ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)
IJDSIR : Dental Publication Service
Available Online at: www.ijdsir.com
Volume – 4, Issue – 1, February - 2021, Page No. : 334 - 342

Effect of addition of lycopene to calcium hydroxide and chlorohexidine combination on radicular dentin matrix

[1]Dr.Lalitha Gunturu, Narayana Dental College, department of conservative dentistry and endodontics, chintareddy palem, Nellore, Andhra Pradesh, India

[2]Dr.Pavan kumar yendluri, Narayana Dental College, department of conservative dentistry and endodontics, chintareddy palem, Nellore, Andhra Pradesh, India

[3]Dr. Lavanya Anumula, Narayana Dental College, department of conservative dentistry and endodontics, chintareddy palem, Nellore, Andhra Pradesh, India

[4]Dr. Suneel Kumar Chinni, Narayana Dental College, department of conservative dentistry and endodontics, chintareddy palem, Nellore, Andhra Pradesh, India

Corresponding Author: Dr.Pavan kumar Yendluri, Assistant Professor, Narayana Dental College and Hospital, Department of conservative dentistry and endodontics, Chinthareddy palem, Nellore, Andhra Pradesh, India

Citation of this Article: Dr.Lalitha Gunturu, Mr. Pavan Kumar Yendluri, Dr. Lavanya Anumula, Dr. Suneel Kumar Chinni,"Effect of addition of lycopene to calcium hydroxide and chlorohexidine combination on radicular dentin matrix", IJDSIR- February - 2021, Vol. – 4, Issue - 1, P. No. 334 – 342.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Objective: The aim of the present study is to evaluate the effect of addition of Lycopene to Calcium hydroxide and chlorhexidine as intra canal medicament on chemical structure of radicular dentin at two different time intervals by using ATR-FTIR Spectroscope.

Materials and Methods: 80 single rooted teeth were taken and decoronated up to Cemento Enamel Junction. After determination of working length, cleaning and shaping was done with Protaper rotary universal instruments. Then the teeth were divided into 4 groups depending upon the medicament to be used.

Group 1: Control (CON, n=20)

Group 2: Calcium hydroxide and Saline (CH, n=20)

Group 3: Calcium hydroxide and Chlorhexidine (CH-CHX, n = 20)

Group 4: Calcium hydroxide, Chlorhexidine and Lycopene (CH-CHX-LP, n=20)

After placing the medicaments in the respective groups, all samples were sealed coronally with Cavit and stored at room temperature. After one week, half of the samples from each group were sectioned longitudinally with the diamond disc. The sectioned specimens were flattened with the Silicone carbide papers and examined under ATR-FTIR Spectroscope. After one month interval remaining Samples were prepared in the same manner.

Results: The data was subjected to One way ANOVA analysis, and post hoc Tukey's HSD test.

There was statistical significant difference between groups both after one week and one month intervals.

Conclusion: From the present study it can be concluded that lycopene when added to calcium hydroxide and Chlorhexidine combination do not the decrease in phosphate amide ratio which occur when calcium hydroxide and chlorhexidine combination without lycopene.

Keywords: Calcium hydroxide, Chlorhexidine, Lycopene, Reactive Oxygen Species.

Introduction

Cleaning and shaping determines the success of endodontic treatment (1). In cases of persistent pain and periapical pathology intracanal medicaments were advocated to eliminate any remaining bacteria after canal instrumentation(2). Calcium hydroxide (CH) and chlorohexidine (CHX) were commonly used intracanal medicaments to disinfect the root-canal system.

CH neutralizes endotoxin by converting the lipid component to fatty acid and amino sugar(3) .The literature also discusses the use of various formulations and provides suggestions for mixing CH powder with other substances(4) .

Studies have proven that CH and CHX combinations have increased the efficacy of CH on reducing microbial growth(5). This combination has also been proven to be effective in inhibiting the growth of E.faecalis by causing damage to its cell wall and plasma membrane(6).

In alkaline environment, chlorohexidine releases reactive oxygen species (ROS), which is further accentuated by the addition of CH. ROS might destroy the root canal microorganisms and it has been demonstrated that mixture of CHX and CH has greater microbial activity than when CH was used alone(7).

Studies conducted by Doyon et al , Andreasen et al, Sahebi et al showed that the long term use of CH may lead to neutralization and denaturation of dentin organic proteins, leaving the root more prone to fracture(8–10). Madhusudhana et al , proved that fracture resistance of radicular dentin is further decreased when CHX was added to CH which could be due to the release of ROS in alkaline environment (11).

To counteract the ROS formation, ROS scavengers/ antioxidants are of prime importance which acts by reacting with ROS and thereby preventing the harmful effects caused by them. Lycopene (LP), a red pigment found in tomato-based products, is an acyclic form of beta-carotene It possess antibacterial, antifungal properties. A study by Madhusudhana et al has proved that addition of LP to CH and CHX combination do not cause decrease in fracture resistance of radicular dentin caused by ROS release after 1-month(11)

However, no previous studies have tried to explore the effect of CH-CHX, CH-CHX-LP on the chemical integrity of radicular dentin. To study the effect of irrigants and intra canal medicaments on the chemical integrity of radicular dentin Attenuated Total Reflection Fourier Transform Spectroscope (ATR-FTIR) is used(12).

Hence, the aim of the present study was to evaluate chemical integrity of radicular dentin when Lycopene was added to combination of Calcium hydroxide and Chlorhexidine medicaments at two different time intervals by using ATR-FTIR Spectroscope.

Materials and methods

Sample collection: 80 freshly extracted human single rooted teeth with straight roots and single canals were selected for this study. After confirmation with radiograph teeth with root cracks, caries, restorations and previous endodontic treatments were excluded. Ultra sonic Scaling was done to remove calculus, and stains from all teeth.

Sample preparation

Teeth were decoronated with the help of low speed diamond saw under copious irrigation with saline. All roots were standardized to have a length of 16 mm. Working length was determined 1 mm short of the apex by using 15 k-file. Later, cleaning and shaping of root canals was done using ProTaper universal rotary instruments (Densply, Maillefer; Ballaigues, Switzerland) till F2. Along with instrumentation, 1 ml of 3% NaOCl (Prime Dental Products; Thane) and 17% EDTA (Prime Dental Products; Thane) were used as an irrigant between use of each succeeding file. Saline (Claris Otsuka Private Limited; Ahmedabad) was used as final irrigant to remove any dentin debris and traces of NaOCl completely, from the canal after instrumentation.

Experimental groups

Teeth were randomly divided into four groups each containing twenty samples after cleaning and shaping.

Group 1, control group (CON, n = 20),

No medicament was used.

Group 2 Calcium hydroxide and Saline (CH, n=20)

1.5 grams of CH powder (Deepa shree Products; Ratnagiri) mixed with 1 ml of saline to paste consistency was used as intra canal medicament.

Group 3 Calcium hydroxide and Chlorhexidine (CH-CHX, n = 20)

1.5 gm of CH powder mixed with 1 ml of 2% CHX to paste consistency (Septodont; Calypso; India) as intra canal medicament.

Group 4 Calcium hydroxide, Chlorhexidine and Lycopene (CH-CHX-LP, n=20)

1.5 gm of CH powder mixed with 1 ml of 2% CHX and 1 ml of 5% LP (Health Vit; Ahmedabad) to paste consistency was used as intra canal medicament. 5% LP solution was prepared by mixing 5 gm of LP in 100 ml of distilled water.

Later, the access openings of all teeth were sealed with Cavit (3M ESPE; Bangalore) and stored at room temperature.

After 1 week, 10 teeth from each group were taken and sectioned longitudinally with a diamond saw (SUZHOU Syndent tool co.Ltd, China) at slow speed. Both the pulpal surface and outer surface of the sectioned roots were flattened with the help of 600silicone carbide paper. Finally, the specimens were rinsed with deionised water to remove surface debris and observed under Attenuated Total Reflection Fourier Transform Infra Red Spectroscope (ATRFTIR Spectroscope) (Analyser Instrument Co. Pvt. Ltd, India).

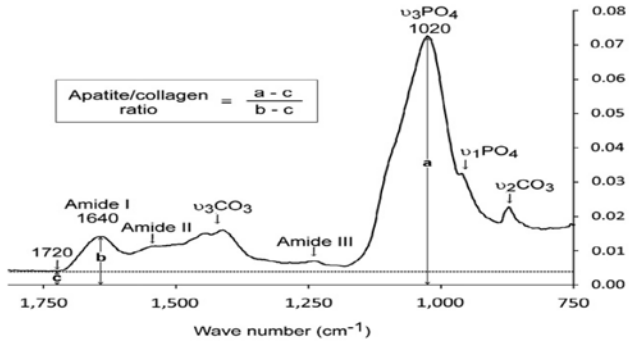
After 1 month interval, the remaining samples in each group were subjected to the same procedure and viewed under ATR- FTIR Spectroscope.

Relative contents of the inorganic and organic components were evaluated with an apatite /collagen ratio between the amide1 peak (1640 cm_1) and the v3 PO4 peak (1020 cm_1) by using the following method.

Apatite/ collagen ratio=Phosphate peak value- base line value

Amide 1 peak value- base line value (as shown in the figure 1)

The mean of the phosphate/amide I ratios derived from the spectra obtained from each group was used for statistical evaluation. Larger ratios represent a greater dentin deproteinization, whereas smaller ratios represent a greater dentin demineralization.



Statistical Analysis

Descriptive analysis, mean and standard deviation were calculated using SPSS 22.0 for windows. Statistical analysis was done by One Way ANOVA which showed the mean phosphate amide ratio of four groups at one week and one month intervals. The inter group difference was calculated using post hoc Tukey's HSD test.

Results

Statistical analysis was done by One Way ANOVA which showed there was statistical significance between the groups after one week and one month intervals.

However, the inter group difference was calculated using post hoc Tukey's HSD test It was observed that there is statistically significant difference in phosphate amide ratio between the control group compared with CH- CHX and CH-CHX- LP at 1 week and there was statistically significant difference between CH group and CH-CHX group in phosphate amide ratio. But the findings were different at 1 month there was statistical significance difference was observed with control group to all the three medicament groups. CH-CHX group showed statistically significant difference when compared to CH and CH-CHX-LP. There is no statistical significant difference between CH and CH-CHX-LP.

Discussion

CH is in regular use in endodontics as intracanal medicament and endodontic regeneration procedures. Its combination with CHX is observed to have high antimicrobial efficacy. But this combination showed to cause radicular dentin demineralization due to the release of ROS. Study conducted by Madhusudhana et al, showed that addition of lycopene to CH-CHX there is no decrease in fracture strength of radicular dentin when compared to CH-CHX intracanal medicament alone. So, the aim of present study was to observe changes in apatite collagen ratio that occurs due to use of CH, CH-CHX and CH-CHX-LP intracanal medicaments at 1week and 1month interval.

In the present study, manipulation of medicaments in CH, CH-CHX, CH-CHX-LP groups was done according to the study by Mageshwaran et al.(6).To simulate the routine clinical conditions, 3% sodium hypochlorite and EDTA have been used between the succeeding files during cleaning and shaping procedure of all the samples.

One week interval was chosen to correlate with clinical conditions as it was the minimum time to get optimal antimicrobial effect of CH. One month interval was chosen because it is often necessary to retain the CH for 4 weeks in case of mature teeth with apical periodontitis(9).

After each time interval, the samples were viewed under ATR-FTIR Spectroscope and effect of three combinations of CH on collagen and apatite composition of surface dentin was evaluated by using the mineral matrix ratio. The mean of the phosphate/ amide ratios derived from the spectra were used for quantitative evaluation. Increased phosphate amide ratios corresponds to a greater Dentin deproteinization, whereas smaller ratios corresponded to a greater dentin demineralization(13).

ATR Spectra after one week showed a significant difference in the phosphate amide ratio in all medicament groups except CH when compared to CON group which indicates deproteinization with similar loss of organic matrix.

After one month, CH group showed much increase in the phosphate amide ratio which denotes deproteinisation and proves the fact that increased exposure to CH for long intervals causes further loss in the matrix. A similar result was obtained in a study by Yassen etal(13) the Due to its smaller molecular weight CH can easily penetrate through apatite encapsulated collagen matrix. It's strong alkalinity denature the phosphate and carboxylate groups and causes changes in tropocollogen structure which is important for

the calcification of dentine, and the interaction between collagen and hydroxyapatite, leading to a collapse of the dentin structure(14). This might cause an accelerated fatigue crack propagation during cyclic stresses and an increase in the susceptibility of root fracture in CH treated root canals (15).

In CH-CHX combination CHX improves contact angle and increases wettability of dentin of CH & have synergistic antibacterial effect.CHX in the presence of CH (pH=12.5) causes release of hydrogen peroxide and superoxide radicals these might cause decalcification of radicular dentin(6).

The cumulative effect of ROS on the dentin and alkalinity of CH causes both demineralization and deproteinization which results in decreased phosphate amide ratio. These findings correlate with a previous study by Madhusudhana et al, which reported decrease in fracture resistance in the CH-CHX group due to excess ROS(11,16).

To counteract the formation of ROS, lycopene was added to CH-CHX. Even though LP counteracts the ROS produced by CHX, It has no effect on the alkalinity of CH and does not cause loss of organic matrix.

Furthermore, it was observed that there was no much difference in the mineral matrix ratio CH-CHX-LP group at both one week and one month time intervals. Although there was slight increase in the deproteinization due to CH, the difference was not significant.

One of the limitations of studying the chemical structure of dentin with FTIR approach is that the depth of penetration of infrared radiation in the FTIR technique is limited to a few microns. Therefore, the spectral data and the phosphate/ amide I ratios reported in our study may only represent the net chemical change of superficial dentin after various treatment protocols rather than the whole chemical change across the total thickness of the dentin specimens(17).

Conclusion

From this study it was observed that addition of Lycopene to combination of CH and CHX inhibited the demineralization of radicular dentin by scavenging on the free radicals generated when combining CH with CHX.

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Legend Table and Figure

Mean Posphate amide ratios of groups after 1 week and 1 month

		N	Mean	SD
Posphate amide ratio1week	CH	10	7.170	.7150
	CH-CHX	10	8.540	.9430
	Control	10	6.320	.8025
	CH-CHX-LP	10	7.750	1.2826
	Total	40	7.445	1.2356
Posphate amide ratio1month	CH	10	9.330	1.6132
	CH-CHX	10	4.940	.5777
	Control	10	6.470	.7543
	CH-CHXLP	10	8.430	1.5959
	Total	40	7.293	2.0941

One way anova was done to compare the mean phosphate amide ratio after one week and one month

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Phosphate amide ratio 1week	Between Groups	26.333	3	8.778	9.516	.000
	Within Groups	33.206	36	.922		
	Total	59.539	39			
Phosphate amide ratio 1mon	Between Groups	116.561	3	38.854	25.680	.000
	Within Groups	54.467	36	1.513		
	Total	171.028	39			

Posthoc Tukey's HSD test was done for intergroup camparison after one week

Pair wise comparison		HSD 0.05 =1.1644 HSD 0.01 =1.4459	Q 0.05 = 3.8088 Q 0.01 = 4.7294
CON:CH	M1= 6.32 M2 = 7.16	0.85	Q= .278(p=0.21968)
CON:CH-CHX	M1 = 6.32 M3 = 8.54	2.23	Q= 7.29(p= 0.00005)
CON:CH-CHX-LP	M1 = 6.32 M4 = 7.75	1.43	Q = 4.68 (p =0.01101)
CH:CH-CHX	M2 = 7.16 M3 = 8.54	1.38	Q = 4.51 (p=0.01486)
CH:CH-CHX-LP	M2 =7.16 M4 = 7.75	0.58	Q= 1.90 (p =.54330)
CH-CHX:CH-CHX-LP	M3 = 8.54 M4 = 7.75	0.80	Q= 2.96(p= 0.2627)

Posthoc Tukey's HSD test was done for intergroup camparison after one month.

Pair wise comparison		HSD 0.05 =1.4815 HSD 0.01 =1.8396	Q 0.05 = 3.8088 Q 0.01 = 4.7294
CON:CH	M1= 6.47 M2 = 9.33	2.86	Q= 7.35 (P=0.00005)
CON:CH-CHX	M1 = 6.47 M3 =4.94	1.53	Q= 3.93 (p= 0.04075)
CON:CH-CHX-LP	M1 = 6.47 M4 = 8.43	1.96	Q = 5.04 (p =0.00557)
CH:CH-CHX	M2 = 9.33 M3 = 4.94	4.39	Q = 11.29 (p =0.000)
CH:CH-CHX-LP	M2 =9.33 M4 = 8.43	0.90	Q= 2.31 (p =.37186)
CH-CHX:CH-CHX-LP	M3 =4.94 M4 = 8.43	3.49	Q= 8.97(p= 0.00000)