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Comparative evaluation of the pH of periapical region following placement of intracanal medicaments along with calcium hydroxide – Ex vivo study

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ABSTRACT:
Background and Objectives: The antimicrobial efficacy of calcium hydroxide combined with chlorhexidine and antibiotics has been reported, but little is known about their influence on the pH of periapical tissues. Hence, the purpose of this study is to assess and compare the changes in pH of the periapical region following the placement of intracanal medicaments along with calcium hydroxide.
Methods: Forty extracted single-rooted premolars were decoronated, instrumented and filled with different intracanal medicaments: Group A: Calcium hydroxide powder + distilled water; Group B: Calcium hydroxide powder + Chlorhexidine gel; Group C: Calcium hydroxide powder + Triple antibiotic paste and Group D: No medicament. These prepared samples were placed in distilled water at 37^0C. The pH of medium was measured at different intervals of 1st day, 2nd day, 3rd day, 7th day, and 14th day.
Results: A rapid rise in pH value was noted in Group A within 2 days, followed by a gradual decrease over 14 days. Group B and C showed slower but sustained release of hydroxyl ions over 14 days. The mean pH values of four groups showed a statistically significant difference at each day of measurement.
Conclusion: Combination of calcium hydroxide with chlorhexidine and triple antibiotic paste seem to be a valuable option for a controlled and sustained release of hydroxyl ions needed for antimicrobial activity and healing of periapical tissue for a prolonged period of time.
KEYWORDS: Calcium hydroxide, Intracanal medicaments, Periapical region, pH, Chlorhexidine, Triple antibiotic paste

I. INTRODUCTION

Root canal therapy aims at control and elimination of microorganisms in infected root canals. Though chemo-mechanical preparation removes a majority of infecting bacteria along with necrotic pulp debris, intracanal medicaments are recommended in infected canal systems to ensure a positive outcome of endodontic therapy.[1] Calcium hydroxide has emerged as the first choice of root canal dressing due to its capacity to release hydroxyl ions. These ions generate an alkaline environment in periapical region which is essential for antibacterial action and repair of altered periapical tissue by inducing hard tissue formation and preventing dissolution of minerals.[2, 3] Most endodontic pathogens except Enterococcus faecalis and Candida albicans are susceptible to calcium hydroxide.[4] Recent studies have shown the antimicrobial efficacy of calcium hydroxide combination with chlorhexidine and antibiotics against these organisms.[5, 6] However, their influence on the pH of surrounding tissues is still obscure.

Objectives:

The present ex vivo study seeks to assess and compare the changes in pH of periapical region following placement of chlorhexidine gel and triple antibiotic paste along with calcium hydroxide in root canal system.

II. METHODS

The ex vivo study was approved by the institutional review committee of D.A. Pandu Memorial R.V. Dental College, Bengaluru.

Sample preparation

Forty single rooted premolars were collected and their external root surfaces were mechanically cleared of debris, calculus and soft

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tissues and stored in normal saline. To facilitate instrumentation, the crown of each tooth was removed at cementoenamel junction using diamond disc. To eliminate root length as a variable, only teeth with roots 12-15 mm long were used. The diameter of apical foramen were evaluated using stereomicroscope under 40X magnification. 1mm short of anatomical length of the root were considered as working length. Root canals were prepared using Protaper files up to apical size F3. The canals were irrigated using 5ml of 3% Sodium Hypochlorite and normal saline after each instrumentation and final irrigation was done with normal saline. All the canals were dried using paper points.

All root surfaces except for the apical 2 mm were covered with modelling wax and randomly divided into 4 groups:

Group A: Calcium hydroxide + distilled water

Group B: Calcium hydroxide + Chlorhexidine gel

Group C: Calcium hydroxide + Triple antibiotic paste

Group D: No medicament placed in root canal (negative control)

These prepared samples were filled with different intracanal medicaments using lentulospiral. While filling the canals, each root was held in a moist gauze pad to prevent the smearing of the medicament on the root and to maintain the tooth in a moist environment. Coronal access was sealed using modelling wax. All samples were placed in plastic containers containing 10 ml of distilled water and incubated at 37^{O}C.

pH measurement

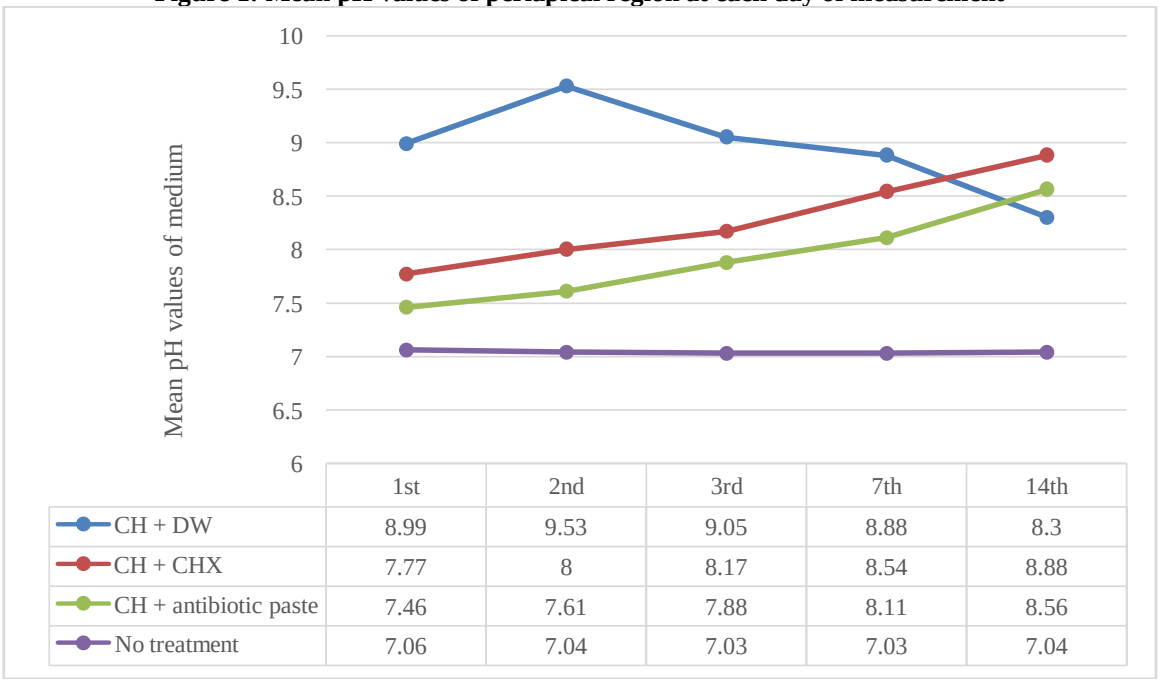
The pH of medium was determined by placing the electrode of a digital pH meter into each container, stirring the solution for 10 seconds and then recording the pH level when the electrode remain submerged in the medium for 30 seconds. The pH readings were taken at different intervals of 1^{st} day, 2^{nd} day, 3^{rd} day, 7^{th} day and 14^{th} day. Three readings per sample were taken and the mean was calculated.

Statistical Ananlysis

Comparison of the mean pH levels between four study groups at different time intervals was done using One-way ANOVA test followed by Tukey's HSD Post hoc Analysis.The level of significance [p-value] will be set at $p<0.05$.\

III. RESULTS

Figure 1: Mean pH values of periapical region at each day of measurement



	1st	2nd	3rd	7th	14th
CH + DW	8.99	9.53	9.05	8.88	8.3
CH + CHX	7.77	8	8.17	8.54	8.88
CH + antibiotic paste	7.46	7.61	7.88	8.11	8.56
No treatment	7.06	7.04	7.03	7.03	7.04

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Table 1: Intergroup comparison of mean pH values in each group on day 1

Groups	Mean±SD	Mean Difference	F	p
A versus B	8.99±0.04 7.77±0.09	1.214	1634.50	<0.001**
A versus C	8.99±0.04 7.46±0.04	1.523		<0.001**
A versus D	8.99±0.04 7.06±0.07	1.928		<0.001**
B versus C	7.77±0.09 7.46±0.04	0.309		<0.001**
B versus D	7.77±0.09 7.06±0.07	0.714		<0.001**
C versus D	7.46±0.04 7.06±0.07	0.405		<0.001**

**Highly significant (p<0.001)

Table 2: Intergroup comparison of mean pH values in each group on day 2

Groups	Mean±SD	Mean Difference	F	p
A versus B	9.53±0.04 8.00±0.03	1.523	5473.93	<0.001**
A versus C	9.53±0.04 7.61±0.04	1.912		<0.001**
A versus D	9.53±0.04 7.04±0.06	2.487		<0.001**
B versus C	8.00±0.03 7.61±0.04	0.389		<0.001**
B versus D	8.00±0.03 7.61±0.04	0.964		<0.001**
C versus D	7.61±0.04 7.61±0.04	0.575		<0.001**

**Highly significant (p<0.001)

Table 3: Intergroup comparison of mean pH values in each group on day 3

Groups	Mean±SD	Mean Difference	F	p
A versus B	9.05±0.05 8.17±0.04	0.877	2610.42	<0.001**
A versus C	9.05±0.05 7.88±0.04	1.161		<0.001**
A versus D	9.05±0.05 7.03±0.07	2.018		<0.001**
B versus C	8.17±0.04 7.88±0.04	0.284		<0.001**
B versus D	8.17±0.04 7.03±0.07	1.141		<0.001**
C versus D	7.88±0.04 7.03±0.07	0.857		<0.001**

**Highly significant (p<0.001)

Table 4: Intergroup comparison of mean pH values in each group on day 7

Groups	Mean±SD	Mean Difference	F	p
A versus B	8.88±0.05 8.54±0.06	0.338		<0.001**
A versus C	8.88±0.05	0.766		<0.001**

	8.11±0.03			
A versus D	8.88±0.05 7.03±0.06	1.845	2308.97	<0.001**
B versus C	8.54±0.06 8.11±0.03	0.428		<0.001**
B versus D	8.54±0.06 7.03±0.06	1.507		<0.001**
C versus D	8.11±0.03 7.03±0.06	1.079		<0.001**

**Highly significant (p<0.001)

Table 5: Intergroup comparison of mean pH values in each group on day 14

Groups	Mean±SD	Mean Difference	F	p
A versus B	8.30±0.07 8.88±0.06	0.581	1740.78	<0.001**
A versus C	8.30±0.07 8.56±0.06	0.268		<0.001**
A versus D	8.30±0.07 7.04±0.06	1.260		<0.001**
B versus C	8.88±0.06 8.56±0.06	0.313		<0.001**
B versus D	8.88±0.06 7.04±0.06	1.841		<0.001**
C versus D	8.56±0.06 7.04±0.06	1.528		<0.001**

**Highly significant (p<0.001)

According to the results, the hydroxyl ion diffusion in group A rose rapidly within a day and reached peak of 9.53 on 2nd day, followed by a decrease in pH value over 14 days. Group B and C showed a slower but significant increase in pH during 14 days. Group D (control) had a mean pH of 7.04 throughout the study period **[Figure 1]**.

Inter group comparison of mean pH values in all four groups at each day of measurement **[Table 1]**, **[Table 2]**, **[Table 3]**, **[Table 4]**and **[Table 5]**showed that there was a highly statistically significant difference between all four groups (p<0.0001) in the rate of hydroxyl ion diffusion at all time periods.

IV. DISCUSSION

The alkalinity of periapical tissues generated by calcium hydroxide is essential for its antimicrobial activity, hard tissue inducing effect and prevention of mineral dissolution. The diffusion of hydroxyl ions through dentinal tubules, apical foramen and accessory canals results in pH alterations in external root surface.[7]Endodontic pathogens, such as Enterococcus faecalis and Candida albicans show resistance to calcium hydroxide, but are susceptible to chlorhexidine and antibiotics.[8, 9]Studies have reported the use of calcium hydroxide in association with chlorhexidine and antibiotics as a valuable option against these organisms.[5, 6] Unlike calcium hydroxide, little is known about the influence of these combinations on the pH of surrounding tissues.

In the present study, all the calcium hydroxide based pastes maintained an alkaline pH over 14 days in comparison with the control group. The pH of group A rose rapidly and reached a maximum of 9.525±0.040 on day 2, followed by a gradual decrease over 14 days. Grover and Shetty reported a similar pH gradient with distilled water as vehicle, where pH rapidly increased from 6.5 to 11.8 at 24 h, followed by a gradual decrease over 30 days (pH = 7.8).[10] On the contrary, Zmener et al. reported that hydroxyl ion release from calcium hydroxide within 24 hours was around 11.24, followed by a continuous but gradual increase from 15 to 30 days.[11]Fulzele et al. demonstrated that calcium hydroxide with distilled water raised the pH to 12.7 after 1 week.[12]

Results of the present study were lower than those from previous studies. This can be explained by the different methodologies used. The above mentioned studies used experimental models (pipette tips) to simulate the natural condition. Another reason can be buffering effect of natural teeth dentin, which is entirely non-existent in case

of glass tubes. Haapasalo et al. demonstrated that dentin powder had a buffering effect on the antimicrobial activity of medicaments (saturated calcium hydroxide solution, 1% sodium hypochlorite, 0.5% and 0.05% chlorhexidine acetate, and 2/4% and 0.2/0.4% iodine potassium iodide).[13] Studies have showed that the pH of 2% chlorhexidine gel was significantly altered by the presence of dentine, raising it to values much higher than optimal for inducing antimicrobial activity.[14, 15]

Chlorhexidine, an antimicrobial agent with wide spectrum of actions and substantivity has been added to calcium hydroxide in an attempt to amplify its antimicrobial effects. The association of chlorhexidine gel with calcium hydroxide has shown a progressive increase in pH of periapical region over a period of 14 days which is consistent with a study conducted by Maniglia-Ferreira et al.[16] However, Signoretti et al. reported that chlorhexidine did not interfere with chemical properties of calcium hydroxide which are needed for antimicrobial activity and healing induction of periapical tissues.[17]

Triple antibiotic paste is a combination of Ciprofloxacin (250 mg), Metronidazole (400 mg), and Minocycline (100 mg) in a ratio of 1:1:1 with propylene glycol as carrier.[18] The combination of calcium hydroxide with triple antibiotic paste showed a gradual increase in pH from 7.463±0.035 to 8.563±0.062 in 14 days. The viscous nature of propylene glycol allows slower diffusion of ions.[19]

Studies have reported that vehicles can have a profound influence on ion release. Pacios et al have reported that aqueous vehicles allow faster diffusion of ions enabling them to achieve higher pH values at shorter periods, whereas other vehicles, such as chlorhexidine and propylene glycol result in satisfactory alkalinity.[20] Simon et al. recommended viscous vehicle for a controlled and sustained release of hydroxyl and calcium ions.[21] These findings are in agreement with the present study.

V. CONCLUSION

Calcium hydroxide in association with chlorhexidine and triple antibiotic paste seems to be a valuable option for inter-appointment root canal medicament. The findings of this exploratory study could be applied to achieve effective outcome of endodontic treatment. Further in vivo investigations are needed to establish the relative antimicrobial efficacy of these combinations.

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