RESEARCH ARTICLE

Intra-canal medication containing silver nanoparticle versus calcium hydroxide in reducing postoperative pain: A randomized clinical trial [version 1; peer review: 1 approved]

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Abstract

Background: Pain of endodontic origin can be annoying for patients and endodontist. Pain relief is more important to the patient than treatment success. Numerous factors such as over instrumentation, over filling, debris extrusion can cause postoperative pain. However, bacteria found in the root canal space is the most important factor. Therefore mechanical preparation is an important step in elimination of micro-organisms from the root canal. It has been reported that micro-organisms can still survive inside the root canal even after mechanical preparation. Hence, the use of intra-canal medicaments in between visits for reduction of bacteria inside the root canal space has been recommended. The aim of this study was to assess the ability of silver nanoparticles versus calcium hydroxide used as intra-canal medication in reducing pain in necrotic teeth with apical periodontitis.

Methods: Thirty-four participants were randomly divided into 2 groups, 17 in each group according to intra-canal medication used silver nanoparticles and calcium hydroxide (AgNPs and Ca(OH)2). Each patient was given pain scale chart numerical rating scale (NRS) in order to record his/her pain level before any intervention followed by placement of intra-canal medicament for 1 week. Postoperative pain was recorded at 4, 12, 24, 48 hours.

Results: Pre-operatively; there was no statistically significant difference between mean pain scores in the two groups. After 4, 12 as well as 24 hours, Ca(OH)2 group showed statistically significantly higher mean pain score than AgNPs group. After 48 hours; there was no statistically significant difference between mean pain scores in the two groups.

Conclusions: There was a statistically significant difference in postoperative pain following 4, 12, and 24 hours where AgNPs group resulted in reduction of pain more than Ca(OH)2 group. At 48 hours, there was no statistically significant difference.

Trial registration: PACTR PACTR201602001444180 26/01/2016
Keywords
calcium hydroxide, silver nanoparticles, intra-canal medication, post-operative pain

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Introduction

Pain in endodontic treatment is a major concern for patients and clinicians. Many factors can cause pain like bacteria, chemical mediators, change in cyclic mediators, change in periapical tissue pressure, and psychological factors. However, the presence of microorganisms as a result of failure to properly disinfect the canal is the most common cause of pain. Infected root canals contain a wide range of microorganisms, which may result in the production of enzymes and endotoxins resulting in persistence of pain.

Calcium hydroxide is the most commonly used intra-canal medicament and is considered the gold standard. However, the ability of calcium hydroxide medication to completely eradicate bacterial species from the root canal has been questioned. Also, Al-Zaka (2007) found that chlorhexidine intra-canal medication significantly reduced postoperative pain more than calcium hydroxide medication, where at 4 hours, 20 of 100 patients in chlorhexidine group had no pain compared to only 4 of 100 patients in calcium hydroxide group, and at 24 hours, 44 of 100 patients in chlorhexidine group had no pain compared to 24 in calcium hydroxide group.

Recently, nano-technology has attracted attention, especially those containing silver. Silver nanoparticles are advantageous as they are biocompatible and have antimicrobial activity, silver ions can cause damage to bacterial cell wall. The antibacterial properties of silver nanoparticles has been demonstrated against both gram-negative as well as gram-positive bacteria, fungi and viruses. Therefore, the aim of the present study was to compare calcium hydroxide and silver nanoparticles in terms of postoperative pain.

Methods

Ethical considerations and trial registration

Each of the protocol, informed consent form, and recruitment materials was reviewed and approved by the Ethical review Committee, Faculty of Dentistry, Cairo University. The approval number is 1637.

Patients were able to discuss anything with the researcher and the whole trial was explained to the patient. The researcher received a written consent from the patients that accepted to participate in the trial prior to starting any dental treatment.

The trial was registered with the Pan African Clinical Trials Registry (PACTR) on 26 January 2016 - PACCTR201602001444180, trial name: comparison between two intra-canal medicament, calcium hydroxide and silver nanoparticle.

Patient selection

34 participants, age range from 18–55 years, males or females, single rooted maxillary or mandibular teeth with: non-vital response of pulp tissue, tenderness to percussion, slight widening of lamina dura, were included in this study, (17 participants in the experimental group (silver nanoparticles: Nanotech, Dreamland Egypt, for its preparation, polyethylene glycol was used to formulate the preparation containing 75% (W/W) silver nanoparticles suspension) and 17 participants in the control group (calcium hydroxide: Metapaste, Meta Biomed, CO, LTD, Korea #107677) representing Egyptian population which were recruited from Endodontic clinic, Faculty of Dentistry, Cairo University, between January 2017–January 2018. The aim of the study, treatment procedures, possible side effects and treatment alternatives were explained to the patients. Patients were asked to follow the general instructions and sign a printed informed consent that explained the aim of the study, and asked the patient to fill the outcomes data charts after the procedures, honestly and accurately and return them to us.

Patients aging more than 55 years old or less than 18 years old, medically compromised patients, patients who had received antibiotic treatment during the last 3 months, pregnant females, teeth that couldn’t be isolated with rubber dam, teeth with abscess, fistula or sinus tract, previously root canal treated teeth, and teeth with periodontal probing depth more than 4 mm were excluded from the study.

Sample size

Based on the previous paper by Singh et al. 2013, the sample size was calculated considering that a minimal clinical difference of 10 in numerical rating scale (NRS) score between two study group was clinically relevant. Using a power of 80%, a level of significance of 5% and considering a standard deviation of 9.0, 11 patients per group would be necessary. This number was increased to 13 to adjust for non-parametric usage and increased again to 17 in each group to compensate for losses during follow up. The sample size was calculated using the PS program (3.1.2 / August 2014).

Intervention

Participants. Clinical examination was done tentatively using a diagnostic mirror and probe, presence of extensive caries or large restoration was detected, percussion with the back of a diagnostic mirror and palpation with the index finger to indicate the presence of any swelling or tenderness were done. An electric pulp tester (Denjoy DY310, Henan) was used to record the response of the affected tooth, with the adjacent and contralateral teeth used as a control. Preoperative pain was recorded, with each patient given pain scale chart (NRS) in order to record his/her pain level before any intervention (Extended data). The pain scale (0–10 scale) consists of a line anchored by two extremes, “No pain” and “the worst pain”, patients were asked to choose the mark that represented their level of pain from 0 to 10. Pain level was assigned as follows:

- 0 reading represents "no pain"
- 1–3 readings represent "mild pain"
- 4–6 readings represent "moderate pain"
- 7–10 readings represent "severe pain"

Radiographic examination was done with a Phosphor Storage Plate (PSP) (Soredex (DIGORA™ Optime DXR-60, Finland) wireless sensor taken using the bisecting angle technique to detect the presence of any widening of the periodontal membrane space.
Final diagnosis revealed necrotic mandibular or maxillary single rooted teeth with apical periodontitis.

**Sequence of endodontic clinical procedures.** Local anesthesia: tooth was anaesthetized using nerve block or infiltration technique by selected local anesthesia (Articaine HCl 4% and Adrenaline 1:100,000).

**Isolation:** teeth were properly isolated with rubber dam.

**Access cavity preparation:** a two stage access cavity preparation was performed. The first stage involved the removal of caries without exposing the pulp chamber using a high speed round diamond (Dentsply, Tulsa Dental, Dentsply Maillefer, USA) bur size 2. In the second stage, gaining access with complete de-roofing using a new round diamond bur size 2 was carried out.

**Root canal instrumentation:** the working length was checked with apex locator (Dent Port Zx J. Morita, Irvine, Japan) and confirmed by digital radiography.

Coronal pre-flaring was performed using Gates-Glidden drills (Mani, Tochigi, Japan) sizes 2, 3 and 4.

Root canal preparation was then done using ProTaper Next rotary (Dentsply, Tulsa Dental, Dentsply Maillefer, TN, USA) system in the following sequence (X1, X2, X3, X4, X5) using an endodontic motor (Dentsply Maillefer, USA) with adjusted torque and speed according to the manufacturer’s instructions using 2.5% sodium hypochlorite (Household cleaning products of Egypt, 10° of Ramadan) and EDTA gel (MD-Chelcream, Meta Biomed Co Ltd, Korea) between each file.

**Intra-canal medication and primary coronal seal:**

- The canals were dried with paper points (Dentsply Maillefer, Ballaigues, Switzerland) and plugged with selected intra-canal medicament.
- The access cavities were properly filled with glass ionomer filling to ensure proper sealing with no leakage of any oral fluids inside the root canal.

The patients were given the pain scale chart (NRS) to record their postoperative pain at 4, 12, 24, & 48 hours and return it back on the 2nd visit. Obturation was then carried out in the 2nd visit which was 7 days following the 1st visit.

**Methods: assignment of interventions**

**Allocation (randomization)**

**a- Sequence generation**

A random sequence was generated by computer software (http://www.random.org/), in the center of evidence-based dentistry (EBD), Cairo University.

The table was kept with the assistant supervisor (G.A.).

**b- Allocation concealment mechanism**

Numbered papers indicating the intra-canal medicament to be used were packed in opaque closed envelopes by G.A., where the patient picked up an envelope. After mechanical preparation, operator F.O. opened the envelope and used the intra-canal medicament assigned to that patient according to the number present inside the envelope.

**c- Implementation**

The assistant supervisor G.A. was the one responsible for the generation of random sequence, assigned the patients to the intervention or control group and the only one who knew which medicament was used for each patient.

**d- Blinding**

- The study was double-blinded (the participants and the assessor).
- Participants did not know which group they were treated with after they chose the opaque envelope which contained the number indicating which intra-canal medicament to be used.
- Assessor, who assessed all results data, did not know which group the participants were related to.

**Harms**

If any harm was seen in the participants either in intervention or control groups they were recorded and reported at the end of the trial. The treatment according to the harm:

- Pain: administration of Analgesics. (Cataflam 60 mg, 1 tablet when needed).
- Swelling: hot fomentation, mouth rinse with salty warm water, antibiotic administration in-case of presence of fever or lymphadenopathy.
- Allergic reaction: referral for a physician for corticosteroid therapy. (Prednisolone 40 mg, 1 tablet/day for 3-10 days)

**Methods: Statistical analysis**

Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Age data showed normal (parametric) distribution, while pain data showed (non-parametric) distribution. Data were presented as mean, median, standard deviation (SD), minimum, maximum and 95% confidence interval (95% CI) for the mean values.

For parametric data; Student’s t-test was used to compare between mean age values in the two groups. For non-parametric data; Mann-Whitney U test was used to compare between the two groups. Friedman’s test was used to study the changes by time in each group. Dunn’s test was used for pair-wise comparisons when Friedman’s test is significant.

Qualitative data were presented as frequencies and percentages. Chi-square test (or Fisher’s Exact test when applicable) were used for comparisons regarding qualitative data. The significance level was set at P ≤ 0.05. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

**Results**

**I-Demographic data**

There was no significant difference between the two groups in terms of age (p=0.539) and no significant difference between the two groups in terms of gender distribution (p=1.000), Table 1.
**Postoperative pain**

The intensity and incidence of preoperative pain and postoperative pain at the predetermined periods were formulated as follows.

*Comparison between the two groups regarding preoperative pain and postoperative pain at each time point: Table 2, Figure 1.* Pre-operatively; there was no statistically significant difference between mean pain scores in the two groups. After 4, 12 as well as 24 hours, Ca(OH)₂ group showed statistically significant higher mean pain score (P<0.001) than AgNPs group. After 48 hours; there was no statistically significant difference between mean pain scores in the two groups.

**Table 1.** Mean, standard deviation (SD), number, percentage and P-value for age and gender distribution.

<table>
<thead>
<tr>
<th></th>
<th>Calcium hydroxide</th>
<th>Silver nanoparticle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean: 30.6</td>
<td>Mean: 29.2</td>
</tr>
<tr>
<td></td>
<td>SD: 6</td>
<td>SD: 6.7</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>N: 9/17</td>
<td>N: 9/17</td>
</tr>
<tr>
<td></td>
<td>%: 52.9</td>
<td>%: 52.9</td>
</tr>
<tr>
<td>Female</td>
<td>N: 8/17</td>
<td>N: 8/17</td>
</tr>
<tr>
<td></td>
<td>%: 47.1</td>
<td>%: 47.1</td>
</tr>
</tbody>
</table>

**Table 2.** the mean, standard deviation (SD) values of Friedman’s test for comparison between pain scores at the predetermined periods.

<table>
<thead>
<tr>
<th>Time/ hours</th>
<th>Ca (OH)₂ group</th>
<th>AgNPs group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Pre-</td>
<td>5.53</td>
<td>0.51</td>
</tr>
<tr>
<td>4</td>
<td>4.29</td>
<td>0.59</td>
</tr>
<tr>
<td>12</td>
<td>7.76</td>
<td>0.66</td>
</tr>
<tr>
<td>24</td>
<td>2.18</td>
<td>1.13</td>
</tr>
<tr>
<td>48</td>
<td>0.35</td>
<td>0.79</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*: Significant at P ≤ 0.05, Different superscripts in the same column are statistically significantly different.

**Figure 1.** bar chart representing mean pain scores in the two groups.
point: Table 3, Figure 2. Pre-operatively; there was no statistically significant difference between intensity of pain in the two groups. After 4 hours, there was a statistically significant difference between the two groups. Ca(OH)$_2$ group showed lower prevalence of mild pain and higher prevalence of moderate pain than AgNPs group. After 12 hours, there was a statistically significant difference between the two groups. All cases in Ca(OH)$_2$ group showed severe pain while AgNPs group showed higher prevalence of mild and moderate pain and no cases with severe pain. After 24 as well as 48 hours; there was no statistically significant difference between incidences of postoperative pain in the two groups.

Table 3. frequencies (n), percentages and results of Fisher’s exact tests for comparison of pain incidence in the two groups.

<table>
<thead>
<tr>
<th>Time/ hour</th>
<th>Ca(OH)$_2$ group</th>
<th>AgNPs group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate pain</td>
<td>17/17 (100%)</td>
<td>13/17 (76.5%)</td>
<td>0.103</td>
</tr>
<tr>
<td>Severe pain</td>
<td>0/17 (0%)</td>
<td>4/17 (23.5%)</td>
<td></td>
</tr>
<tr>
<td>4 Hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild pain</td>
<td>1/17 (5.9%)</td>
<td>8/17 (47.1%)</td>
<td>0.017*</td>
</tr>
<tr>
<td>Moderate pain</td>
<td>16/17 (94.1%)</td>
<td>9/17 (52.9%)</td>
<td></td>
</tr>
<tr>
<td>12 Hours</td>
<td></td>
<td></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mild pain</td>
<td>0/17 (0%)</td>
<td>16/17 (94.1%)</td>
<td></td>
</tr>
<tr>
<td>Moderate pain</td>
<td>0/17 (0%)</td>
<td>1/17 (5.9%)</td>
<td></td>
</tr>
<tr>
<td>Severe pain</td>
<td>17/17 (100%)</td>
<td>0/17 (0%)</td>
<td></td>
</tr>
<tr>
<td>24 Hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No pain</td>
<td>2/17 (11.8%)</td>
<td>5/17 (29.4%)</td>
<td>0.398</td>
</tr>
<tr>
<td>Mild pain</td>
<td>14/17 (82.4%)</td>
<td>12/17 (70.6%)</td>
<td></td>
</tr>
<tr>
<td>Moderate pain</td>
<td>1/17 (5.9%)</td>
<td>0/17 (0%)</td>
<td></td>
</tr>
<tr>
<td>48 Hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No pain</td>
<td>13/17 (76.5%)</td>
<td>13/17 (76.5%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Mild pain</td>
<td>4/17 (23.5%)</td>
<td>4/17 (23.5%)</td>
<td></td>
</tr>
</tbody>
</table>

*: Significant at P ≤ 0.05

Figure 2. bar chart representing pain incidence % in the two group at the postoperative periods.
Discussion
Effective control of intra-canal microbial load before obturation is the key element to the high success of root canal treatment. Bacteria can be eliminated from the root canal space by mechanical instrumentation of the root canal which resulted in 50% reduction in endotoxins level in infected root canals. Endotoxins are recognized as foreign by the immune system which will result in a potent immune response resulting in an increased release of pro-inflammatory cytokines which lead to pain and the development of apical periodontitis. Numerical rating scale (NRS) is a subjective method for scoring preoperative and postoperative pain. This is a valid and reliable method which has been widely used in endodontic literature, owing to its standardized assessment measure and reported better compliance when compared to other scales. In addition, it was preferred by the majority of patients amongst different demographic backgrounds. Preoperative pain was recorded for each patient before starting treatment as it was considered a risk factor that can affect the postoperative pain. Although calcium hydroxide is considered the gold standard for disinfecting the root canals, and has been widely used as an intra-canal medicament since 1920’s, its role in eliminating bacteria associated with apical infections is controversial. Microorganisms have the ability to invade the dentinal tubules and buffer the high pH produced by calcium hydroxide compromising its antimicrobial activity. Nanoparticles such as metallic, polymeric and bioactive were recently used as antimicrobial agents in medical and dental fields. Silver nanoparticles bind to the negatively charged bacterial cell membrane, which affect the permeability and respiration of the bacteria causing its rupture and death. Silver nanoparticles release silver ions which lead to bacterial death when it comes in contact with an aqueous media. Silver nanoparticles intra-canal medicament was used in the present study as its antibacterial effect was the most commonly considered in the recent literature than the other types. The incidence and intensity of postoperative pain, was recorded at 4, 12, 24, 48 hours after placement of intra-canal medication. Control group calcium hydroxide showed statistically significant higher mean pain score than silver nanoparticles group. After 48 hours, there was no statistically significant difference between mean pain scores in the two groups. There was a statistically significant increase in mean pain score from 12 to 24 hours, as from 24 to 48 hours there was a statistically significant decrease in mean pain score. Regarding the intensity of postoperative pain at the predetermined time periods, the two groups showed a statistically significant difference at 4, 12, 24 hours, whereas at 48 hours, the results were not statistically significant. Comparing preoperative and postoperative pain between both groups showed no statistically significant difference, neither in intensity nor incidence of pain at different follow up periods. In the present study, the assumption that patients who have acute preoperative pain are likely to experience more severe postoperative pain pre-operatively; as pain was present in all patients in both groups. However, there was no statistically significant difference between mean pain scores between the two groups. Silver in the form of nanoparticles has been used in various forms for treating burns and severe bacterial infected wounds and injuries and as an antimicrobial agent. In this scenario the materials in nano-scale have been used as an anti-microbial agent due to their physical and chemical properties. Silver nanoparticles when used in small doses and with particles at 10 nm, appeared to have anti-inflammatory characteristics, and speed up the healing process of wounds, as well as modulate cytokines, and the induction and production of peripheral blood cells. Calcium hydroxide showed higher mean pain score at 4, 12 and 24 hours compared to silver nanoparticles group. This may be attributed to the combined effect of silver nanoparticles as being anti-inflammatory and anti-microbial. Calcium hydroxide release hydroxyl ions which increase alkalinity leading to death of bacteria and microorganisms inside the root canal. This could explain the reduction of postoperative pain at 48 hour time period, however, Anjaneyulu and Niveditha, concluded in their systematic review that the effect of calcium hydroxide in reducing postoperative pain can be increased when it is combined with other medications such as camphorated mono-chlorophenol or chlorhexidine and the use of calcium hydroxide alone isn’t effective to reduce postoperative pain. Orstavik and Hapassalo, Yesiloy et al. found that certain bacteria present in the root canal system were resistant to the high pH environment produced by calcium hydroxide. Future studies can be directed to identify the the type of bacteria present in root canals, especially the harbored bacteria along full length of dentinal tubules to define the proper effect of the intra-canal medication used.

Conclusions
There was a statistically significant difference in postoperative pain following 4, 12, and 24 hours where AgNPs group resulted in reduction of pain more than Ca(OH)2 group. At 48 hours, there was no statistically significant difference.

Data availability
Underlying data is available from figshare

Figshare: Dataset 1. Silver nanoparticle versus calcium hydroxide intra-canal medication https://doi.org/10.6084/m9.figshare.744133

Licence: CCO 1.0 Universal (CC0 1.0) Public Domain Dedication

Extend data
Figshare: Extended data. pain scale chart https://doi.org/10.6084/m9.figshare.744133

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Reporting guidelines

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Reham Hassan
Department of Endodontics, Faculty of Dentistry, Minia University, Minya, Egypt

The present article compares the postoperative pain after using either calcium hydroxide or silver nanoparticles as intra-canal medication.

The success of root canal therapy depends on the reduction or eradication of the microbial load inside root canals. However, total elimination of the bacterial population inside the root canal is difficult to accomplish.

It's well known that Ca(OH)₂ does not show complete effectiveness in cases of persistent root canal infections, therefore different nano scale materials have been recently used as an antimicrobial agent, the post operative pain after using new materials is considerably an important factor.

The authors definitely spent a lot of time carrying out the study and writing down their findings.

The Introduction section contains an adequate background regarding the use of silver nanoparticles for intra-canal medication.

Regarding the methodology, several aspects should be pointed out:
- Patients inclusion and exclusion criteria were clearly stated
- Ethics committee approval and randomization procedure were provided
- The method of introduction of the silver nanoparticle to fill of canal space was not clearly mentioned

Results section shows the main results clearly.
Generally discussion section is good and deliberate.
The provided CONSORT checklist and flowchart for this article is quit beneficial.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Endodontics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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