Original Article

Post-treatment Flare-up Incidence after Using Nano Zinc Oxide Eugenol Sealer in Mandibular First Molars with Irreversible Pulpitis

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KEY WORDS
Flare-up;
Nano sealer;
AH-26 Sealer;

ABSTRACT
Statement of the Problem: Some patients may report moderate-to-severe pain and/or swelling following root canal treatment that is undesirable for both the patient and dentist and may requires an unscheduled emergency visit by patients to relieve the symptoms.

Purpose: The aim of this study was to evaluate the post-treatment flare-up incidence following application of nano zinc oxide-eugenol (NZOE) sealer in mandibular first molars with irreversible pulpitis.

Materials and Method: This single-blinded study was performed on 60 patients having mandibular first molars with irreversible pulpitis. After signing the written consent form, the patients were randomly divided into two groups considering their age range (20-34 and 35-50 years). Individuals without systemic diseases and with a first mandibular molar diagnosed with irreversible pulpitis due to caries, no sinus track and abscess, normal periapical radiographic appearance, no spontaneous pre-treatment pain, not having taken any medication for at least 8 h before the treatment visit were included in this study. Patients of both sexes ranging from 20 to 50 years of age were selected.

In order to obturate the root canal space, AH26 sealer was used in the one group and synthesized NZOE was applied in another group. The patients were then given questionnaires to report the severity of pain and presence of swelling during 6, 18, 24 and 48 hours of follow-up. The data was analyzed using SPSS v.19 software applying repeated measures ANOVA. The significance level was set at 0.05.

Results: The severity of pain was significantly lower in NZOE group at 24 hours post-treatment \((p=0.003)\) Patients reported no symptoms of swelling in any group.

Conclusion: NZOE sealer manifested satisfactory results and could be regarded as a promising substitute to routine sealers due to the fact that patients may experience less pain during the first hours after receiving root canal therapy.

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Introduction
The main objectives of root canal therapy are to achieve long-term comfort, function, and aesthetics for the patients and prevention of reinfection of tooth. These objectives are provided through complete cleaning, shaping and obturation of canals of affected teeth [1]. However, some patients may report moderate-to-severe pain and/or swelling following root canal treatment that is undesirable for both the patient and the dentist and may requires an unscheduled emergency visit by patients to relieve the symptoms. Flare-up following RCT has been reported to range from 2.53% to 58% [2]. Pack et al.
reported 40% of the patients had post-operative pain 24 hours after RCT and this decreased to 11% after one week [3]. Although the exact reasons for flare-up are not clearly understood, it is accepted that flare-up is a multi-factorial phenomenon and there is no single reason for its occurrence [4]. Microbiological, chemical, mechanical, and host variables can inducing pain and swelling following root canal treatment [5]. In addition, preoperative pain, gender and age, the pre-operative pulp status, the type of tooth, the type of treatment (initial or re-treatment), and a history of allergy may all influence flare-up [6]. Also, some studies have demonstrated the effect of several factors such as instrumentation technique, type of analgesic, method of analgesic prescription, type of rotary instrument, an aesthetic solution, occlusal reduction and method of root canal filling on flare up [7-8].

In obturation of the root canal system, sealer is using to prevent penetration of microorganisms and their by-products. In the other hand, sealer is in direct contact with periapical tissues and may cause inflammation, tissue degeneration and delay in wound healing. Therefore, the ideal root canal sealer should be non-cytotoxic, non-mutagenic and immunologically compatible with periapical tissues [9]. Currently, a large variety of sealers with different formulas and physical properties are available for use. However, they all have their limitations. It is difficult to produce a sealer with proper physicochemical properties while being biocompatible for long-term. For many years, zinc oxide-eugenol (ZOE)-based sealers have been widely used in endodontic practice. The use of nanotechnology has allowed many developments in dentistry and advances in oral-health-related nano material and therapeutic methods [10]. Some of the advantages of using nano particles in endodontic sealers include improving their physicochemical characteristics, enhancing the antibacterial property, better penetration into the dental tubules, decreasing microleakage, and increasing biocompatibility [11-12]. It has been shown that incorporating zinc oxide nano particles enhances the physicochemical characteristics (setting time, flow, solubility, dimensional stability and radiopacity) of Grossman sealer [12]. Kesler Shvero et al. [13] demonstrated that epoxy resin-based surfaces with cationic nano particles attracted and sacrificed Enterococcus faecalis. DaSilva et al. [14] showed that incorporating chitosan nano particles into ZOE sealer reduced the formation of biofilm within the sealer-dentin interface. Also, it has been reported that nano-ceramic sealer had better cytocompatibility than Endoseal MTA considering the effects on cell spreading and proliferation [15].

Recently, a new endodontic sealer with nano-sized ZO powder particles (NZOE) has been developed in the Dental Material Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. This sealer is similar to various ZOE-based sealers, but with different sizes of ZOE nanoparticles. In previously published articles, we showed that NZOE sealer that had less microleakage and better antibacterial property in comparison with Pulpdent and AH-26 sealers [16]. In an animal study, it was observed that the histocompatibility properties of NZOE were comparable to the above-mentioned commercial sealers [17]. Also, the cytotoxicity of NZOE on murine L929 cell line was comparable to that of Pulpdent and was lower than AH-26 sealer [18]. In another study the cytotoxicity of NZOE sealer on human gingival fibroblasts isolated from healthy subjects was evaluated and NZOE exhibited lower cytotoxicity compared to Pulpdent and AH-26 [19]. So, after in-vitro and in-vivo experimental study, this study was the first clinical study that was performed on this new sealer.

ZOE -based sealers are the oldest used in endodontic therapy. Zinc-oxide is a valuable component of these sealers that is very effective as an antimicrobial agent. Many reforms have been done on this sealers in order to improve their property and also many commercial models are available [20]. In addition, ZOE-based cements have been found to possess favorable characteristics in terms of biocompatibility. These were the reasons for selecting ZnO as the base of a nano-sealer in the present study.

To the best of our knowledge, the use of nano-structured materials as sealers in root canal therapy is limited to two or three types of nano-structured hydroxyapatite alone or in combination with epoxy resin (Nanosel) [20-22].

No evidence-based investigations have evaluated postoperative pain and swelling when different sealers were used for obturation. The aim of this study was to assess postoperative pain and swelling in a prospective randomized clinical trial following the use of AH-26.
and NZOE as a sealer in mandibular molars with irreversible pulpitis that were treated in one visit.

Materials and Method
The protocol of this study was approved by the Ethics Committee of Mashhad University of Medical Sciences (Ir.mums.dentistry.rec.1397.044 and Iranian Registry of Clinical Trials ID No. IRCT2018071136542).

Synthesis of ZnO nano-particles
In this work ZnO nano-powders were prepared by a modified sol-gel method, using gelatin [23].

To prepare 5 g of the final product, first a solution of gelatin (type B from bovine skin, Sigma Aldrich) was prepared by dissolving 10 g of gelatin in 150 mL of deionized water at 60°C. Then, appropriate amounts of zinc nitrate (Zn(NO₃)₂·6H₂O, Merck %99) were dissolved in minimum deionized water at room temperature. Then, the two solutions were mixed and stirred for 8 hours while the temperature was kept at 80°C. Finally, the pure resins were calcined at different temperatures of 500, 600 and 700°C to obtain ZnO nano-powders. Morphological and structural properties of the prepared ZnO were characterized by x-ray diffraction (XRD) and transmission electron microscopy (TEM) techniques.

Procedural steps
The sample size was calculated based on an alpha of 0.05 with power of 80%, indicating that 27 patients would be required in each group but after considering the dropout rate of 10%, the sample size increase to 30 patients in each group.

Inclusion criteria were as follows: individuals without systemic diseases and with a first mandibular molar diagnosed with irreversible pulpitis due to caries, no sinus track and abscess, normal periapical radiographic appearance, no spontaneous pre-treatment pain, not having taken any medication for at least 8 h before the treatment visit. Patients of both sexes ranging from 20 to 50 years of age were selected. During the root canal procedure, teeth that could not be treated in a single visit or patients who interrupted treatment were excluded. Also, the patient with severe periodontal disease, over instrumentation or overfilling beyond the root canal space, teeth that could not be isolated with rubber dam, root canal calcification, root resorption and teeth that were not suitable for further restoration were excluded.

All the treatment procedures were performed by a postgraduate student in the Endodontic Department, Mashhad Dental School, Iran, from May to August 2018. A total of 60 teeth were randomly assigned to two groups using a random-digit table. Before initiating treatment, the patients were asked to rate their pre-treatment pain on a visual analogue scale from 0 to 9. For each number, one of the sealers was selected. Each patient chose one of the number, and based on the number, the nurse supplied one of the sealer for the treatment visit. Due to differentiation in apparent characteristic of two sealers, the practitioner could not be blind. Prior to beginning of the root canal treatment, inferior alveolar nerve block injection was done with 2% lidocaine with 1: 80 000 epinephrine. After isolation with a rubber dam, followed by access cavity preparation, working length was determined with a Raypex6 apex locator (VDW, Germany) and confirmed radiographically. After preparing the root canals with hand instruments to at least a size 15 K-file (Mani, Togichi, Japan), Hero rotary instruments (Micro-Mega, Besancon Cedex, France) were used for root canal preparation. Apical patency was performed with a size 10 K-file between each rotary instrument. The apical preparation was completed up to size 30 .04 taper. Between instruments, the root canals were irrigated with 3 mL of 5.25% NaOCl with a 30-gauge side perforated needle (Endo-Top, PPH Cerkamed, Stalowa Wola, Poland). During irrigation, the needle was repeatedly moved up and down to prevent locking in the canals. The smear layer was removed by irrigating with 3 mL 17% ethylene diamine tetra acetic acid (EDTA) (Asia Chimi Teb Co., Tehran, Iran) followed by 5 mL normal saline irrigation at the end of root canal preparation. The root canals were dried with paper points (Meta Biomed Co., Chungcheongbuk, Korea). Then, in group 1 the canals were filled with gutta-percha (Meta Biomed Co.) and AH26 (Dentsply De Tery, Konstanz, Germany) sealer and group 2 was filled with gutta-percha and NZOE sealer using the cold lateral condensation technique. Each patient was given a numerical visual analogue scale (VAS) form to record the severity of pain from 0 to 9 during the 6, 18, 24, and 48-hours period following treatment, and the other form was used to record swelling. In the second form, the following criteria were used: 0, none swelling; 1, swelling. The data was analyzed using SPSS v.19 software applying Chi-square
and Kendall’s Tau-b. The significance level was set at 0.05.

Results
Based on the inclusion and exclusion criteria, a total of 64 patients were eligible to participate in the study. 4 patients from both groups were excluded due to: 1) treatment could not be completed in one visit for one patient; 2) two patients did not return the VAS form and 3) one patient had partial necrosis pulp. Finally, 60 patients were included for data analysis (30 in each group). The age and sex distribution of patient in each group was reported in Table 1. The two groups were similar according to distribution of age and gender of the patients. None of the patients had pain on palpation, percussion and also spontaneous pain at the treatment visit. The results showed that at the 24 h following treatment, the patients receiving NZOE as the sealer had significantly lower pain compared to those who had AH 26 (p= 0.003); no significant difference was found between the group for the rest of the study period (Table 2). None of the patient in both groups had swelling during the 48 h period following treatment.

Discussion
In the present study, the results of single-visit root canal treatment of mandibular first molars with irreversible pulpitis with the use of AH26 and NZOE as root canal sealers showed that the NZOE was associated with significantly less pain at the 24 h after treatment. Also postoperative pain intensity decreased with time within both groups that was in agreement with the findings of Parirokh et al. [8]. In both experimental groups, no swelling was found during the 48 h follow up period of study.

In the present study, to prevention of possible bias, the patients with similar preoperative conditions was included. As the preoperative pain had significant effect on postoperative pain [24], only patients with no spontaneous pain were included. Also, because pre-treatment analgesic consumption has effect on postoperative pain following root canal treatment [25], all patients were asked about the medication they used and should not have taken any medication for at least 8 h before the treatment visit. According to systematic reviews, single-visit root canal treatment had no significant difference or significantly lower postoperative pain compared to multi-visit treatment [26]. So, in the present study, single-visit root canal treatment was undertaken. In this study, the distribution of age and sex was the same for both groups due to the relationship of these variables to endodontic flare-up. All the treatments carried on mandibular first molars with irreversible pulpits and instrumentation of the root canals was completed in a crown-down technique using rotary nickel titanium files as it has shown to be associated with less debris and irritant extrusion apically, which has a clinical implication in decreasing the incidence of postoperative pain [27]. Also attempt was done to avoid over-instrumentation of root canal during filing. Therefore, both experimental groups were homogenous in terms of age, gender, tooth type, preoperative pain intensity, irrigation solution, instrumentation, and obturation technique. The results of a systematic review showed that during the fi-

**Table 1:** Frequency distribution of sex and age in sealer groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>AH-26 N(%)</th>
<th>NZOE N(%)</th>
<th>Total N(%)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-34 years</td>
<td>16(53.3)</td>
<td>16(53.3)</td>
<td>32(53.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>35-50 years</td>
<td>14(46.7)</td>
<td>14(46.7)</td>
<td>28(46.7)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td>0.796</td>
</tr>
<tr>
<td>Male</td>
<td>15(50)</td>
<td>14(46.7)</td>
<td>29(48.3)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>15(50)</td>
<td>16(46.7)</td>
<td>31(51.7)</td>
<td></td>
</tr>
</tbody>
</table>

N:Number

**Table 2:** Effect of AH 26 and NZOE on pain in different periods

<table>
<thead>
<tr>
<th>Time</th>
<th>Sealer</th>
<th>No pain n(%)</th>
<th>Very low n(%)</th>
<th>Low n(%)</th>
<th>Moderate n(%)</th>
<th>Severe n(%)</th>
<th>Very severe n(%)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6h</td>
<td>AH26</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>8(44.4)</td>
<td>10(55.6)</td>
<td>0(0.0)</td>
<td>0.872</td>
</tr>
<tr>
<td></td>
<td>NZOE</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>5(41.7)</td>
<td>7(58.3)</td>
<td></td>
</tr>
<tr>
<td>18h</td>
<td>AH26</td>
<td>0(0.0)</td>
<td>1(5.6)</td>
<td>7(38.9)</td>
<td>10(55.6)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0.107</td>
</tr>
<tr>
<td></td>
<td>NZOE</td>
<td>1(8.3)</td>
<td>5(41.7)</td>
<td>18(8.3)</td>
<td>4(33.3)</td>
<td>1(8.3)</td>
<td>0(0.0)</td>
<td></td>
</tr>
<tr>
<td>24h</td>
<td>AH26</td>
<td>0(0.0)</td>
<td>5(27.8)</td>
<td>13(72.2)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>NZOE</td>
<td>0(0.0)</td>
<td>7(58.3)</td>
<td>5(41.7)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td></td>
</tr>
<tr>
<td>48h</td>
<td>AH26</td>
<td>11(61.1)</td>
<td>7(38.9)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0.119</td>
</tr>
<tr>
<td></td>
<td>NZOE</td>
<td>3(25.0)</td>
<td>9(75.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td></td>
</tr>
</tbody>
</table>
rst 48 h after root canal treatment, post-operative pain significantly decreased [28]. Therefore, we limited our research to the first 48 h after treatment.

Various methods were used for evaluating the effect of different variables on postoperative endodontic pain. In the present study, a VAS was used to compare post-operative pain intensity because it is a simple tool with high reliability and validity and more sensitive to small changes than descriptive ordinal scales [8].

There are some studies on the effect of irrigation type [29] and irrigation concentrations [30] on endodontic flare up but no study was evaluated the relationship of endodontic sealers and flare up. In the other hand, some researchers evaluated the effects of sealer extrusion into the periapical tissues. Sometimes, extrusion of sealers or diffusion of toxic components from the sealers may occur into the surrounding anatomical structures and periapical tissues during root canal obturation. Although small material extrusions are generally well tolerated by the periradicular tissues and had no significant effect on periapical healing and endodontic outcome, clinical symptoms such as pain and swelling may appear. Toxic effects of the products and a pressure phenomenon from the presence of sealer can initiate an inflammatory process and tissue damage and lead to flare up [31].

Over-instrumentation, excessive amount of sealer, the excessive compaction force, hydrostatic pressure, the use of lentulo spirals, immature canal apices or root tip resorption can increase the risk of sealer extrusion [32].

In this study, the most post-operative pain was reported by AH 26 group during the 24 h after treatment. This can be explain due to mild-to-moderate irritating effects of AH26 when freshly prepared and the toxicity is due to the release of a small amount of formaldehyde, epoxide bisphenol resin, or amines during the chemical setting process [33]. Also, Ruparel et al. [34] revealed that resin-based sealer can directly activate trigeminal nociceptors and leading to release of CGRP and may therefore cause pain and neurogenic inflammation. In another study, Huang [35] reported that AH26 sealer had significant cytotoxicity in the periapical tissues by inducing inflammatory mediators such as receptor activator of nuclear factor kappa B ligand, nitric oxide synthase, cyclooxygenase-2, and reactive oxygen species. The direct activation along with the immunologic response may underlie flare-up occurrences. On the other hand, two in-vitro study reported that the cytotoxicity of NZOE on murine L929 cell line [18] and on human gingival fibroblasts [19] was lower than AH-26 sealer.

According to the result of the present study and since the less microleakage and better antibacterial property of NZOE in comparison to AH-26 sealers [16] and also lower cytotoxicity of NZOE than AH-26 sealer [17-19], NZOE sealer can be used as endodontic sealer for root canal obturation.

Conclusion

Up to 6 h following root canal treatment, the use of NZOE sealer was associated with significantly less pain than the use of AH26.

Acknowledgment

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Post-treatment flare-up

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