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Research Article

Ability of Sodium Hypochlorite to Clean Dentinal Tubules by Manual or Sonic Activation at Varying Temperature: A Confocal Laser Scanning Microscopic Study

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Abstract

Aim: To compare Ability of Sodium Hypochlorite to clean dentinal tubules by manual or sonic activation at varying temperature: A Confocal Laser Scanning Microscopic Study.

Introduction: The primary aim of endodontic treatment is to eliminate the microorganism within the root canal system and prevent recontamination. According to Weine, untreated and unfilled lateral canals can retain enough irritants in a protected sanctuary high enough to cause endodontic treatment failure. To achieve the complete cleaning and disinfection, sodium hypochlorite is used as a potent irrigating solution. Studies have been reported that preheating sodium hypochlorite enhances its tissue solubility and disinfection properties. Cavitation and acoustic streaming of the irrigant contribute to the biological chemical activity for maximum effectiveness Many studies have been reported that has studied the effectiveness of sonic instrument on the cleanliness of the canal with sodium hypochlorite. A literature search did not identify any studies evaluating the penetration depth of preheated sodium hypochlorite using sonic instrument by confocal laser electron microscopy.

Methodology: 40 freshly extracted mandibular premolar were collected. Routine endodontic treatment was performed in all teeth of four groups. Teeth were randomly divided into 4 group:

1. Group 1- Sodium hypochlorite with hand activation
2. Group 2- Sodium hypochlorite with sonic activation
3. Group 3- Warm sodium hypochlorite with hand activation
4. Group 4- Warm sodium hypochlorite with sonic activation.

Following irrigation, all roots were sectioned horizontally at 2mm and 5mm from the apex and examined under a confocal laser scanning microscopy for evaluating the penetration depth of sodium hypochlorite.

Results: Irrigant penetration ability was greater in the middle section then in the apical section.

Sonic irrigation when used with warm sodium hypochlorite had better penetrability in the dentinal tubules of middle and apical then the conventional irrigation.

Keywords: Sodium Hypochlorite; Confocal Laser Scanning; Endodontic Treatment

Introduction

The main aim of the endodontics is complete elimination of bacteria and their byproducts from the root canal system with instrumentation and chemical cleaning. This is followed by filling the root canal with an inert material in order to maintain the health of periradicular tissues [1]. Bacteria are causative factor for pulpal and periapical changes and mainly occur in areas such as lateral or accessory canals, apical deltas, isthmus, ramification and dentinal tubules [2]. Various studies have reported 300μm of bacterial penetration into dentinal tubules [3]. Enterococcus faecalis has a penetration depth of 500μm into dentin, with a front of infection may reach 1000 μm [4]. For complete eradication of the pulpal tissue, dentinal shavings and bacteria from the root canal, mechanical preparation and shaping is done followed by irrigation. Sodium hypochlorite (NaOCL) is considered as the potent endodontic irrigant. Also, sodium hypochloite shows no residual activity [5]. Its properties can be enhanced by thermodynamic changes within the root canal [6]. Sirtes., *et al*. stated that 1% NaOCl solution heated to 45°C equals the tissue dissolution capacity of a 5.25% NaOCL solution at 20°C.The tissue dissolution capacity of the 1% solution was even higher at 60°C [5]. Traditionally, Syringes are used for effective irrigation. It can only deliver irrigant up to 1mm deeper than the needle tip. due to creation of vapor lock at the apical area [7]. Several studies have found that apical vapor lock has adverse effect [8]. Throughout the history of endodontics, modifications have been made to develop a more effective irrigant delivery and agitations technique for root canal system. These systems are divided into manual agitation and machine-assisted agitation devices [7]. Among these techniques, machine assisted agitation has been considered more effective. Machine assisted agitation is further divided into sonic and ultrasonic.Activation with sonic devices produces frequency ranging from 1 to 6 KHz with mechanical oscillation [9].

Aim of the Study

The aim of this study is to evaluate the penetration depth of sodium hypochlorite in dentin using manual and sonic agitation techniques at two different temperatures. The null hypothesis is that there is no difference in the penetration of 5.25% NaOCl solution into dentinal tubules activated by manual agitation or by sonic agitation at different temperatures.

Materials and Methods

Forty freshly extracted single rooted human first premolar teeth with single canal were selected for the study as per the inclusion criteria in this study. The teeth were collected, stored and handled as per the OSHA and CDC guidelines (2003 report 17). Forty teeth were randomly divided into four groups having 10 teeth in each group (n=10). The teeth were decoronated at 15mm to standardize the root length. After the establishment of canal patency by size #10 K-file (Mani, Inc., Tochigi, Japan), shaping and cleaning was done till F1 using ProTaper Gold (Dentsply Maillefer, Ballaigues, Switzerland) file system. Samples were randomly further divided into four group. Each group was subjected to different irrigation protocols.

- Group IA- Sodium hypochlorite with hand activation.
- Group IB- Sodium hypochlorite with sonic activation for 10 sec.
- Group IIA- Warm sodium hypochlorite with hand activation.
- Group IIB- Warm sodium hypochlorite with sonic activation for 10 sec.

Samples were rinsed with normal saline. They were then flushed with 5 ml of 17% EDTA (Dental Avenue India Pvt. Ltd. Mumbai India), solution after root canal preparation for smear layer removal. Two coats of nail polish were applied around the root surface, and modelling wax was used to seal the apex. 5.25% sodium hypochlorite (Prime Dental Products Pvt. Ltd., India) was than mixed with 0.01% rhodamine B isothiocyanate dye (Merck, Darmstadt, Germany) and irrigated into the root canal. Samples were mounted vertically in the acrylic block and sectioned horizontally at 2 mm (apical) and 5 mm (middle) from the apical foramen, removing 1 mm of thickness with a low speed water cooled 0.3 mm blade Isomet saw (Buehler, Lake Bluff, IL, USA) The coronal surfaces of slices were polished with silicon abrasive carbide paper to remove dentin debris created during the cutting procedures. The slides were then examined using a confocal laser scanning microscope at 10X magnification with a wavelength of 543 nm. The dentinal tubule penetration area was measured as micrometers (mm) and converted to square millimeters (mm^2) for the statistical analysis.

Statistical analysis

Data were analysed with IBM SPSS Statistics 22 software (PASW Statistics 20; SPSS Inc., Chicago, IL, USA). Unpaired t test were used to compare the penetration depth of each group in middle and apical section. Anova F test was used to compare the depth of irrigant penetration in apical section with different irrigation techniques at different temperature at each level. Intergroup comparison was done by Tukey's Post hoc test. The significance level for all analyses was set at $P < 0.05$.

Results

Statistical analysis indicated a significant effect on penetration depth of different groups in middle and apical sections (Table 1). Group IIB showed higher significance ($p < 0.001$) when compared to other groups. Group IA (sodium hypochlorite with hand activation) showed the lowest penetration in middle section and apical section and Group IIB (warm sodium hypochlorite with hand activation) showed highest penetration. Table 2 showed Intergroup comparison of different group in middle section. Group IA was compared to Group IB, IIA, IIB. Group IB (sodium hypochlorite with sonic activation) did not show significant difference. Table 3 compared penetration depth in apical section Group IA was compared with IB, IIA and IIB. Group IA did not show significant difference when compared to Group IB.

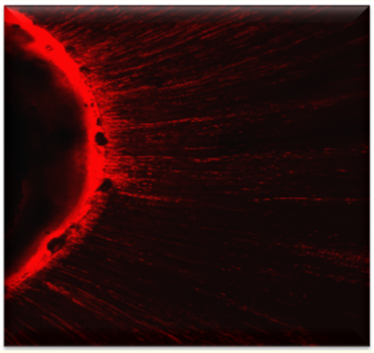

Figure 1: Group IA-sodium hypochlorite with hand activation middle.

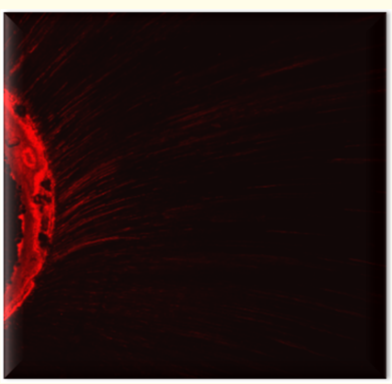

Figure 2: Group IB-sodium hypochlorite with hand activation apical.

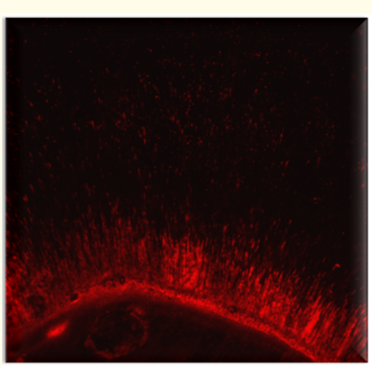

Figure 3: Group IIA-sodium hypochlorite with sonic activation middle.

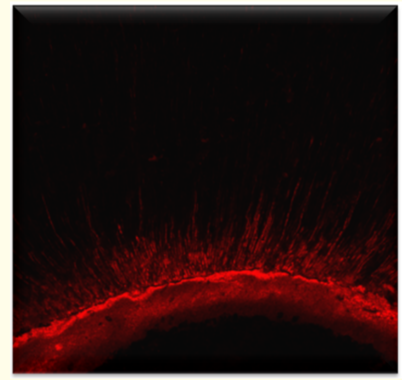

Figure 4: Group IIB-sodium hypochlorite with sonic activation apical.

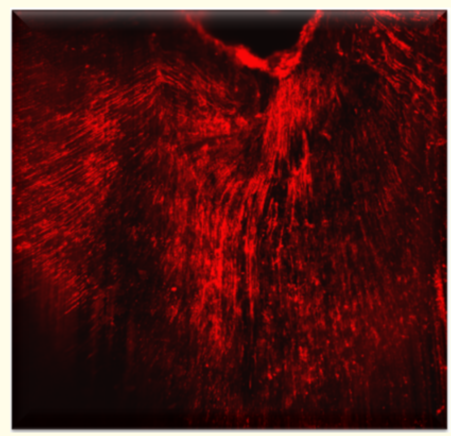

Figure 5: Group IIIA-warm sodium hypochlorite with hand activation middle.

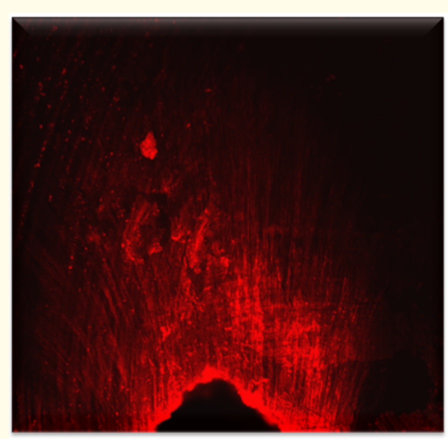

Figure 6: Group IIIB-warm sodium hypochlorite with hand activation apical.

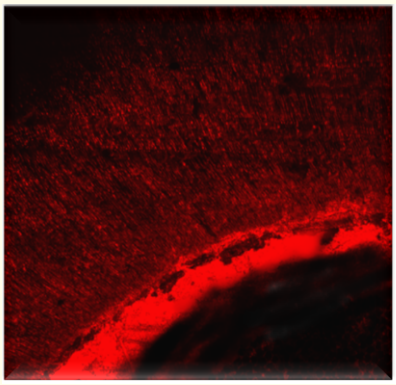

Figure 7: Group IVA-warm sodium hypochlorite with sonic activation middle.

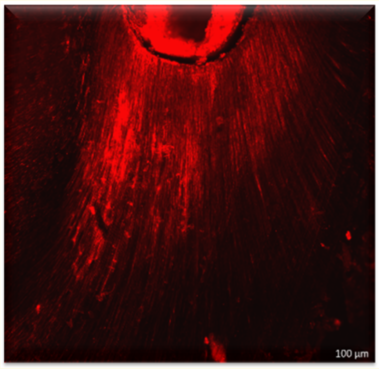

Figure 8: Group IVB-warm sodium hypochlorite with sonic activation apical.

	Middle section mean (SD)	Apical section mean (SD)	Unpaired t test	p value, Significance
Group IA (Sodium Hypochlorite + Hand activation)	48.75 (2.98)	32.50 (15.54)	t = 2.053	p = 0.086
Group IB (Sodium Hypochlorite + Sonic activation)	429.5 (87.94)	387.5 (85.39)	t = 0.658	p = 0.519
Group IIA (Warm Sodium Hypochlorite + Hand activation)	1662.5 (268.87)	1212.5 (278)	t = 2.327	p =0.059
Group IIB (Warm Sodium Hypochlorite + Sonic activation)	2887.5 (143.61)	1800 (70.71)	t = 13.587	p <0.001**

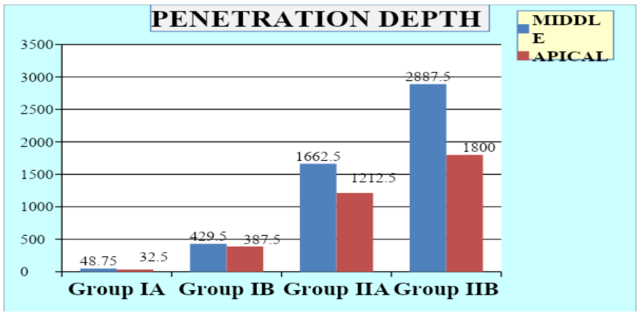

Table 1: Comparison of penetration depth of each group in middle section as compared to apical section of root canal.

p > 0.05 – not significant; *p < 0.05 – significant; **p < 0.001 – highly significant.

Middle Section	Mean	S.D	Anova F Test	p value, Significance
Group IA (Sodium Hypochlorite + Hand activation)	48.75	2.98	F = 263.201	p < 0.001**
Group IB (Sodium Hypochlorite + Sonic activation)	429.5	87.94		
Group IIA (Warm Sodium Hypochlorite + Hand activation)	1662.5	268.87		
Group IIB (Warm Sodium Hypochlorite + Sonic activation)	2887.5	143.61		
Tukey's post hoc test to find pairwise comparison				
Group	**Comparison group**	**Mean difference**		**p value, Significance**
Group IA	Group IB	380.75		p = 0.024*
	Group IIA	1613.75		p < 0.001**
	Group IIB	2838.0		p < 0.001**

Group IB	Group IIA	1233.0	p < 0.001**
	Group IIB	2458.0	p < 0.001**
Group IIA	Group IIB	1225.0	p < 0.001**

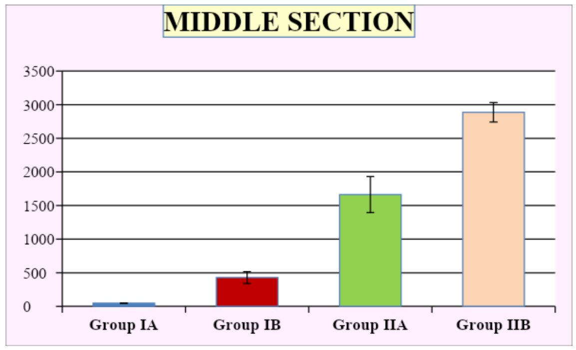

Table 2: Intergroup comparison of penetration depth among four study group in middle section of root canal.

p > 0.05 - Not significant; *p < 0.05 - Significant; **p < 0.001 - Highly significant.

Apical section	Mean	S.D	Anova F Test	p value, Significance
Group IA (Sodium Hypochlorite + Hand activation)	32.5	15.54	F = 113.753	p < 0.001**
Group IB (Sodium Hypochlorite + Sonic activation)	387.5	85.39		
Group IIA (Warm Sodium Hypochlorite + Hand activation)	1212.5	278.01		
Group IIB (Warm Sodium Hypochlorite + Sonic activation)	1800.0	70.71		
Tukey's post hoc test to find pairwise comparison				
Group	**Comparison group**	**Mean difference**	**p value, Significance**	
Group IA	Group IB	355.0	p = 0.026*	
	Group IIA	1180.0	p < 0.001**	
	Group IIB	1767.5	p < 0.001**	
Group IB	Group IIA	825.0	p < 0.001**	
	Group IIB	1412.5	p < 0.001**	
Group IIA	Group IIB	587.5	p < 0.001**	

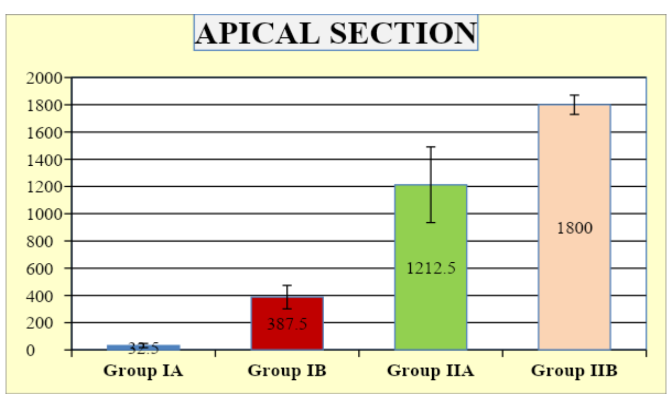

Table 3: Intergroup comparison of penetration depth among four study group in apical section of root canal.

p > 0.05 - Not significant; *p < 0.05 - Significant; **p < 0.001 - Highly significant.

Discussion

The main cause of post treatment failure is the presence of microorganism within the root canal system [10]. Bacterial invasion occurs deeper within the dentinal tubules and is important for the persistence of infection. Hence the depth of penetration of irrigant is evaluated as the antibacterial property of the chemical irrigants which is critical to prevent reinfection [11].

Sodium Hypochlorite acts as a solvent in presence of organic tissue by releasing chlorine which forms chloramines by combining with the protein amino groups. The efficacy of sodium hypochlorite can be increased by increasing the concentration, contact time, volume and temperature [12]. When temperature of 1% NaOCL is raised to 45 can cause tissue dissolution equivalent to 5.25% sodium hypochlorite at 20 [6]. Waltimo., *et al*. stated that NaOCl solution at 50 ppm can kill microorganism within 30s at 60°C [13]. It has been found that increasing the concentration will decrease the sensitivity of the experiments in invitro studies between manual activation and machine activation of the irrigant [14]. Needle irrigation being one of the most commonly employed technique in clinics [15]. It can produce adequate canal debridement but it fails to create any hydrodynamic turbulence energy within the irrigants [7]. To overcome this problem, various agitation methods are advocated. Gutta Percha master cone is used to manually agitate the irrigants within the canal [7].

In the present study passive sonic agitation when used with warm sodium hypochlorite has proven to achieve maximum disinfection by removing intra-radicular biofilm significantly compared to other methods in the coronal third then the apical third. The results are attributed to the fact that tubules within the coronal and middle third of the root are more permeable [16]. Tubules in the apical third are impermeable due to the presence of tubular sclerosis, smaller diameter, and a reduced number of dentinal tubules in this region [16].

Sonic Activation can produce powerful hydrodynamic intracanal waves. These waves create bubbles which will oscillate within the given solution. These bubbles will expand and collapses as an implosion that can lead to radiating shockwaves that dissipates at a speed of 25,000 - 30,000 times per second. These shock waves detaches the biofilm from the root canal surfaces [15]. Yan shen., *et al*. reported the bioacoustics effect of sonic irrigation where the shockwaves created by sonic agitation transports disinfecting agents deep into the biofilms by breaking its protective mechanism which leads to bacterial death. This action leads to inefficient cleaning of the particles from the middle and apical third of the root canals [17].

In this study manual dynamic agitation has not performed as effectively as sonic agitation technique. The reason behind this is because of the energy created by push-pull motion of manual activation is (3.3 Hz) much lesser than sonic energy of 1 - 6 kHz [15].

Numerous microscopic techniques such as stereomicroscopy, scanning electron microscopy (SEM) and CLSM have been advocated to evaluate irrigant penetration into dentinal tubules. CLSM provides detailed information about the presence and distribution of irrigants inside the dentinal tubules along the circumference of the canal walls. It provides image acquisition from several sections, which is further reconstructed to obtain final image [11].

Guo X in 2014 stated that the smear layer can be removed effectively even without any activation by using combination of 60°C 3% NaOCl and 17% EDTA. The results of this study showed better penetration in the middle third then the apical third. In accordance to the study done by Kucuk. M., *et al*. in 2018 it was concluded that the penetration into dentinal tubules is significantly greater in middle section than at apical section of root canal [11]. The current results of this study is similar to that of furkan., *et al* [18]. There are other studies which found no significant difference in conventional syringe irrigation when compared to other methods of activation [19]. Future investigations are necessary to validate the kinds of conclusions that can be drawn from this study.

Conclusion

Thus, within the limitations of the study following conclusions were drawn:

- The penetration of sodium hypochlorite increases with increase in the temperature and with use of sonic irrigation method.
- Irrigant penetration into dentinal tubules was significantly greater in the middle section than on the apical section of the root canal.

Acknowledgements

The authors deny any conflict of interest related to this study.

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