Effect of dentin on the antimicrobial efficacy of 3% sodium hypochlorite, 2% chlorhexidine, 17% ethylenediaminetetraacetic acid, and 18% etidronic acid on Candida albicans: An in vitro study

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Abstract

Aim: The aim of this study was to evaluate the effect of dentin on the antimicrobial efficacy of 3% sodium hypochlorite, 2% chlorhexidine, 17% EDTA and 18% etidronic acid against C. albicans.

Methodology: Dentin powder was prepared from mandibular first premolar using electrical grinder and sterilized. 3% NaOCl, 2% CHX, 17% EDTA and 18% etidronic acid were tested against C. albicans in the presence and absence of dentin, in eppendorf tubes. Group 1 (presence of dentin): - 100ul dentin powder + 100ul C. albicans suspension + 100ul irrigating solution.

Group 2 (absence of dentin): - 100ul C. albicans suspension + 100ul irrigating solution.

Control group: - 100ul C. albicans suspension. + 100ul sterile saline Suspension was thoroughly mixed, submitted for serial dilution upto10-5 after 1 min and colony forming units were counted.

Results: In group 2 (without dentin powder), 3% NaOCl and 2% CHX showed the lowest bacterial count compared to group 1 (with dentin powder). Dentin had a significant inhibitory effect on 3% NaOCl (P<0.001) and 2% CHX (P<0.001). 17% EDTA showed lowest bacterial count in group 1 (with dentin powder) compared to group 2 (without dentin powder). 18% Etidronic acid showed similar bacterial counts in the both the groups. No reduction was observed in the control group.

Conclusion: NaOCl & EDTA showed measurable antimicrobial effect even in the presence of dentin which can be promising in the reduction of C. albicans in root canal therapy.

Keywords: Candida albicans; chlorhexidine; dentin; ethylenediaminetetraacetic acid; etidronic acid; sodium hypochlorite

INTRODUCTION

It has been accepted for many years that microorganisms are considered as a main etiologic agent for periapical infection. For this reason, endodontic treatment aimed at chemomechanically reducing the microorganisms in the root canal system and sealing the root canal space to prevent future recontamination.[2]

Candida albicans is the most commonly isolated yeast in the root canal system infections. Studies have reported the occurrence of C. albicans in 21% of the samples from primary root canal infections.[2]

The effectiveness of routinely used intracanal irrigants, i.e, sodium hypochlorite (NaOCl), chlorhexidine (CHX), and ethylenediaminetetraacetic acid (EDTA) against C. albicans have been quoted in many studies.[3-5]

NaOCl is the powerful necrotic pulp tissue solvent due to the proteolytic effect of free chlorine available.[6] The high
pH of NaOCl interferes in cytoplasmic membrane integrity, biosynthetic alterations in cell metabolism, and also phospholipid destruction.[7]

CHX is a stronger antiseptic and has a longer antimicrobial activity. It is used as a final irrigant but cannot be used as a main irrigant because it cannot dissolve the necrotic tissue.[8] It has been suggested as an intracanal irrigant because of its substantivity and antimicrobial effect via prolonged binding to hydroxypatite.[9]

EDTA is a chelating agent mainly used to increase cleaning efficacy during root canal instrumentation. It reacts with calcium ions in the dentine and forms calcium chelates.[10] It removes smear layer from root canal walls, and hence, the adaptation of the canal filling to the root canal wall is much improved.[11]

However, it can result in excessive peritubular and intertubular dentinal erosion that results in decreased microhardness of root dentin and also strongly interacts with NaOCl, reducing the free available chlorine.[12-14]

Etidronic acid (also known as 1-hydroxyethylidene-1,1-bisphosphonate or HEBP) is a good biocompatible chelator.[13] It can be used in combination with NaOCl without interfering its properties.[14] It also has very less impact on the mineral content of root dentin. Hence, it has recently been suggested as a possible alternative to EDTA.[15]

In vitro studies have shown that dentin and organic materials within root canals can affect the antibacterial effect of irrigants.[16]

Therefore, this in vitro study was aimed to evaluate the effect of dentin on the antimicrobial efficacy of 3% NaOCl, 2% CHX, 17% EDTA, and 18% HEBP on C. albicans.

The null hypothesis in the study was that there would be no difference in the antibacterial effect of the above irrigants in eliminating C. albicans in the presence and absence of dentin.

**MATERIALS AND METHODS**

**Sample preparation**
Dentin powder was prepared by crushing and grinding radicular dentin of 25 mandibular premolars using an electronic grinder (Figure 1). The dentin powder was suspended in distilled water to 28 mg per aliquot of 50 µl as described by Haapasalo et al.[16] Aliquots were placed in Eppendorf Tubes, sterilized in an autoclave, and stored at 4°C until used.

**Irrigants**
Irrigants tested were 3% NaOCl (Prime Dental Products Pvt. Ltd., Pune, India), 2% CHX (Neelkanth Healthcare Pvt. Ltd., Rajasthan, India), 17% EDTA (Pulpdent Corporation, USA), and 18% HEBP (Aqua Diagnostic Labs, Bengaluru, India). The sterile saline solution was used as a negative control.

**Test organism**
C. albicans was used as a test microorganism. Sabouraud dextrose agar was used for the growth of microorganisms as it is a selective medium that is formulated to allow growth of fungi and inhibit the growth of bacteria. After incubation for 5 days, the microbial suspension was prepared and adjusted by a spectrophotometer to a cell density of 1.5 × 10⁹ CFU/ml.

Samples (n = 100) were divided into following groups:
- Group 1 (presence of dentin) (n = 40)
  - 100 µl dentin powder + 100 µl C. albicans suspension + 100 µl irrigating solution in Eppendorf Tubes
- Group 2 (absence of dentin) (n = 40)
  - 100 µl C. albicans suspension + 100 µl irrigating solution in Eppendorf Tubes
- Control group (presence of dentin) (n = 10)
  - 100 µl dentin powder + 100 µl sterile saline + 100 µl C. albicans suspension in Eppendorf Tubes
- Control group (absence of dentin) (n = 10)

***Graph 1: Antimicrobial efficacy of 3% sodium hypochlorite in both groups (presence and absence of dentin powder)***
Karale, et al.: Antimicrobial efficacy in the presence of dentin

The inactivators used to reduce the carryover effect of the CHX were 3% tween 80 + 0.3% alpha lecithin (Aqua Diagnostic Labs, Bengaluru, India), and for NaOCl, inactivator used was 0.5% NaOCl (Aqua Diagnostic Labs, Bengaluru, India) as described by Pappen et al.[17]

RESULTS

Table 1 shows the comparison of pre- and post-microbial count in both groups (presence and absence of dentin) in the presence of 3% NaOCl, 2% CHX, 17% EDTA, and 18% HEBP.

Table 2 shows the comparison of the postmicrobial count in both groups (presence and absence of dentin) in the presence of 3% NaOCl, 2% CHX, 17% EDTA, and 18% HEBP. In group 2 (without dentin powder), 3% NaOCl and 2% CHX showed the lowest bacterial count compared to group 1 (with dentin powder). Dentin had a significant inhibitory effect on 3% NaOCl (P < 0.001) as shown in graph 1 and 2% CHX (P < 0.001) as shown in graph 2. 17% EDTA showed lowest bacterial count in group 1 (with dentin powder) compared to group 2 (without dentin powder) as shown in graph 3. 18% Etidronic acid showed similar bacterial counts in the both the groups as shown in graph 4. No reduction was observed in the control group.

Mann–Whitney test was carried out for pair-wise significant differences in the absence of dentin powder at “Post” time interval [Table 3]. Statistically significant difference in the mean microbial count was found between NaOCl and HEBP (P < 0.05), NaOCl and EDTA (P < 0.05), NaOCl and control (P < 0.05), CHX and HEBP (P < 0.01), CHX and EDTA (P < 0.01), CHX and control (P < 0.001) as well as between HEBP and control (P < 0.01).

Mann–Whitney test was carried out for pair-wise significant differences in the presence of dentin powder at “Post” time interval [Table 4]. Statistically significant difference in the mean microbial count was found between NaOCl and CHX (P < 0.01), NaOCl and HEBP (P < 0.01), NaOCl and control (P < 0.01), CHX and HEBP (P < 0.05), CHX and control (P < 0.01), HEBP and control (P < 0.05) as well as between EDTA and control (P < 0.01).

Statistical software

The statistical software namely SAS 9.2 (SAS Institute Inc., Cary, NC, USA), SPSS 15.0 (SPSS Inc. Chicago, USA), Stata 10.1 (StataCorp LP Texas, USA), MedCalc 9.0.1 (MedCalc Software, Belgium), Systat (Systat Software Inc, London, UK), R Enviroment version 2.11.1 (R studio, Boston) were used for the analysis of the data, and Microsoft Word and Excel have been used to generate graphs, tables, etc.

Colony-forming units per milliliter were counted in all the groups after a period of 1 min.

Graph 2: Antimicrobial efficacy of 2% chlorhexidine in both groups (presence and absence of dentin powder)

Graph 3: Antimicrobial efficacy of 17% ethylenediaminetetraacetic acid in both groups (presence and absence of dentin powder)

Graph 4: Antimicrobial efficacy of 18% etidronic acid in both groups (presence and absence of dentin powder)
**DISCUSSION**

*C. albicans* is the most commonly detected yeast species from infected root canals. Various virulence factors enable *C. albicans* to adhere to and penetrate into dentine. Furthermore, *C. albicans* can survive in harsh ecological conditions including high alkalinity.[3]

NaOCl is the most widely used root canal irrigant, has many advantageous properties for the endodontist. In our study, 3% NaOCl was the most effective irrigant. It presented with the lowest bacterial count in both groups (absence and presence of dentin).

This is consistent with the previous studies demonstrating that concentrated NaOCl was superior to other irrigants on the basis of time taken to eliminate all bacteria.[3]

Dentin had a significant inhibitory effect on 3% NaOCl. Similar results were also seen in other studies where dentin powder showed an inhibitory effect on 1% NaOCl. This depends on the concentration of the medicament as well as on the period for which the medicament was kept in contact with dentine powder before microorganisms are added.[16]

However, NaOCl can cause severe irritation when extruded beyond the periapical region.[3]

CHX gluconate is a cationic agent that exhibits antibacterial activity. At the bacterial surface, cationic ions combine with the anionic ions, capable of altering its integrity. The antimicrobial activity of CHX is pH-dependent, with an optimum pH range between 5.5 and 7.0.[4]

In endodontics, 2% CHX is mainly used, and the same was followed in our study. In the absence of dentin powder, CHX showed better results in effectiveness after NaOCl, while in the presence of dentin powder CHX was less effective, when compared to NaOCl and EDTA. Dentin had a significant inhibitory effect on 2% CHX.

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### Table 1: A comparison of pre- and post-microbial count (log CFU/ml)

| Irrigants          | Groups                  | Premicrobial count (log CFU/ml) | Postmicrobial count (log CFU/ml) | Difference | t-test | P     |
|--------------------|-------------------------|---------------------------------|----------------------------------|------------|--------|-------|
| Sodium hypochlorite| Presence of dentin powder | 11.86±1.44                      | 3.66±2.02                        | 8.200      | 18.860 | <0.001** |
|                    | Absence of dentin powder | 11.06±2.63                      | 2.30±1.78                        | 8.763      | 12.181 | <0.001** |
| Chlorhexidine      | Presence of dentin powder | 12.60±1.17                      | 9.61±2.86                        | 2.992      | 4.158  | 0.002** |
|                    | Absence of dentin powder | 11.64±0.81                      | 6.42±1.50                        | 5.224      | 12.253 | <0.001** |
| EDTA               | Presence of dentin powder | 5.89±0.60                       | 8.14±2.70                        | 2.241      | 2.505  | 0.034*  |
|                    | Absence of dentin powder | 11.23±1.24                      | 8.87±1.44                        | 2.366      | 7.493  | <0.001** |
| Etidronic acid     | Presence of dentin powder | 11.97±1.44                      | 10.66±2.08                       | 1.307      | 3.804  | 0.004** |
|                    | Absence of dentin powder | 11.01±1.98                      | 10.00±2.08                       | 0.101      | 1.964  | 0.081   |

*Significant (P<0.05). EDTA: Ethylenediaminetetraacetic acid, CI: Confidence interval, HEBP: Etidronic acid, CHX: Chlorhexidine.

### Table 2: Difference in both groups with paired t-test

| Irrigants          | Presence of dentin powder | Absence of dentin powder | t     | P     | Mean difference |
|--------------------|---------------------------|--------------------------|-------|-------|-----------------|
| Sodium hypochlorite| 3.66±2.02                 | 2.30±1.78                | 1.5973| 0.1276| 1.36            |
| Chlorhexidine      | 9.61±2.86                 | 6.42±1.50                | 3.1236| 0.0058| 3.19            |
| EDTA               | 5.89±0.60                 | 8.87±1.44                | -6.04076| 0.000010| -2.98          |
| Etidronic acid     | 10.66±1.29                | 10.00±2.08               | 0.8527| 0.4050| 0.66            |

EDTA: Ethylenediaminetetraacetic acid

### Table 3: Mann–Whitney U-test was carried out for pair-wise significant differences in the presence of dentin powder at “Post” time interval

| Irrigant (I)       | Irrigant (J)  | Mean difference (I-J) | P     | 95% CI          |
|--------------------|--------------|-----------------------|-------|-----------------|
| Sodium hypochlorite| CHX          | -1.195                | 0.234 | -2.650, 0.260   |
|                    | EDTA         | -2.58                 | 0.011*| -4.204, -1.294  |
|                    | Control      | -3.445                | 0.011*| -4.900, -1.990  |
| CHX                | HEBP         | -1.554                | 0.002*| -2.542, -0.565  |
|                    | EDTA         | -1.063                | 0.004*| -2.051, -0.074  |
|                    | Control      | -2.250                | <0.001*| -3.239, -1.262  |
| HEBP               | EDTA         | 0.491                 | 0.363 | -0.497, 1.480   |
|                    | Control      | -0.696                | 0.500 | -1.685, 0.292   |
| EDTA               | Control      | -1.187                | 0.001*| -2.176, -0.199  |

*Moderately significant (0.01<P<0.05). EDTA: Ethylenediaminetetraacetic acid, CI: Confidence interval, HEBP: Etidronic acid, CHX: Chlorhexidine.

### Table 4: Mann-Whitney U-test was carried out for pair-wise significant differences in the presence of dentin powder at “Post” time interval

| Irrigant (I)       | Irrigant (J)  | Mean difference (I-J) | P     | 95% CI          |
|--------------------|--------------|-----------------------|-------|-----------------|
| Sodium hypochlorite| CHX          | -1.841                | 0.002*| -3.314, -0.368  |
|                    | HEBP         | -2.701                | 0.001*| -4.174, -1.229  |
|                    | EDTA         | -1.104                | 0.063 | -2.577, 0.368   |
|                    | Control      | -3.122                | 0.001*| -4.595, -1.650  |
| CHX                | HEBP         | -0.860                | 0.045*| -2.197, 0.476   |
|                    | EDTA         | 0.737                 | 0.495 | -0.600, 2.073   |
|                    | Control      | -1.281                | 0.002*| -2.618, 0.055   |
| HEBP               | EDTA         | 1.597                 | 0.041*| 0.261, 2.934    |
|                    | Control      | -0.421                | 0.112 | -1.758, 0.916   |
| EDTA               | Control      | -2.018                | 0.002*| -3.355, -0.682  |

*Moderately significant (0.01<P<0.05). EDTA: Ethylenediaminetetraacetic acid, CI: Confidence interval, HEBP: Etidronic acid, CHX: Chlorhexidine.
Other studies had also shown the significant inhibitory effect of dentin powder on 0.5% and 0.05% CHX acetate.\cite{16}

This is contrary to the results of other studies which indicated that liquid CHX is much more effective in eliminating C. albicans when compared to the NaOCl.\cite{4}

These controversial results can be explained by differences in methodology, form of CHX used, and also the contact period which was 1 min in our study.

EDTA showed less reduction in the absence of dentine, while in the presence of dentine, EDTA demonstrated the most effective antifungal activity after NaOCl.

The reason attributed to this is that there are two particular ways through which EDTA can show its activity: Anti-colonization and antigrowth properties by chelating calcium ions in the dentin, thus preventing binding of C. albicans to the dentin wall. In the other process, EDTA works by removing calcium from the cell walls, causing cell wall collapse of C. albicans and by inhibiting enzyme reaction. It was suggested that there is a direct relation between adherence of C. albicans to the dentinal wall and its ability to colonize and cause disease.\cite{5}

It is also evident that C. albicans can use dentin as a source of nutrition using collagen and calcium from the dentinal wall. The presence of calcium ions has shown to have a critical role in the control of morphogenesis and adherence capacity of C. albicans to many extracellular matrix proteins.\cite{5}

Therefore, calcium removal from the environment (e.g., root canals) will be more critical in decreasing C. albicans metabolism and pathogenesis.

HEBP is a highly biocompatible chelator with adequate calcium-chelating capacity. Hence, there was possibility that they may have a similar type of antifungal activity which was seen with EDTA and also these solutions need around 300 s to completely remove the smear layer, but it has fewer effects on the dentine structure, and hence it was selected.\cite{13}

HEBP was least effective in both groups. It can even be mixed with NaOCl solution without interfering with its antimicrobial property.\cite{13}

EDTA retains its calcium-complexing ability when it is mixed with NaOCl but causes NaOCl to reduce its tissue-dissolving capacity.\cite{19}

In a study done by Haapasalo et al., 1% NaOCl, 0.5% CHX, and 2–4% potassium iodide were tested against Enterococcus faecalis in the presence of dentin powder. The main difference between the present study and Haapasalo’s study is that 3% NaOCl, 2% CHX, 17% EDTA, and 18% HEBP were tested against C. albicans in the presence of dentin powder.\cite{16}

Haapasalo et al. explained irrigant inhibition by dentin to be the result of dentin buffering effect. The root canal is a complex system with a variety of organic and inorganic compounds. Hydroxyapatite, the main component of dentine, is supposed to be mainly responsible for the buffering effect of dentine. However, the fact that the whole dentine is a more effective buffer than hydroxyapatite alone, suggests that other inorganic and even organic components such as tissue remnants also contribute to the buffering action.\cite{20}

In addition, inflammatory exudates which enter the apical portions in purulent infections, is rich in proteins such as albumin, that also contributes to the buffering effect and, as a result, decrease in effectiveness of the intracanal irrigants.\cite{20}

The buffering effect may explain that products such as Ca(OH)$_2$ or NaOCl are inhibited.\cite{21-24}

For this reason, it is possible that having more organic or inorganic substrate interacting with the irrigant solution may inherently delay the elimination of bacteria.

The null hypothesis was rejected in the present study as there was statistically significant difference in the reduction of C. albicans in both groups (presence and absence of dentin powder) with 3% NaOCl and 2% CHX. Dentin increases antimicrobial efficacy of 17% EDTA. No statistically significant difference was observed with 18% HEBP.

The present study was carried out with C. albicans and irrigants such as NaOCl, CHX, EDTA, and HEBP. Further, in vivo studies are required to evaluate the effect of dentin on antimicrobial efficacy of different irrigants.

**CONCLUSION**

NaOCl and EDTA showed a measurable antimicrobial effect even in the presence of dentin which can be promising in the reduction of C. albicans in root canal therapy.

HEBP showed antimicrobial efficacy with fewer effects on dentin structure and can be mixed with NaOCl without interfering with its properties. However, a longer contact time will be needed clinically for the beneficial effects of HEBP.
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Conflicts of interest
There are no conflicts of interest.

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