ejpmr, 2018,5(3), 589-593

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article
ISSN 2394-3211
EJPMR

A COMPARISON OF PENETRATION OF SODIUM HYPOCHLORITE LABELED WITH ALIZARIN RED FLUORESCENT DYE INTO DENTINAL TUBULES OF ROOT CANAL SYSTEM USING THREE DIFFERENT TECHNIQUES OF IRRIGATION: AN IN VITRO FLUORESCENT MICROSCOPE STUDY

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Article Received on 20/01/2018 **Article Revised on 09/02/2018** **Article Accepted on 02/03/2018**

ABSTRACT

Aim and objectives: To compare the effect of three different techniques of irrigation on penetration of Sodium hypochlorite into dentinal tubules of root canal system using alizarin red fluorescent dye labeled with sodium hypochlorite. **Materials and Methods**: Forty eight extracted maxillary anterior teeth were instrumented up to Pro-taper finishing file F5. They were randomly divided into four groups (N = 12). Group 1 was the control group, rest all the groups (Group 2, 3, & 4) were experimental groups. After removing the smear layer using EDTA Gel (*Glyde, Dentsply*) followed by 5ml of normal saline, A final rinse of 5 ml of 3% NaOCl labeled with a fluorescent dye 0.2% alizarin red was done using syringe irrigation with a 24-Gauge syringe in Group 2, Intermittent passive ultrasonic irrigation (IPUI) in Group 3 and Continuous passive ultrasonic irrigation (CPUI) in Group 4. Control Group (Group 1) had a final rinse of 5 ml of plane 3% NaOCl sodium hypochlorite i.e.; not labeled with 0.2% alizarin red dye using a 24-Gauge syringe. The Specimens were sectioned at 1, 3 & 5 mm from the apex in 1-mm thick sections, ground and prepared for fluorescence microscopy. NaOCl penetration into dentinal tubules was analyzed using Student t- test analysis. **Results**: At 1 mm from the apex, the penetration of NaOCl was significantly higher ($p<0.05$) for Group 4 (CPUI group). **Conclusion**: CPUI appears to be more effective than IUPI and syringe irrigation techniques.

KEYWORDS: passive ultrasonic irrigation, alizarin red, fluorescent microscope, sodium hypochlorite.

INTRODUCTION

Microorganisms and their end products are considered the major cause of Pulp and Periapical Diseases [1]and their removal by biomechanical procedures is critical because of the anatomic complexities of many root canal systems.[2] Among all the irrigating solution used in endodontics, sodium hypochlorite is quite satisfactory as it dissolves necrotic tissue[3], inactivates the endotoxins of bacteria[4], disintegrates endodontic biofilms[5], and kills sessile endodontic pathogens in biofilm.[6] Alizarin red is a fluorescent organic compound used in biomorphologic assays for quantifying the presence of calcific depositions.[7] Ultrasonic was introduced to Endodontics by Richman in 1957.[8]

Two types of ultrasonic irrigation have been described in the literature: 1. First type is combination of simultaneous ultrasonic instrumentation and irrigation (UI). 2. Second type commonly termed as passive ultrasonic irrigation (PUI) which operates without simultaneous instrumentation.[9] The purpose of this study was to assess the penetration of 5% NaOCl labeled with 0.2% alizarin red into dentinal tubules when used in root canals with three different agitation protocols. The null hypothesis was set as if there was no significant difference (*$p>0.05$*) between all three techniques.

MATERIALS AND METHODS

Forty eight extracted maxillary anterior teeth were selected for the study. The exclusion criteria were teeth with caries, fracture or any root fissure. All teeth were stored in normal saline at 4 degree temperature and were used within 30 days after extraction. After conventional access cavity preparation, a size 10 K-type file was inserted into each canal until it was seen through the apical foramen. The working length was established by reducing this length by 0.5mm (fig. 1).

They were instrumented up to Pro-taper finishing file F-5. 5 ml of 3% sodium hypochlorite (NaOCl) was performed during instrumentation using a syringe with a 24-Gauge needle. Smear layer was removed with application of EDTA Gel (*Glyde, Dentsply*) for 2 minutes followed by 5 ml of normal saline (fig.2a & 2b). After removing smear layer, Samples were divided randomly in 4 groups (N = 12 for each group) for final rinse procedure:

Group 1: Control Group.
Group 2: Syringe irrigation Group.
Group 3: Intermittent Passive Ultrasonic Irrigation (IUPI) Group.
Group 4: Continuous Passive Ultrasonic Irrigation (CUPI) Group.

The exterior part of the apical third of each root was covered with wax to prevent irrigant from dripping through the apical foramen (fig.3). NaOCl was labeled with alizarin red in a Biochemistry lab (fig.4). To standardize the irrigation technique, the irrigation needle was placed at 5 mm from the apex and the rate of irrigation was kept 1mL/30 seconds for 150 seconds for all the groups.

Group 1: Teeth of Control Group were irrigated with 5ml of sodium hypochlorite (NaOCl) using syringe (24-Gauge) irrigation using sodium hypochlorite (NaOCl) *without* labeling with alizarin red (fig.5).

Group 2: Teeth of Syringe irrigation Group were irrigated using 5ml of sodium hypochlorite (NaOCl) labeled with 0.2% alizarin red using syringe (24-Gauge) (fig.6).

Group 3: Teeth of Intermittent Passive Ultrasonic Irrigation (IPUI) Group were irrigated using 5 ml sodium hypochlorite (NaOCl) labeled with 0.2% alizarin red intermittently. Power setting was kept at 5 for 20 seconds (fig.7).

Group 4: Teeth of Continuous Passive Ultrasonic Irrigation (CPUI) Group were irrigated using 5 ml of sodium hypochlorite (NaOCl) labeled with 0.2% alizarin red continuously. Power setting was kept at 5 for 20 seconds (fig.8).

• After drying the canal with sterile paper points, each specimen was cut into three sections of 1 mm. thickness at 1, 3 and 5mm. from the root apex respectively. These sections were bonded onto a glass slide and ground subsequently with wet silicon carbide papers to approximately 40µm size (fig. 9a, 9b, 9c). The slides were then examined with a fluorescence light microscope (*Olympus*) at 100 x with a wavelength of 540 to 570nm. If the whole canal could not fit completely in one image, two or more partial images were taken to create a mosaic using Adobe Photoshop CS3 (*adobe systems, italia, Milan, Italy*).

Scoring criteria

- ‘‘0’’= no visible alizarin red
- ‘‘1’’ = minor traces of alizarin red
- ‘‘2’’= traces of alizarin red along the whole intraradicular surface of the canal
- ‘‘3’’= penetration of alizarin red in <50% of the dentinal tubules
- ‘‘4’’ = penetration of alizarin red in >50%of the tubules.

RESULTS

Significant difference was found among all the techniques at 1mm from the root apex ($p<0.05$). The F – Value was Calculated by one way ANOVA = 16.94. At 1 mm from the apex Group 4 (CPUI) showed better results than the other Groups (fig.10a, 10b, 10c, and 10d).

Table 1: Mean and Median.

Distance from the root apex (mm)	Group 1 Mean (Median)	Group 2 Mean (Median)	Group 3 Mean (Median)	Group 4 Mean (Median)
1	0	2(2)	3(3)	4(4)
3	0	3(3)	3.75(4)	4(4)
5	0	4(4)	4(4)	4(4)

DISCUSSION

Although NaOCl is an effective disinfectant, when used in conjunction with nickel-titanium instruments it produced clean and debris-free dentin surface, but not in the apical third of the canal.[10] In this study alizarin red dye was used to enable us to trace the NaOCl within the Dentinal tubules of root canal. Results demonstrated that Group 4 (CPUI) showed better penetration into Dentinal tubules. A possible explanation for this is the better current flow and increased irrigant volume associated with Ultrasonic agitation. PUI increases the action of NaOCl by pushing it into the dentinal tubules and by generating heat in the solution and studies have also shown that 1min of Continuous PUI produced significantly cleaner canals in both vital and necrotic teeth.[11]

CONCLUSION

In our study, Continuous PUI (CPUI) resulted in better penetration in the dentinal tubules; still, a ‘well-controlled’ and ‘evidenced based study’ has to be done to correlate the clinical efficacy of these devices.

Fig 1.

Fig 2a.

Fig.2b.

Fig 3.

Fig 4.

Fig. 5.

Fig. 6

Fig. 7

Fig. 8

Fig. 9a

Fig. 9b

Fig. 9c

Fig. 10a

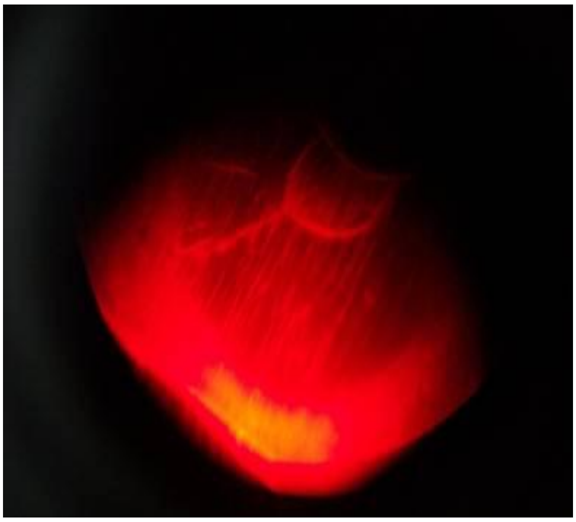

Fig. 10b

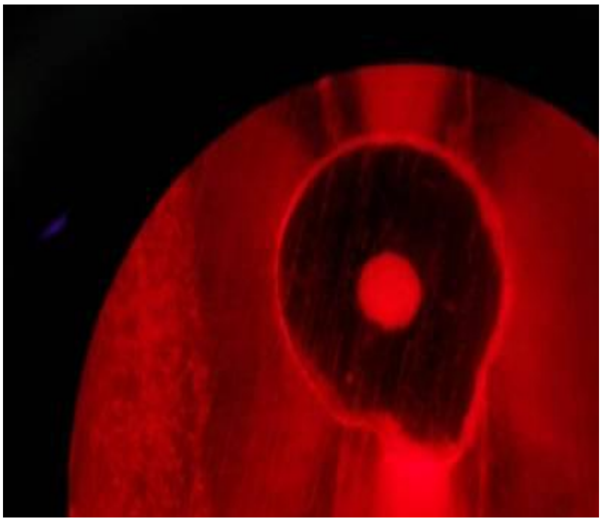

Fig. 10c

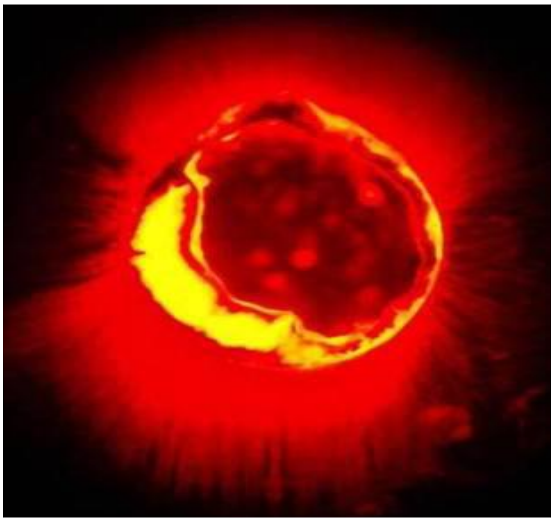

Fig.10d

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