Original Article

Evaluation of postoperative pain in endodontic retreatment with apical periodontitis using ozonated 2% chlorhexidine and 0.1% octenidine application: A randomized clinical trial

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Abstract

Introduction: This study aimed to evaluate and compare postoperative pain (PP) in single-visit nonsurgical endodontic retreatment (SV NSER) with 2% chlorhexidine (CHX), 0.1% octenidine (OCT) with or without ozone, and 5.25% sodium hypochlorite (NaOCl).

Materials and Methods: In this randomized, parallel, prospective, double-blind, clinical trial, 132 single-rooted, root-filled teeth with symptomatic apical periodontitis (AP) requiring NSER were allocated into six groups randomly (n = 22/group): 2% CHX with NaOCl (CHXH), 2% Ozonated CHX without NaOCl (OCHX), 2% Ozonated CHX with NaOCl (OCHXH), 0.1% OCT with NaOCl (OCTH), 0.1% Ozonated OCT without NaOCl (OOCT), and 0.1% Ozonated OCT with NaOCl (OOCTH). Standard NSER protocol was followed groups were irrigated with 15 ml of ozonated or nonozonated irrigant (CHX/OCT) for 3–5 min with ultrasonic agitation. PP at baseline, after 6, 12, 24, 48 h, and 7 days was recorded using the Visual Analog Scale (VAS). Logistic regression of predictor variables was compared using the Chi-square test. For group-wise and time-wise comparisons, a two-way analysis of variance followed by the *post hoc* Bonferroni test was carried out.

Results: None of the patient-related variables in logistic regression obtained a statistically significant ($P > 0.05$) role in PP. The VAS score after 6 h was OCHX (4.72) > OOCT (4.42) > CHXH (4.23) > OCTH (3.95) > OCHXH (3.42) > OOCTH (3.21). OOCTH and OCHXH groups demonstrated statistically significant reductions in VAS scores at various time intervals ($P < 0.05$).

Conclusion: SV NSER with ozonated OCT, CHX irrigation, and NaOCl resulted in lesser PP at all time intervals, i.e., 6, 12, 24, 48 h, and 7 days in patients with symptomatic AP.

Keywords: Apical periodontitis; chlorhexidine; ozone; pain; retreatment

INTRODUCTION

Substantial advancement has occurred in contemporary endodontics concerning disinfection of radicular spaces, but root canal reinfections and their associated apical periodontitis (AP) lesions are remarkably prevalent. Fifty-six percent of adults globally have undergone at least one endodontic treatment, yet 24%–65% of these teeth remain associated with persistent AP.[1,2] Furthermore, there has been an increase in the occurrence of AP from 35% to 41% in endodontically treated teeth worldwide from 2012 to 2020.[3] Prevention of further rise in these cases would require a significant improvement in endodontic treatment.

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Our understanding of AP in failed cases points toward the cumulative pathogenicity of the multi-species microbial community. These act as a unit in biofilm adhering to anatomical complexities contributing to refractory infection and postoperative pain (PP).[4] Clinical strategies focus on "canal shaping" to radiographically impeccable levels rather than the critical aspect of "chemomechanical disinfection," which prevents reinfection. Irrigation plays a primary role in removing pulp tissue, reducing the microbial load to a subcritical level, resolving periapical disease, and helping healing.[5]

Sodium hypochlorite (NaOCl) is the gold-standard endodontic irrigant because of its antibacterial properties, lubricant action, and high tissue dissolution. However, its inadequacies include its cytotoxicity, high surface tension, and reduced antibacterial potential on mature biofilms (>3 weeks old) in the presence of organic tissue.[6,7]

Chlorhexidine (CHX) is the irrigant of choice in retreatment cases due to its substantivity and bactericidal action against resistant microorganisms (effectively reduces bacteria by 99.61% and endotoxin by 60.6%).[8] However, poor tissue dissolution, limited action on Gram-negative bacteria, and cytotoxicity limit its usage as a stand-alone irrigant.[9,10] Since no single irrigant is ideal, a need for an optimal multi-irrigant regimen that predictably achieves safe and efficacious irrigation is needed.

Octenidine (OCT) dihydrochloride is an emerging irrigant with broad-spectrum activity against Gram-positive and Gram-negative bacteria, chlamydia, and fungi.[11,12] It is unique owing to its noncytotoxic nature and microbicidal efficacy, which is ten times greater than CHX. OCT can be an alternative irrigant, but the lack of *in vivo* studies has prevented its widespread usage.[13]

Ozone is a potent and selective oxidant that rapidly dissociates into water and releases a reactive form of oxygen that oxidizes cells, hence having antimicrobial and anti-inflammatory efficacy with low cytotoxicity and high biocompatibility with oral tissues.[14] The antimicrobial effect of a single ozone molecule is equivalent to 3000–10000 chlorine molecules, thereby acting around 3500 times faster. Owing to these properties, this novel technique of ozonation of irrigants attempts to assess its efficacy in combination with other irrigants. This randomized, parallel, prospective, double-blind clinical trial evaluated and compared PP after nonsurgical endodontic retreatment (NSER) using ozonated or nonozonated, 0.1% OCT, and 2% CHX with or without 5.25% NaOCl application in teeth with posttreatment AP.

Objectives

The primary objective was to evaluate and compare PP in single-visit (SV) NSER following ozonated or nonozonated irrigation with CHX or OCT. The secondary objective was to assess the effect of NaOCl and ozonated CHX, OCT on PP at baseline, 6, 12, 24, 48 h, and 7 days.

MATERIALS AND METHODS

Ethics and reporting guidelines

Prior approval (PDCH/21/EC-286) from the research ethical committee was procured. Signed informed consent of all the participants was obtained after discussing alternative treatment options and the pros and cons associated with the procedure. During the trial Preferred Reporting Items for Randomized Trial in Endodontics 2020 guidelines (Nagendrababu *et al*. 2020) were followed.[15]

Selection of subjects

The study was conducted from February 2022 to March 2023, and it included patients requiring NSER reporting to the Department of Conservative Dentistry and Endodontics, XXX Dental College and Hospital. It was registered in the Clinical Trials Registry, India (CTRI/2022/02/040315).

Sample size determination

A pilot study was conducted on 48 subjects among the six groups (eight subjects per group) to estimate the sample size using G Power (version 3.19.7) software. Based on the mean and pooled standard deviation, effect size *F* was 0.32. The sample size was estimated as 132 (22 subjects per group) at a 95% confidence interval (CI) with a power of 80%.

Inclusion criteria

Patients with single-rooted, root-filled teeth with persistent AP (symptomatic teeth) requiring NSER with radiographic evidence of previous endodontic treatment done >3 years ago, sufficient coronal tooth structure for adequate isolation with a rubber dam, gutta-percha (GP) material within 4–7 mm of the radiographic apex and no exposure of obturating material to the oral cavity.

Exclusion criteria

Patients who were medically compromised, allergic to the local anesthetic agent, irrigants, and ozone, with a recent history of myocardial infarction, acute alcohol intoxication, active bleeding, uncontrolled hyperthyroidism, thrombocytopenia, and glucose six phosphatase deficiency.

Patients under 18 years, with swelling, acute apical abscess, antibiotic, corticosteroid, anti-inflammatory, analgesic medication taken within 7 days before treatment, multiple teeth (to eliminate the possibility of pain referral), periodontal pocket >4 mm, and intraradicular posts were excluded from the study.

Subject allocation and randomization

A secondary investigator not involved in the trial assessed the eligibility of patients, based on their medical and dental history; and clinical and radiographic assessment for random allocation. To ensure randomization and allocation concealment, patients picked a computer generated, randomly sequenced, sealed opaque envelope with a unique five-digit code (containing group information). Patients were randomly allocated into groups (allocation ratio 5:1)-

- Group 1 – 2% CHX (Chlor X, Prevest DenPro Ltd., Jammu, India) with 5.25% NaOCl (CHXH) (Control Group)
- Group 2 – 2% Ozonated CHX (OCHX)
- Group 3 – 2% Ozonated CHX with 5.25% NaOCl (OCHXH)
- Group 4 – 0.1% OCT (Octenisept, Norderstedt, Germany) with 5.25% NaOCl (OCTH)
- Group 5 – 0.1% Ozonated OCT (OOCT)
- Group 6 – 0.1% Ozonated OCT with 5.25% NaOCl (OOCTH).

Only the secondary investigator was aware of the allocated group of patients and provided operator irrigants according to the group with opaque labels. However, complete blinding was impossible because of hypochlorite and ozone odor. Visual Analog Scale (VAS) scale was employed to assess pain at baseline, 6, 12, 24, 48 h, and 7 days. A sole operator with more than 15 years of experience performed retreatment of all the patients following standard and uniform treatment protocol. The group information was kept confidential during the data collection period to ensure the blinding of the patient and investigator. On study completion, a blinded assessor and statistician filled the master chart with the VAS scores of each patient and analyzed the data without identifying their actual group.

Non-surgical endodontic retreatment procedure

One hundred and eighty-seven patients requiring NSER were assessed based on eligibility criteria, of which 43 did not meet inclusion criteria, and 12 were unwilling to participate in the trial. Hundred and thirty-two patients were enrolled and randomly divided into six groups [Figure 1]. NSER followed contemporary standard protocol. Each patient was anesthetized using 2% Lidocaine with 80,000 adrenaline (Lignox, Indoco, Warren, Mumbai, India) followed by rubber dam isolation. Previous coronal restorations and GP were removed using rotary retreatment files (ProTaper Universal, Dentsply Maillefer), following manufacturer instructions. Hedstrom, K-files were used to retrieve apical GP with copious irrigation with a 27-G side vented needle (Densply. Tulsa, OK) (Group 1-CHX, Group 2 and 3-OCHX, Group 4-OCT, Group 5 and 6-OOCT) but without chemical solvents. CHXH, OCHXH, OCTH, and OOCTH groups were irrigated with 5.25% NaOCl after 10 ml saline irrigation to remove residues of NaOCl and prevent the formation of para-chloroaniline (with CHX in CHX, OCHX groups).[16,17] CHX and OCT groups were irrigated with 2% CHX and 0.1% OCT, respectively. Working length (WL) was determined using an apex locater (Root ZX mini, J Morita Corp, Tokyo, Japan). Root canal cleanliness (removal of GP) and WL were confirmed with intraoral periapical radiographs. This was followed by apical patency, glide path creation, and cleaning and shaping (crown-down technique). Apical preparation ranged from # 35 to # 60 based on the initial apical gauging. After completion of instrumentation, canals were irrigated with saline and dried with paper points to prevent adverse interaction between irrigants before final irrigation with CHX and OCT. Ozonated CHX, OCT was obtained by bubbling ozone in a gaseous state (4 mg/ml) in 2% CHX and 0.1% OCT for 15 min using an ozone generator (Dentozone, Analytical and medical tech. Mumbai, Maharashtra). Fifteen milliliters of ozonated CHX, OCT (based on the group) were delivered for 3–5 min, keeping the syringe tip two mm short of WL. All the irrigants were ultrasonically activated at the power setting three, with file ISO size #10 (Satelec Acteon Group, Merignac Cedex, France) following three cycles of 20 s each. Obturation and core build-up were completed in the same appointment, but no postoperative analgesics were advised to the patients. In case of severe pain, a backup phone number and a reserve analgesic ibuprofen; and if there was an emergency or if the pain did not respond to analgesics; reporting back was suggested. Patients who took the reserve medication within 7 days of treatment were excluded from the study.

Postendodontic pain assessment

The VAS scale used for pain assessment consisted of a 10-cm line anchored by two extremes with "no pain" at the start and "pain as bad as it could be" at the end. PP levels at baseline, after 6, 12, 24, 48 h, and 7 days post-treatment, were recorded by patients based on their pain perception. Patients were reminded to fill in the VAS scores at stated time intervals with reminder phone calls.

Follow-up and outcome assessments

Patients were recalled for follow-up after 1 week, for their clinical evaluation and pain diary collection. The recorded pain levels were tabulated and subjected to statistical analysis.

Statistical analysis

After data collection, it was coded and entered into Microsoft Excel 2019. Descriptive analysis was presented as mean and standard deviation. Due to the normal distribution of data, parametric tests were employed to compare the means. Two-way analysis of variance was used to compare mean differences among the groups and at various time intervals, followed by a *post hoc* Bonferroni test. For statistical analysis, Statistical Package for Social

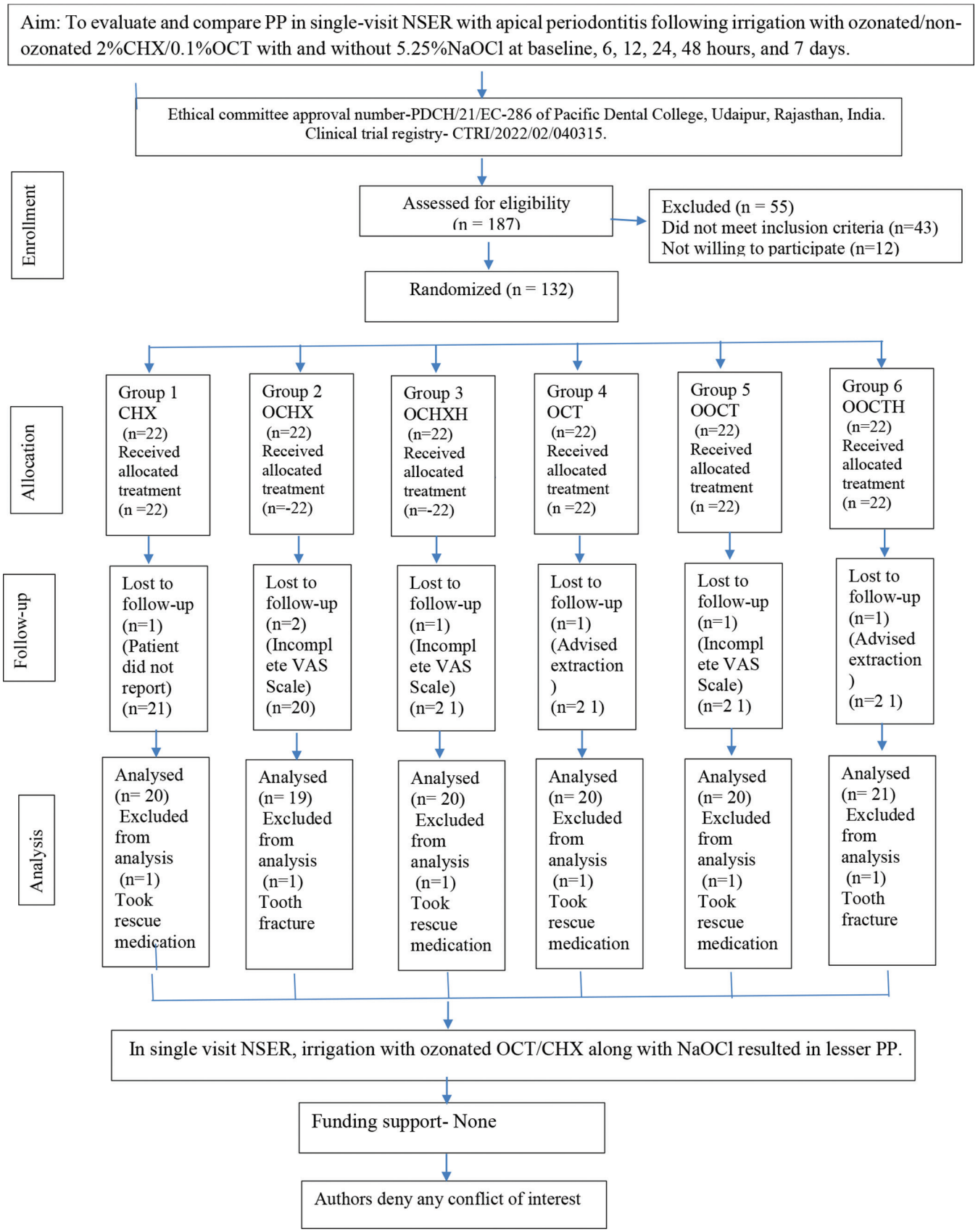


Figure 1: Preferred reporting items for a randomized trial in endodontic 2020 flow diagram

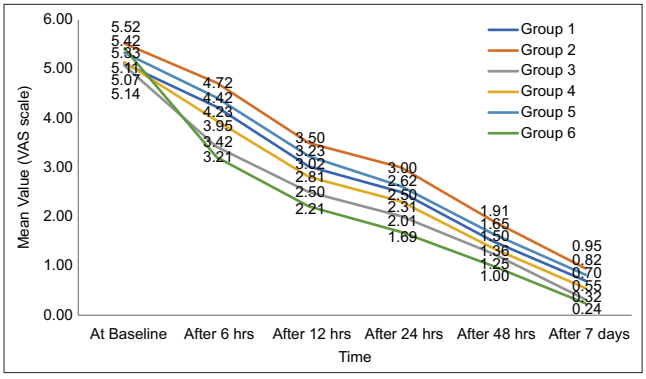
Sciences (SPSS version 22, IBM cooperation, Armonk, NY, USA) was used, with a 5% level of significance.

Logistic regression of demographic data was presented as a proportion [Tables 1 and 2] and compared using the Chi-square test. Relationship between predictors variables such as age groups, gender (male/female), tooth (incisors/canine/premolars), arch (maxillary/mandibular), pain during previous treatment (yes/no), duration of prior endodontic treatment (years >4 years), PAI score (>3), obturation length (over/under/adequate), obturation density (adequate/inadequate), sinus tract (present/absent), and swelling (present/absent) with the dependent variable of pain (VAS score 0–2 = no pain, 3–6 = pain) was analyzed by logistic regression model with an enter method. A crude odds ratio with a 95% CI of risk of pain, was calculated and was considered statistically significant if $P < 0.05$.

RESULTS

Hundred and thirty-two patients randomly assigned into six groups participated in the trial, of which there were twelve drop out, one from group six, three from group two, and two from groups one, three, four, and five each, due to complications during retreatment, use of analgesics, antibiotics, extraction, and incomplete forms, making the final sample size 120 [Figure 1]. The majority of patients requiring retreatment was females (52.5%), predominantly maxillary teeth (56.6%), and in the age group of 35–49 years (40%). Under-obturation 48% (58) and inadequate obturation density 75% (89) represented the primary cause of failure. Eight patients reported preoperative swelling (6.6%), and 18 had sinus tract (15%). None of the patient-related variables studied in the logistic regression showed any statistically significant role concerning PP [Table 1].

Baseline (preoperative) VAS scores of groups 1–6 were 5.11, 5.52, 5.07, 5.14, 5.33, and 5.42, respectively, showing homogeneity ($P > 0.05$) [Graph 1]. Intergroup comparison of PP indicated a significant decrease in VAS scores between baseline and at all time intervals in all the groups [Tables 2, 3 and Graph 1]. The most severe PP was recorded after 6 h in OCHX (4.72) followed by OOCT (4.42) and least with OOCTH (3.21) followed by OCHXH (3.42) group [Graph 1].



Graph 1: Mean score of time for different groups

Table 1: Logistic regression for predictors variables of pain on Visual Analog Scale

Variables	OR	95% CI	*P*
Age groups (years)			0.71
34–49	0.91	0.29–2.84	0.87
>49	1.38	0.37–5.09	0.63
Gender (female)	0.83	0.37–1.84	0.64
Tooth type (canine and premolars)	0.85	0.34–2.15	0.73
Arch (mandibular)	1.06	0.43–2.61	0.91
Previous treatment (<4 years)	1.44	0.49–4.29	0.51
Pain during previous treatment (yes)	0.79	3.43–1.81	0.57
Size of lesion (>5 mm)	2.12	0.65–6.92	0.21
PAI score (> 3)	0.83	0.26–2.66	0.75
Obturation length			0.95
Under	1.61	0.39–3.14	0.79
Adequate	1.28	0.26–6.29	0.76
Obturation density (inadequate)	1.02	0.34–2.84	0.96
Sinus tract (present)	1.02	0.27–3.67	0.97
Swelling (present)	1.62	0.29–9.06	0.58
Constant	0.88		0.88

P: Probability value (level of significance - 0.05). CI: Confidence interval. PAI: Periapical index. OR: Odds ratio

Table 2: Pairwise comparisons between groups by *post hoc* Bonferroni test

	Mean difference	*P*	95% CI
Groups			
Group 1 versus Group 2	−0.42	<0.001**	−0.60–−0.24
Group 1 versus Group 3	0.42	<0.001**	0.24–0.59
Group 1 versus Group 4	0.16	0.14	−0.02–0.34
Group 1 versus Group 5	−0.17	0.10	−0.34–0.01
Group 1 versus Group 6	0.55	<0.001**	0.37–0.73
Group 2 versus Group 3	0.84	<0.001**	0.66–1.02
Group 2 versus Group 4	0.58	<0.001**	0.40–0.76
Group 2 versus Group 5	0.26	<0.001**	0.08–0.43
Group 2 versus Group 6	0.98	<0.001**	0.79–1.54
Group 3 versus Group 4	−0.26	<0.001**	−0.44–−0.08
Group 3 versus Group 5	−0.58	<0.001**	−0.76–−0.40
Group 3 versus Group 6	0.14	0.37	−0.04–0.31
Group 4 versus Group 5	−0.32	<0.001**	−0.50–−0.14
Group 4 versus Group 6	0.40	<0.001**	0.22–0.57
Group 5 versus Group 6	0.72	<0.001**	0.54–0.89
Time interval			
Baseline versus after 6 h	1.27	<0.001**	1.09–1.45
Baseline versus after 12 h	2.38	<0.001**	2.20–2.56
Baseline versus after 24 h	2.91	<0.001**	2.73–3.08
Baseline versus after 48 h	3.82	<0.001**	3.64–3.99
Baseline versus after 7 days	4.67	<0.001**	4.49–4.85
After 6 h versus after 12 h	1.11	<0.001**	0.93–1.29
After 6 h versus after 24 h	1.64	<0.001**	1.46–1.81
After 6 h versus after 48 h	2.54	<0.001**	2.36–2.72
After 6 h versus after 7 days	3.40	<0.001**	3.21–3.58
After 12 h versus after 24 h	0.53	<0.001**	0.35–0.70
After 12 h versus after 48 h	1.43	<0.001**	1.25–1.61
After 12 h versus after 7 days	2.29	<0.001**	2.11–2.47
After 24 h versus after 48 h	0.91	<0.001**	0.73–1.08
After 24 h versus after 7 days	1.76	<0.001**	1.58–1.94
After 48 h versus after 7 days	0.85	<0.001**	0.68–1.03

**Highly significant. P: Probability value (level of significance - 0.05). CI: Confidence interval

A statistically significant reduction in VAS scores occurred in the OOCTH and OCHXH groups in comparison to other groups [Table 2]. The OOCTH group had marginally lower VAS scores than the OCHXH group in all periods, but there was no statistically significant ($P > 0.05$) difference between them [Table 2]. VAS scores for the groups were in the order OCHX > OOCT > CHXH > OCTH > OCHXH > OOCTH [Graph 1]. The OOCTH group demonstrated the lowest pain scores (5.42, 3.21, 2.21, 1.69, 1.00, 0.24) at 6, 12, 24, 48 h, and 7 days, respectively [Graph 1]. There were no reported side effects during the trial.

DISCUSSION

Successful NSER requires a complex analysis of root canal anatomy, microbiota, efficient procedural practice, and meticulous treatment protocol. SV NSER has become a more practical and predictable option with reported favorable periapical healing and a success rate of 81%–85%.[18,19] However, it can be more challenging due to an eight-fold more elevated (13.6%) incidence of PP.[20]

PP has a multifactorial etiology and a possible pathogenic association (higher occurrence - *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Prevotella intermedia*, deeper penetration of residual polymicrobial colonies) with acute periapical inflammation, secondary to mechanical (over-instrumentation), and chemical (incomplete canal disinfection, apical extrusion of irrigants) damage occurring during treatment.[21] To achieve efficacious irrigation and resolve PP, a combination of frequently used and novel irrigants (ozonated CHX, OCT) were investigated in this study.

We observed that PP was moderate during the first 6 h but dropped significantly within 24 h of treatment and continued to reduce to minimal levels in 7 days. Pak and White reported a similar pain reduction (40%–11%) from the first to the 7th day.[22] Pain within the first 24 h is typically related to apical instrumentation, discomfort due to extended mouth opening, and pre-existing, ongoing inflammatory processes. A gradual decrease in intensity with time is logical and expected as a natural healing course commences after thorough canal disinfection, reducing microbial load and inflammation.[22]

After 6 h, VAS scores of OCHX (4.72) were higher than CHXH (4.23), probably because of the limited efficacy of ozonated CHX in the absence of NaOCl. CHX's inability to dissolve vital and necrotic tissue, disrupt biofilm, and reduce activity in the presence of periapical inflammatory exudate (frequently present in therapy-resistant AP) seems to be the possible reason.[23] Okino *et al*. reported that CHX (solution and gel) showed no vital tissue dissolution after 6 h.[24] Another *ex vivo* study on apical dentine biofilms concluded that six percent NaOCl completely disrupted and removed biofilm, one percent NaOCl disrupted it but could not eliminate bacteria, whereas two percent CHX presented no disruption.[25] Various studies have suggested irrigation with both CHX and NaOCl with an intermediary irrigant between them to take advantage of the substantivity of the former and exceptional tissue dissolution of the latter.[23,26]

More notable pain in ozonated groups (OCHX, OOCT) could be due to reduced activity of ozone without NaOCl. Ozone acts by oxidation of microbial cellular components by forming reactive lipid oxidation compounds, ozonides. Oxygen radicals are dramatically toxic for microaerophiles and Gram-negative anaerobic bacteria predominantly present in primary endodontic infections. Contrarily, residual facultative Gram-positive bacteria in NSER can remain, adapt, and tolerate these conditions as they contain enzymes to secure them from oxygen toxicity, probably resulting in lower efficacy of ozone.[27] Estrela *et al*. detected no significant reduction in cell viability in biofilm incubated for 240 s with ozonated water which could be ascribed to ozone alone.[27] Limited antibacterial action of ozone on *Enterococcus faecalis* embedded in biofilms was observed by Hems *et al*. unless they were displaced into the surroundings by agitation.[28] Contrarily, in an *ex vivo* study by Nagayoshi *et al*., ozonated water demonstrated similar antimicrobial activity against *E. faecalis* and *Streptococcus mutans* as 2.5% NaOCl when combined with ultrasonication.[29] Noites *et al*. were among the first authors to report synergism of CHX with Ozone, resulting in the complete elimination of *E. faecalis* and *Candida albicans* by depolarization of cells in a dose-dependent manner. They proposed this combination use, particularly in posttreatment disease.[30]

After 6 h, OOCTH (3.21) showed lower pain scores than OCHXH (3.42), which could be due to the more potent

Table 3: Mean values of groups at different time intervals

Time interval	Groups					
	Group 1 (n=20)	Group 2 (n=20)	Group 3 (n=20)	Group 4 (n=20)	Group 5 (n=20)	Group 6 (n=20)
Baseline	5.11±0.56	5.52±0.61	5.07±0.41	5.14±0.45	5.33±0.57	5.42±0.52
After 6 h	4.23±0.57	4.72±0.65	3.42±0.62	3.95±0.55	4.42±0.65	3.21±0.53
After 12 h	3.02±0.57	3.50±0.63	2.50±0.48	2.81±0.45	3.23±0.57	2.21±0.51
After 24 h	2.50±0.41	3.00±0.61	2.01±0.47	2.31±0.36	2.62±0.47	1.69±0.48
After 48 h	1.50±0.20	1.91±0.53	1.25±0.27	1.36±0.36	1.65±0.36	1.00±0.29
After 7 days	0.70±0.20	0.95±0.30	0.32±0.17	0.55±0.15	0.82±0.32	0.24±0.20

Data presented as mean±SD. *n*: Number of subjects, SD: Standard deviation

disruption action of OCT on mature biofilms (99% of mature biofilm within 8 min) and endodontic pathogens.[11,12] The antiadhesive property of OCT effectively inhibits bacterial coaggregation critical for biofilm formation. OCT produces a pH-independent action, being stable in a broader pH range of (1.6–12.2) compared to CHX (5.5–7). OCT possesses a cation-active structure and readily binds to cardiolipins of bacterial cell walls, leading to cell death. Its other advantages include activity in the presence of serum protein, better penetration, and easy movement within the canal due to its lesser shear viscosity and surface tension.[31]

OCT has good tissue tolerability with minimal adverse effects because it binds to chondroitin sulfate, which reduces its cytotoxicity. While maintaining its antimicrobial efficacy, explaining the lowest pain scores of the OOCTH group.[32] As previously proposed, the supposition that OCT can be a substitute for NaOCl is unsupported by the results of the present trial, presumably due to its nontissue dissolving ability.[31]

In our study, CHX (CHXH-4.23, OCHXH-3.42) and OCT (OCTH-3.95, OOCTH-3.21) groups when ozonated showed lower VAS scores when used along with NaOCl. For maximum beneficial therapeutic effect, ozone should be applied at the end of cleaning and shaping when minimal organic debris remains in the canal, so in our study, ozonated irrigants were used as the final irrigant.[12] Favorable therapeutic effects shown by ozonation could be due to its antimicrobial, anti-inflammatory, analgesic, and noninvasive properties and the relative absence of discomfort. Unique properties of ozone include immunostimulation, bioenergetic, biosynthetic actions, angiogenesis stimulation, and high oxidizing power. Huth *et al*. ascribed its anti-inflammatory and immune-modulatory capacities to its inhibitory effects on the NF-kB system. Ozone more significantly enhances bone regeneration and tissue healing due to its deeper penetration through the apical foramen into the surrounding bone.[33] Its anti-hypoxic effect improves the metabolism of inflamed tissues and the synthesis of biologically active substances such as interleukins. This improves tissue oxygenation and nutrient supply in the area, thus promoting healing.[34]

The salient feature of the current study design includes the following:

First, symptomatic teeth were solely selected in the trial, as they are significant predictors of PP. Moreover, they are more challenging for clinicians and frequently require endodontic therapy.[35] Second, to prevent over-instrumentation, one of the chief causes of PP, both radiographic and electronic root canal measurements of WL were considered.[36] Third, sustained efforts were placed to achieve unbiased and comparable outcomes by eliminating all probable anticipated causes of pain at every stage of the study, with the difference being only in the irrigation protocol for precise evaluation of their efficacy. Fourth, limit the variations caused by (i) anatomical factors, only single-rooted teeth were included in the study, (ii) technical factors such as impact and velocity of filing motion and maintenance of apical patency, which could influence pain, a sole operator performed all the procedures.[37] Fifth, ultrasonic agitation further optimized root canal cleaning efficiency in the apical third, providing better penetration of irrigants.[38,39] Finally, to reduce the probability of errors while recording VAS scores patients were individually assisted during baseline pain scoring. Reminder phone calls were made at relevant times to improve patient compliance, making scoring more accurate.

Limitations

Owing to the *in vivo* nature of the study, even though standardization remained the intended goal, many inevitable factors such as specific dimensions of the root canal, complicated pain mechanism, local adaptation syndrome, microorganisms, host, and psychological factors may have affected the outcome. Some unavoidable biases were (i) blinding of the primary investigator could not be achieved due to the odor of NaOCl and ozone and (ii) the Unequal age and gender distribution of patients in the groups.

Future studies focusing on possible synergism observed in the present study and exploring the efficacy of the irrigant combination in varied clinical scenarios such as curved root canals and primary and regenerative endodontics are recommended. Further investigation regarding ozonated OCT, CHX mode of action, dose-dependent effectiveness, and optimal regimens is warranted.

CONCLUSION

The outcome indicates that OOCTH and OCHXH can provide effective pain control in single-visit NSER with AP. From the results of this study, it may be prudent to assume that the potent antimicrobial property of OCT coupled with high antimicrobial action and biosynthetic and bioenergetics properties of ozone makes the combination a potential option in regenerative endodontics, cases with an increased possibility of irrigant extrusion such as open apices, perforation, resorption, and flare up where pain control is critical. Furthermore, the addition of 5.25% NaOCl to ozonated CHX and OCT displayed a further reduction in PP scores at baseline, 6, 12, 24, 48 h, and 7 days in both the irrigants.

Hence, the proposed irrigation regimen can serve as a promising clinical alternative, fulfilling most of the requirements of an ideal irrigant and resolving some challenges of pain management faced by dental practitioners and specialists.

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Conflicts of interest
There are no conflicts of interest.

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