Clinical and Radiographic Evaluation of Calcium Hydroxide and Bi-antibiotic paste as Anti-microbial Dressing in Revascularization of Non-Vital Immature Permanent Anterior Teeth: A Randomized Clinical Trial

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ABSTRACT:
Objectives: To evaluate the clinical and radiographic performance of the effectiveness of Calcium Hydroxide as intracanal medication in revascularization of immature teeth with necrotic pulp.

Participants and Methods: Regenerative endodontic treatment was performed in (56) immature necrotic anterior permanent teeth which were randomly allocated to two equal groups. Calcium Hydroxide intracanal medication was used at the end of the first visit in
Group I, while bi-antibiotic paste intracanal medication was used in Group II. After resolution of the clinical signs and symptoms in the second visit, mineral trioxide aggregate was placed as coronal plug in both groups following blood clot formation. All the treated teeth were evaluated clinically and radiographically at 3, 6, 9, and 12 months of time interval using predetermined criteria.

**Results:** The overall clinical and radiographic success rate of Groups I and II at the end of the 12 months follow-up period was 92% and 100% respectively and there was no statistically significant difference between the two groups (P-value equal to 0.157).

**Conclusions:** Both Calcium Hydroxide and bi-antibiotic paste can be used as intracanal medication in regenerative endodontic treatment.

**Keywords:** Bi-antibiotic paste; Calcium Hydroxide; Intra-canal medication; Necrotic immature permanent teeth; Regenerative endodontic treatment.

**INTRODUCTION:**
Dentists face many challenges during the endodontic treatment of infected immature permanent teeth. This is mainly due to the thin dentinal walls of the root and blunderbuss apices that make these teeth susceptible to fracture during and after treatment (1).

Apexification is the traditional treatment protocol for immature permanent teeth with pulp necrosis/apical periodontitis. Calcium hydroxide (Ca(OH)2) and mineral trioxide aggregate (MTA) are commonly used for apexification. The major drawbacks associated with calcium hydroxide are reduction in root strength, possibility of root fracture, and several treatment visits over a long period of time. In comparison with calcium hydroxide, MTA apexification requires only short treatment time. But neither of these methods encourages root maturation, which is the thickening of root canal walls and/or closure of apex (2).

Regenerative endodonic treatment (RET) is a biologically based procedure designed to replace damaged tooth structures, including dentine and root structures, as well as cells
of the pulp–dentine complex. In clinical endodontics, this approach is referred to as a “paradigm shift.” Even though repair instead of true regeneration is achieved with current protocols, it is hoped that further research in the area of stem-cell-based tissue engineering will allow for true regeneration and improved treatment outcomes (3).

The biological concept of RET involves the triad of stem cells, scaffold and signaling molecules. The European Society of Endodontology (ESE, 2016) and the American Association for Endodontics (AAE, 2018) have released position statements and clinical considerations for regenerative endodontics. Clinically, regenerative endodontic involves disinfection of the root canal system without damaging the endogenous stem cell potential present in the apical papilla and other tissues. These stems cells are introduced into the root canal space by inducing a blood clot followed by placement of an intracanal barrier to prevent microleakage (4).

For proper disinfection of the root canal system, different antibiotic pastes were applied locally as intracanal dressing for 2-3 weeks. However, the recent recommendations by the ESE advices the use of Ca(OH)2 as intracanal medication instead of intracanal antibiotics. Because of some concerns regarding the use of antibiotics which include: cytotoxicity to stem cells needed for the revascularization process, risk of antibiotic resistance and the risk of sensitization (5).

Thus, this study aimed to compare the effectiveness of Ca(OH)2 to bi-antibiotic paste in disinfecting the root canal system and the effect of the selection of intracanal medication on the clinical and radiographic outcomes of the revascularization of immature permanent anterior teeth.

**PARTICIPANTS AND METHODS**

**Ethical consideration and approval:**

Ethical approval of the current study was obtained from the Research Ethics Committee, Faculty of Dentistry - Cairo University with an approval number (18613).
The study was conducted in the Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Cairo University. Once the child was identified as eligible to the study by the clinical investigator, the trial procedures, benefits from the study and expected harms were clearly discussed with the parent or the child’s legal guardian. Verbal assent was obtained orally from the eligible child and the written consent translated into Arabic, agreeing to the clinical procedures, was signed by the child’s guardian.

**Registration:**

The proposal was registered on clinical trial website, https://www.clinicaltrials.gov, with ID: NCT03504332 and was verified on April 2018.

**Study design:**

The present study is a parallel, randomized clinical trial with a 1:1 allocation. It was a triple blinded clinical trial, where children and their parents, radiographic outcomes assessor and the statistician were blinded.

**Sample Size Calculation:**

Sample size was calculated by an expert statistician using G* power 3.1.7 software based on the previous research by Poorni et al. (2017) (6). In order to reject the null hypothesis and to detect a 40% difference in success rate between the two intra canal medicaments assuming a type I error of 0.05 and a power of 0.8, a total sample size of 50 teeth, 25 teeth for each group was found to be sufficient for detecting a statistically significant difference between the groups. To compensate for a 10% possible drop-out rate calculated using Chi square test, more six teeth were recruited in the study (three teeth in each group) with a total sample size of 56 teeth, with 28 teeth in each group.

**Participant’s selection:**

Inclusion and exclusion criteria of the study have been determined according to the ESE position statement about revitalization procedures, 2016 (7). Children who participated in the present study aged between 8 and 14 years with a mean age of 8.8 ± 1.5 years.
All children presented with immature necrotic anterior teeth with open apices regardless the cause of pulpal involvement: Caries, trauma or congenital anomalies. CONSORT flow chart outlining this study design (Figure 1).

Children with medically compromising conditions, allergic to the materials used in the study or presenting with laxative traumatic dental injuries were excluded from the study. Fifty-six immature necrotic permanent anterior teeth were selected and randomly divided into two equal groups with 28 teeth in each group as follows:

Group I: teeth were treated with MTA (cirkamed, Poland) revascularization after using Ca(OH)2 (JK dental vision, Egypt) as intra-canal medicament.

Group II: teeth were treated with MTA revascularization after using bi-antibiotic paste, Ciprofloxacin (European Egyptian Pharmaceutical Industries, El Ameriya, Alexandria. Egypt) and Flagyl (Sanofi-Aventis, Cairo, Egypt) as intra-canal medicament.

**Allocation concealments:**

Eligible consented participants were assigned to either the intervention or comparator group according to a sequence generated on a Microsoft Excel sheet where the intervention (I) and the control (C) were simply randomized. The table of sequence generation was kept with the co-supervisor and concealed from the main researcher. Once the study consent was signed, a phone call was made to the co-supervisor to assign the participant to either group according to the generated random sequence.

**Steps of operative procedure:**

1. Diagnosis of the cases was performed according to the pre-operative diagnostic checklist of the ESE and a custom-made diagnostic chart was used for the documentation of each case. The treatment procedures were discussed with the parents and the informed consent were signed.

2. Clinical examination, conventional pre-operative periapical radiographs and intra-oral clinical photographs were taken. Individualized radiographic stent was fabricated for each patient and used in combination with an Extension Cone
Paralleling (XCP) dental x-ray film positioning devices for standardized digital radiographic recording of the eligible cases.

3. In the first appointment, the involved tooth was anesthetized using 4% articaine (Inibsa Dental S.L.U, Spain) and isolated. Access cavity and deroofing of the pulp chamber were performed using a water-cooled high-speed carbide bur.

4. Necrotic pulpal tissues were then removed using manual endodontic k-files (Poldent, Poland), with no instrumentation of the root canal walls. Root length was determined by the aid of the endodontic file positioned loosely in the canal and using conventional periapical radiographs.

5. Using side double vented needles (Bibodent, Egypt), the root canals were passively irrigated first with 1.5% NaOCl irrigant (Calix, Dharma, USA), followed by sterile physiological saline (FIBCO, Egypt) and finally with 20mL of 17% EDTA (Calix, Dharma, USA). The irrigation needle was placed 2mm from the root apex (Figure 2).

6. The root canals were dried using paper points and the intracanal medication was placed according to the tooth allocated in which group, either Ca(OH)$_2$ paste (group I) or bi-antibiotic paste (group II):

- Calcium hydroxide paste was prepared by mixing pure Ca(OH)$_2$ powder with saline with 1:1 powder: liquid mixing ratio. The homogenous paste was then loaded inside a sterile plastic syringe to insert the medication into the root canal.

- For the other group, bi-antibiotic paste (Metronidazole 500 mg + ciprofloxacin 500) mg was prepared. The tablets of ciprofloxacin and metronidazole were individually grounded for each patient. equal amounts of ciprofloxacin and metronidazole powder with a ratio 1:1 was mixed with equal amount of saline. The homogenous paste was then loaded inside a sterile plastic syringe to insert the medication into the root canal.
7. A dry sterile cotton pellet was then placed to cover the root canal orifice and the access filled with a temporary restorative material, Riva self-cure (SDI Riva, Melbourne, Australia). The intra-canal medication was left for 2-4 weeks.

8. In the second appointment, if there were no signs or symptoms of persistent infection, 3% mepivacaine (Alexandria Co. for pharmaceutical, Alexandria, Egypt) was used to anesthetize the involved tooth. After isolation with rubber dam, the access cavity was re-opened.

9. Copious irrigation to remove intracanal medication was performed using side, double vented needles and EDTA (20mL, 5 min) followed by a flush of physiological saline (5mL).

10. Bleeding was induced by mechanical irritation of the periapical tissues and rotational movement with a pre-bent endodontic H-file, size #40. A wet cotton was placed then in the access cavity for 15 min till the formation of blood clot (figure 3).

11. A resorbable collagen matrix (Collaplug, Zimmer Biomet, CA, USA) was then cut to a diameter larger than the coronal part of the root canal and a height of 2–3 mm and placed over the formed blood clot. A bond brush was used for the gentle adaptation of the collagen matrix (figure 4 A, B & C).

12. The entire contents of the MTA powder glass vial were mixed with 1 to 2 drops of the MTA liquid on a mixing plate for 30 s until the compound reaches a consistence of moist sand. The mix was placed over the collagen matrix 2 mm beneath the CEJ using amalgam carrier.

13. The access cavity was then sealed with resin modified glass ionomer, Riva self-cure. Immediate post-operative radiograph was obtained using standardized paralleling technique by the (XCP) alignment system, acrylic radiographic stent and the size 2, 3x4 cm, phosphor storage plates (PSPs) imaging plate (Soredex, Finland). (Figure 5)

Follow-up protocol:
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All patients were planned to be recalled for clinical follow up after one months, three months, six months and 12 months. Radiographic follow up was planned to be at 6 month and 12 months. However, Due to the impact of Covid-19 lock down, some patients were not able to attend to the clinic for follow up at three and six months and were telephone screened to follow up them.

As for the root length measurements, software of the Digora system (Digora Optime, Soredex, Tuusula, Finland) was used. The root length measurements were performed as a straight line from the CEJ to the radiographic apex of the tooth in millimeters. The root length from both the pre-operative and 12-month follow-up images was measured, and the increase in root length (mm) (figure 6).

**Statistical analysis:**

The results of the current study were interpreted using per-protocol analysis. Quantitative data were expressed as mean and standard deviation (SD) values. To test the significant differences between quantitative data, t-test was used for analysis. Qualitative data were presented as frequencies and percentages. To test the significant differences between qualitative data, Chi square (X2) was used for analysis. The P-value was considered significant at level P ≤ 0.05. Statistical analysis performed with IBM SPSS Statistics Version 20 for Windows.
Figure 1: CONSORT flow chart outlining the study design

Figure 2: Irrigation of the root canal using side vented needle
Figure 3: Bleeding induction

Figure 4: Application of the collagen matrix; A: Collaplug, B: Collagen matrix to be cut, C: Collagen matrix placed over the formed blood clot
RESULTS:
Table 1 shows the percentage of clinical success among both groups and there was no statistically significant difference between the two groups with a P-value equal to 0.157 distribution according to clinical outcomes in both groups.
Table 2 shows that there was no statistically significant regarding the root lengthening difference between the two groups with a P-value equal to 0.814.
Table 3 shows that there was no statistically significant difference in the distribution of discoloration of the treated teeth. 12 teeth (46%) in group I and 13 teeth (52%) in group II showed crown discoloration at the end of 12 months follow-up period. Table 4 shows that there was no statistically significant difference between the two groups with a P-value equal to 0.157. Overall clinical and radiographic success for group I was 92% while group II was 100% by the end of the 12 months follow-up period.

Table 1: Showing the percentage of clinical success and patient distribution according to clinical outcomes in both groups

<table>
<thead>
<tr>
<th>Clinical parameter</th>
<th>Group I</th>
<th>Group II</th>
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<tbody>
<tr>
<td>Resolution of pain (%)</td>
<td>92%</td>
<td>100%</td>
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<tr>
<td>Resolution of pain on percussion (%)</td>
<td>92%</td>
<td>100%</td>
</tr>
<tr>
<td>Resolution of swelling (%)</td>
<td>92%</td>
<td>100%</td>
</tr>
<tr>
<td>Resolution of fistula (%)</td>
<td>92%</td>
<td>100%</td>
</tr>
<tr>
<td>Resolution of tooth mobility (%)</td>
<td>92%</td>
<td>100%</td>
</tr>
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Table 2: The mean and standard deviation (SD) of root lengthening in mm among both groups

<table>
<thead>
<tr>
<th>Increase in root length in mm</th>
<th>Group I</th>
<th>Group II</th>
<th>P -value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>0.87 (±0.89)</td>
<td>0.81 (±0.88)</td>
<td>0.814 (ns)</td>
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Table 3: Number (N) and percentage (%) crown discoloration of the treated teeth in both groups

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DISCUSSION:
Regenerative endodontic treatment is biologically based procedure designed to physiologically replace damaged pulp-dentin complex which overall helps in maintaining skeletal and dental development and it is superior to apexification techniques in terms of stimulation of root maturation (8). Thus, RET was used to treat immature necrotic permanent teeth enrolled in this study.

The worldwide concern about over-prescription of antibiotics and the associated risk of antibiotic resistance has led to guidelines from the National Institute of Health and Care Excellence to reduce the use of antibiotics within the United Kingdom. These guidelines apply across healthcare, including dentistry, and have implications for antibiotic use in RET (9,10). Thus, the current trial was conducted to evaluated the use of non-setting Ca(OH)2 intracanal medicament as a replacement for antibiotic pastes to create a disinfected environment for the pulp space in RET as recommended by ESE (7).

The main treatment objectives were resolution of clinical signs and symptoms and further root maturation. Pain reported from the patient was selected as the primary outcome because the ideal treatment for an adolescent presenting with a non-vital

<table>
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<th>Table 4: Overall clinical and radiographic success</th>
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<td><img src="table.png" alt="Table 4: Overall clinical and radiographic success" /></td>
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<tr>
<th>Discoloration</th>
<th>Group I</th>
<th>Group II</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present N (%)</td>
<td>12 (46%)</td>
<td>13 (52%)</td>
<td>0.676 (ns)</td>
</tr>
<tr>
<td>Absent N (%)</td>
<td>14 (54%)</td>
<td>12 (48%)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Overall clinical and radiographic success N (%)</th>
<th>Group I</th>
<th>Group II</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 (92%)</td>
<td>25 (100%)</td>
<td>0.157 (ns)</td>
</tr>
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</table>
immature tooth is to prevent pain followed by retention of the tooth for function and optimum dental development (10).

Fifty-one teeth completed the follow up period. The loss to follow up rate was as the anticipated proportions of dropouts calculated in the sample size. Three cases missed the follow up visits due to lack of understanding and engagement while two cases dropped out due to the fear of exposure to COVID-19. The drop-out cases were excluded from the final analysis which was interpreted y per-protocol analysis.

The results of clinical outcomes in this study showed there was no statistically significant differences between the two groups in resolution of clinical signs and symptoms (P-value equal to 0.157). However, two teeth (8%) in group I showed the presence of signs and symptoms of persistent infection in terms of the presence of pain on percussion, swelling, sinus or fistula and tooth mobility. On the other hand, all teeth (100%) in group II showed resolution of clinical signs and symptoms.

In both groups, the elimination of clinical signs of infection occurred early at the second visit as reported by Chueh et al., (2009) and Kharchi et al., (2020) suggesting the initial healing period for a patient is relatively rapid (10,11).

Group I showed 92% clinical success rate which was disagreed with Chueh et al., (2009); Bose et al., (2009); Nagata et al., (2014) and Song et al., (2017) who reported 100% success rate on the basis of the regression of signs and symptoms associated with infected necrotic teeth when using Ca(OH)2 paste as an intracanal medication in RET(11–14). In all studies, disinfection was established in addition to Ca(OH)2 intracanal medication by the use of a NaOCl irrigant (2.5-6%) while in current study, a lower concentration of 1.5% NaOCl was used which may explain the difference in results. In addition, Nagata et al., 2014, used 2% chlorhexidine in the preparation Ca(OH)2 paste which may explain their different results (13).

The failure detected in group I could be explained by the use an inert vehicle to prepare the Ca(OH)2 paste which may be ineffective to kill E. faecalis, a bacterium commonly isolated in persistent root canal infections and presenting resistance to alkaline pH (15).
Furthermore, this microorganism’s capacity for adhering, colonizing, and forming biofilms favors associations between species, increasing their resistance to antimicrobial intracanal medication. In addition, Arruda et al., (2018) reported that bacteria can still be detected in some canals even after one week or more of Ca(OH)2 medication (16).

As regard for discoloration following RET, few teeth in both groups (12 teeth (46%) in group I and 13 teeth (52%) in group II) showed coronal discoloration with no statistically significant difference between the two groups (P-value equal to 0.676). More observed coronal discoloration was reported by Chrepa et al., 2020 where the rate of discoloration was 62% - 97% of all teeth treated with RET and MTA coronal seal regardless of the used intracanal medicament (17).

Coronal discoloration could be explained by oxidation of the heavy metal oxides (iron, bismuth or manganese) contained in calcium silicate cements. Also, the interaction of bismuth oxide with collagen which is converted into black precipitate. In addition, Fagogeni et al., (2019) reported that discoloration of calcium silicate cement may be due to its contact with blood as the porosities in calcium silicate cement can absorb blood components and cause discoloration (18).

Regarding the radiographic assessment of root lengthening, no statistically significant difference was detected at the end of 12 months between both study groups regarding the root lengthening (P -value equal to 0.873). The percentage increase in root length and SD in group I was (7.38% ± 0.08) while in group II was (7.03% ± 0.08). These findings were supported by the results of Saoud et al., 2014 in which the percent change in root length was 5% at the end of 12 months follow up (19). In addition, Aly et al., (2019) reported that the percent of root lengthening over 12- month follow- up was 5.02 (±1.65)% when MTA was used as a coronal plug (20).

Almutairi et al., (2019) suggested that teeth with long-standing periapical infection have shown healing of periapical pathosis without continued root development due to the irreversible damage to the HERS and its interaction with SCAP cells caused by either
long-standing infection or as a result of the severity of traumatic injury incurred (21). However, Tong et al., (2020) and Ong et al., (2020) suggested that 20% threshold of the radiographic increment of root length to be of clinical significance to avoid overestimation of the benefits of RET. However, this 20% threshold is an arbitrary figure and it has not been yet tested scientifically (22,23).

The percent of root lengthening was not correlated with the initial apical diameter in agreement with Estefan et al., (2016) (24). This was justified recently by the study of Chrepa et al., (2020) which reported that age was a more significant predictor for root development than the apical diameter (17).

The results of the current study showed that the overall clinical and radiographic success rate for group I was 92% while for group II was 100% by the end of the 12 months follow-up period in agreement with previously published reports by Nagata et al., (2014); Saoud et al., (2014); Nazzal & Duggal, (2017); Torabinejad et al., (2018); Nazzal et al., (2018) and Koç & Del Fabbro, (2020) which reported that, despite the used intracanal medication, the overall success rate of RET is over 90% (13,19,25–28).

**CONCLUSIONS:**

Based on the results of the current study, the followings could be concluded:

1. The application of intracanal medication for two weeks was sufficient for the resolution of clinical signs and symptoms associated with pulp necrosis.

2. Both calcium hydroxide and bi-antibiotic pastes can be used successfully as intracanal medications for root canal disinfection in regenerative endodontic treatment with comparable results (no statistically significant difference was detected between the two study groups).

3. Coronal discoloration as an undesirable side effect of regenerative endodontic treatment following the use of both calcium hydroxide and bi-antibiotic paste intracanal medication was detected in few teeth (there was no statistically significant difference between the two study groups regarding coronal discoloration).
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FUNDING:
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CONFLICT OF INTEREST:
No potential conflict of interest relevant to this study was reported.

REGULATORY STATEMENT:
This study was conducted by all the provisions of the local human subjects oversight committee guidelines and policies of the ethics committee of scientific research - Faculty of Dentistry - Cairo University - Egypt. The approval code for this study is 18613.

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