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Original Research Paper

Endodontics

EFFICACY OF CHLORHEXIDINE, QMIX AND OCTENIDINE DIHYDROCHLORIDE AGAINST E.FAECALIS CONTAMINATED GUTTA PERCHA CONES

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ABSTRACT

Aim: The study aimed to evaluate and compare the effectiveness of different disinfecting agents against E.faecalis contaminated gutta percha cones. **Objective:** The objective of the study was to evaluate and compare the efficacy of Qmix, chlorhexidine and octinidine dihydrochloride against E.faecalis contaminated GP cones after rapid chemical disinfection procedure against E.faecalis. **Material & Method:** The study used #25 size, 80 standardized GP cones (Diadent) which were artificially contaminated by immersing in E.faecalis. Three chemical agents were used: 0.12% chlorhexidine, Qmix and octenidine. GP cones were immersed in the solution for 30 sec. After disinfection, GP cones were placed in tubes containing thioglycolate media and were incubated at 37°C for 7 days. And then media was subcultured and colony-forming units were counted. The data generated were analyzed using Pearson chi-square test, $p<0.05$. **Result:** There statistically significant difference was found in disinfection ability between the irrigation solution used on GP cones contaminated with E.faecalis ($p<0.001$). 0.12% chlorhexidine was unable to eliminate E.faecalis in the exposure time of 30 sec. While Qmix and octinidine dihydrochloride were unable to eliminate bacteria completely. **Conclusion:** Based on the result it was concluded that 0.12% chlorhexidine was found to be an effective agent for rapid disinfection of GP cones and may be used for chairside disinfection.

KEYWORDS : Disinfection, Gutta percha, E.faecalis, chlorhexidine, qmix, octenidine dihydrochloride

INTRODUCTION

The purpose of endodontic treatment is the cleaning, shaping and disinfection of the root canal, followed by the obturation of the endodontic system so that the tooth can be restored to function. The presence of microbes inside the canal is the main reason for post- treatment infection[1].Microorganisms might survive despite thorough biomechanical preparation or may invade the root canal system through contaminated instruments or materials.

Obturation during the endodontic treatment eliminates the root canal space by introducing a root-filling material combined with a sealer. Gutta percha (GP) cones are the most widely used material for this purpose. GP cones comes in sealed packages, but as they are exposed to the dental office environment or even by handling, they get contaminated by microorganisms.

As gutta percha cannot be sterilized by dry or moist heat, the best way to sterilize them is by cold sterilization using disinfectants. Various chemical agents have been proposed as GP disinfectants, including sodium hypochlorite (NaOCl), glutaraldehyde, alcohol, iodine compounds, chlorhexidine and hydrogen peroxide[2].

The ideal disinfectant should be the one that can be used routinely in dental clinics, providing rapid chairside disinfection without modifying the structure of gutta percha. Octenidine dihydrochloride is a bis-pyridine antimicrobial compound which demonstrates a broad antimicrobial effect and is highly biocompatible[3]. Chlorhexidine has been used in dentistry for a long time due to its antimicrobial properties, high substantivity and low toxicity[4]. Qmix(Dentsply Sirona) is a solution containing 2% Chlorhexidine and 17% Ethylenediamine tetraacetic acid(EDTA) which has shown good smear layer removal and antimicrobial properties[5].

To accomplish the appropriate decontamination of the cones, the disinfectant agent has to be effective against different bacterial species. Studies have reported that Enterococcus faecalis is the most common bacteria associated with post-treatment infection of the root canal system and has been found to survive for a longer period in the root canal system[6,7]. It has a prevalence of 40% in primary endodontic infection and 24-77% in secondary or persistent endodontic infection[7,8]. And it is most resistant to elimination by various disinfecting agents. For this reason, E.faecalis was chosen in this study.

There have been studies using the aforementioned disinfectants for rapid decontamination of gutta percha, but there is no study where the efficacy of Octenidine dihydrochloride, Chlorhexidine, and Qmix have been simultaneously compared for rapid chairside disinfection of GP cones. Therefore, this study aimed at evaluating the efficacy of these agents against E. faecalis-contaminated gutta percha cones for rapid decontamination.

Summary:

This was an experimental study designed as a non-randomised controlled trial which was conducted in a Tertiary Care Centre after approval from the Institutional Ethics Committee to evaluate the efficacy of different disinfecting agents which can be used for rapid decontamination of GP cones at chairside.

The study used #25 size, 80 standardized GP cones (Diadent) which were artificially contaminated by immersing in E.faecalis. Three chemical agents were used: 0.12% chlorhexidine, QMix and octenoxa. GP cones were immersed in the solution for 30 sec. After disinfection, GP cones were placed in tubes containing the thioglycolate media and were incubated at 37°C for 7 days. And then media was subcultured and colony-forming units were counted. The data generated were analyzed using Pearson chi-square test, $p<0.05$.

There statistically significant difference was found in disinfection quality between the irrigation solutions used on GP cones contaminated with E. faecalis ($p<0.001$). 0.12% chlorhexidine was able to eliminate E.faecalis in the exposure time of 30 sec. While QMix and octenidine dihydrochloride were unable to eliminate bacteria.

Based on the result it was concluded that 0.12% chlorhexidine was found to be an effective agent for rapid disinfection of GP cones as a well-known irrigation solution followed by QMix. Octenidine was found to be the least effective.

MATERIALS & METHOD

This is an experimental study designed as non randomized controlled trial which was conducted in Tertiary Care Centre after approval from Institutional Ethics Committee.

Inclusion Criteria

- GP cones from freshly opened box
- Straight GP cones

Exclusion Criteria

- Curved GP cones
- Used or contaminated GP cones

In this study #25 size, 80 standardized GP cones (Diadent) were used from freshly opened boxes and were divided in 4 groups (n=20 each group).

- Group 1:(G1)n=20, the positive control group.
- Group 2:(G2)n=20, 0.12% chlorhexidine group.
- Group 3:(G3)n=20,0.1% w/v octenidine dihydrochloride group
- Group 4:(G4)n=20, Qmix group\

• Artificial contamination of GP cones

Microbial suspension of E.faecalis of approximately 10^8CFU/ml in peptone water was used for this study. GP cones from Groups 1,2,3 and 4 were contaminated by immersing them in sterile tubes with the help of tweezers containing 20ml of microbial suspension for 30 minutes. The cones were then transferred to sterile gauze and were allowed to air dry for 10 minutes.

• Disinfection of GP cones

Disinfecting agents used for this study were:

- Chlorhexidine 0.12%
- Octenidine dihydrochloride 0.1% w/v
- Qmix

All the contaminated GP cones from each group were then placed one by one in container containing 1ml of disinfecting agents for 30 sec as follows.
Group 1:(G1) Contaminated GP cones were immersed in distilled water.
Group 2:(G2) GP cones were placed for 30 sec in 0.12% chlorhexidine.
Group 3:(G3) GP cones were placed for 30 sec in 0.1% w/v octenidine dihydrochloride
Group 4:(G4) GP cones were placed for 30 sec in Qmix.

After the points were dipped in the disinfectant, they were kept for drying on a sterile gauze piece for 10 minutes to remove excess solution and then were placed in culture tubes containing 10 ml of sterile thioglycollate media and incubated at 37 °C for 7 days. The whole experiment was done under aseptic conditions.

Then thioglycollate media was subcultured on chocolate agar and colony forming units were counted using semi quantitative standard loop technique. The results were statistically analysed using Chi square test.

OBSERVATIONS & RESULTS:

The effect of the disinfectants on the contaminated cones after being soaked for 30 seconds is shown in Table 1 and the same is graphically represented in Figure 1. To compare the efficacy of experimental solutions Colony Forming Units were categorize into three categories.

Table 1a: Comparison between disinfection efficacy of all the groups

CFU Categories * Group Crosstabulation							
			Group				Total
			Group 1	Group 2	Group 3	Group 4	Total
CFU Categories	No growth	Count	0	20	6	8	34
		% within Group	0.0%	100.0 %	30.0 %	40.0 %	42.5 %
	10000-100000	Count	0	0	10	12	22
		% within Group	0.0%	0.0%	50.0 %	60.0 %	27.5 %
	>=100000	Count	20	0	4	0	24
		% within Group	100.0 %	0.0%	20.0 %	0.0%	30.0 %
Total		Count	20	20	20	20	80
		% within Group	100.0 %	100.0 %	100.0 %	100.0 %	100. 0%

Table 1b:

Chi-Square Tests			
	Value	df	P value (<0.05 is significant)
Pearson Chi- Square	92.520	6	<0.001

As shown in table 1, the best response was found in cones that were exposed to 0.12% chlorhexidine wherein no colony forming units were present which indicates the complete elimination of E. faecalis. QMix was found to be the second most effective disinfecting solution while octenidine dihydrochloride was least effective among the solutions tested. Statistically significant difference was observed between all the groups as their P value suggest <0.001(Table 1b).

Graph demonstrating the effectiveness of the chemical agents on the contaminated cones is shown in Fig 1.

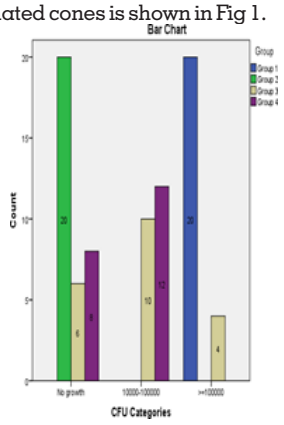

Figure 1: Graphical representation of counts and E.faecalis colonies of all the groups.

DISCUSSION:

Despite following stringent methods and treatment standards, recurrent failure of endodontic treatment occurs. The main cause behind this is the presence or persistence of microorganisms in the root canal[1]. One possible explanation for this problem may be the introduction of contaminated gutta percha cones into the root canal. Many studies have shown the presence of cultivable microorganisms in 5-19% of freshly opened packages[9].Gomes et al. in their study reported that 5.5% of GP cones removed from their boxes were contaminated[10]. For the success of endodontic treatment, it is

necessary to eliminate bacteria from the infected canals or to prevent reinfection. In this context, GP cones should be sterilized to avoid canal contamination. As GP cones are thermolabile they can't be sterilized by wet or dry heat so without choice, rapid and effective chair-side decontamination using a chemical agent should be performed to maintain the aseptic chain. In the current study, disinfection treatment was done for 30 sec, as it is the minimum chair side time required for the same.

In this study, the cones were artificially contaminated with E.faecalis. The present study uses CFU/ml as an indicator of microbial growth for bacterial quantification as it is one of the most frequently used methods to assess the antimicrobial activity of endodontic decontamination protocols[11].

Chlorhexidine is used widely as an endodontic irrigant and medicament. It is a potent antimicrobial agent that is particularly effective against Enterococcus faecalis[10]. Because of its antimicrobial properties, substantivity and low toxicity it has been used for a long time in dentistry. The antimicrobial property is due to the cationic molecule binding to extra-microbial complexes and negatively charged microbial cell walls, thereby altering the cell's osmotic equilibrium. This increases the permeability of the cell wall. It kills the bacteria by disrupting the membrane integrity and inducing the precipitation of the cytoplasm. Studies have shown that in liquid form CHX takes 30 s or less to kill microorganisms[12].

Gomes et al. in their study found that 2% chlorhexidine takes less than 30 seconds to completely disinfect E.faecalis contaminated GP cones10. But CS Carvalho et al, in their study reported that one of the replicates of chlorhexidine with exposure of 30 seconds showed turbidity13. The result of our study showed no bacterial growth when cones were treated with 0.12% chlorhexidine for 30 seconds.

QMix 2 in 1 solution (Dentsply Sirona) contains chlorhexidine, EDTA and a detergent (surface-active agent). The solution has demonstrated both antibacterial and smear layer removal properties which are shown by chlorhexidine and EDTA respectively. And detergent has increases its wettability5,14. This might be the reason behind its good antimicrobial activity.

MHM Schmidt et al, reported that chlorhexidine and QMix were successful in eliminating E. faecalis from GP cones[15]. SA Turker et al, in their study, investigated that Qmix was effective to sterilize GP cones at an exposure time of 5 and 10 minutes but still few samples showed growth[16]. Also, Pachalla M Sailaja et al, showed QMix to be a good disinfecting agent against E.faecalis but some amount of turbidity was observed[9]. In this study, Qmix was found to be the next good disinfecting agent among all experimental solutions. 60% of samples showed in the current study showed bacterial growth. The reason behind its less effectiveness than CHX might be that for complete effectiveness it requires longer exposure of more than 60 seconds.

Octenoxa (octenidine dihydrochloride) which belongs to the bipyridines carrying two cationic active centres per molecule and demonstrates broad spectrum antimicrobial effects. Octenidine acts by interfering with cell walls and membranes of bacteria/fungi[3]. Butylated hydroxytoluene (BHT) an phenol derivate, is a preservative component in Octenoxa which has shown added advantage by acting antioxidant[17]. G Ôahinkesen et al, found that 2% CHX was more effective than 0.05% octenisept against E.faecalis at time periods of 1, 5 and 10 minutes[18]. In the present study, it was found to be least effective in completely eliminating E.faecalis from gp cones. Effectiveness of octenidine over different disinfecting agents has been documented in literature but very few studies to date has shoen efficacy of octenidine in disinfecting gp cones. So more studies are required to evaluate the efficacy of octenidine in disinfecting contaminated cones.

CONCLUSION:

The disinfection of GP cones is of utmost necessity for successful biomechanical preparation. Based on statistical analysis, we concluded that immersion of GP cones in the solution of 0.12% chlorhexidine for 30 sec was found to be most effective in eliminating E.faecalis followed by QMix which was the second-best disinfectant among the tested agents. Octnidine dihydrochloride was the least effective for disinfection of GP cones among the experimental solutions. So, based on our result, 0.12% chlorhexidine may be used for rapid disinfection of GP cones for 30 sec.

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