al 2015.

In middle third, both sonic and NaviTips showed similar results. Sonic Endoactivator uses hydrodynamic phenomena, a negative apical pressure. The vibrating polymer tip causes irrigant activation, produces intracanal waves which causes production of bubbles or cavitation that oscillates in the irrigant. Sonic activation removed more dentin debris than ultrasonic activation in apical third.[23] However, in some *in-vitro* tests the sonic activation methods and PUI were reported to have statistically similar ability of irrigant penetration, and resulted in better removal in apical third of root canal than syringe irrigation method.[24]

Use of mechanical devices for agitation of irrigant solution seems to improve the removal of intracanal medicaments. NaviTips are effective in cleaning coronal, middle third of root canals. The lateral vent percolates fluid in and around the canal, increasing the irrigating solution which shows positive result in coronal cleaning.[25]

But canal irregularities may cause breakage of NaviTip in canal which resist the placement of gutta percha points and enhance apical leakage of materials.

Syringe irrigation is described as positive pressure irrigation.[26] Side vented needles have lateral vent but pressure is not exerted in apical part so it did not remove the medicament in apical third. Needles with side or beveled openings did not present advantages of irrigation in the apical portion of root.[27]

The conventional needle irrigation can deliver solutions no more than 0-1 mm beyond the needle tip and a vapor lock also hinders the exchange of debris. Another reason might be lesser diameter of dentinal tubules and more sclerotic dentin in the apical third also contributes for lesser removal of medicaments.[22]

The limitations of our study are the longitudinal sectioning with a diamond disk creates a smear layer, produces niches and also removes the medicament and care should be taken not to penetrate root canals.[28] The scoring criteria and Stereomicroscope assessment is subjective. Scanning Electron Microscope could have given a clearer idea of these different irrigation techniques.

5. Conclusion

Novel techniques of irrigation have been used for effective removal of intracanal medicaments. Though none of the irrigation techniques completely removed the intracanal medicament, use of irrigant activation devices are more effective than manual irrigation using different needles. Within the limitations of this study, passive ultrasonic irrigation effectively removed N-acetyl cysteine intra canal medicament in coronal and middle third of the canal. Whereas in apical third, sonic irrigation was better compared to other techniques.

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None.

7. Conflict of Interest

None.

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