Original Article

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Dentistry Section

Effects of Ozonated Water and Sodium Hypochlorite as an Irrigant on Postoperative Pain in Patients with Symptomatic Apical Periodontitis: A Split Mouth Randomised Clinical Trial

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ABSTRACT

Introduction: Even after prompt administration of local anaesthesia during root canal/endodontic treatment procedures, postoperative pain resulting from improper irrigation techniques remains a major issue in endodontics. Nonetheless, serious efforts were made for incorporating effective irrigation protocols using conventional irrigation methods such as Sodium Hypochlorite (NaOCl) and aqueous ozone. Even though they have demonstrated strong bactericidal properties and numerous other advantages, the effects of these methods on patients with symptomatic apical periodontitis undergoing single-visit root canal therapy are not well understood.

Aim: To determine the effects of ozonated water and NaOCl as an irrigant on postoperative pain in patients with symptomatic apical periodontitis undergoing single-visit root canal therapy using the Visual Analogue Scale (VAS).

Materials and Methods: The present single-centre, prospective, double-blinded, parallel-group, equivalency, split-mouth, randomised clinical trial was conducted during the period of June 2022-May 2025 at Department of Conservative Dentistry and Endodontics, SDS, KVV, Karad, Maharashtra, India, on a total of 25 patients (n=25) and 50 teeth (n=50) that were randomly assigned to two groups by computer-generated Sequentially Numbered, Opaque Sealed Envelopes (SNOSE) method. Following the conventional procedure for biomechanical tooth preparation, one arch irrigated with 5.25% NaOCl was designated as "Group A" and opposite arch irrigated with ozone water and then subjected to 20 seconds of Ultrasonic Activation (UA) was designated as "Group B". To assess the precision of the root canal obturation, a periapical radiograph was obtained after the procedure. Finally, the level of postoperative discomfort was measured at 6 hour, 12 hour, 24 hour, 48 hour, 72 hour, and 7-day period using a VAS. Statistical Package for Social Sciences {Statistical Package for Social Sciences (SPSS), Version 21.0} was used to analyse the data. The Kolmogorov-Smirnov test and Pearson's chi-square test were used to compare means and analyse categorical data.

Results: A mean pain score of 3.64 (SD±0.95), 2.68 (SD±0.90), 1.44 (SD±0.58), 0.76 (SD±0.52), 0.56 (SD±0.51) and 0.00 (SD±0) for Group A and 4.12 (SD±0.97), 3.08 (SD±0.81), 1.68 (SD±0.69), 0.72 (SD±0.54), 0.52 (SD±0.51) and 0.00 (SD±0) for Group B was obtained at 6 hr, 12 hr, 24 hr, 48 hr, 72 hr and 7-day period respectively. Group B showed a slightly higher mean pain score than Group A but the difference was non-significant (p>0.05).

Conclusion: Intracanal irrigation with 5.25% NaOCl or aqueous ozone, ultrasonically triggered, reduced postoperative discomfort during a 7-day period. However, there was no significant difference in pain reduction between the two groups.

Keywords: Endodontic treatment, Endodontic irrigant, Irrigation techniques, Ozone therapy

INTRODUCTION

In endodontics, managing postoperative pain, particularly following root canal therapy, is a challenging and daunting patient-centered approach faced by majority of clinicians in their day-to-day practice [1]. The subjective nature of pain and its relatable physiological/psychological factors further complicate its management, making the implementation of a standard technique for postoperative pain management difficult [2-4]. Therefore, comprehensive cleaning/irrigation techniques with effective irrigation methods and efficient endodontic irrigants are crucial for the overall success of root canal therapy [5].

NaOCl is the most widely used and gold standard endodontic irrigant due to its broad-spectrum antimicrobial and antibiofilm properties. Acting as a strong base organic solvent with a pH over 11, it is used in concentration between 0.5% and 6%. It breaks down amino acids and forms chloramine and effectively neutralises bacteria by interfering with Deoxyribonucleic Acid (DNA) synthesis and bacterial oxidative phosphorylation [6,7]. Evidence based scientific study conducted by Gomes BP et al., (2001), Fidalgo TK et al., (2010) and Neelakantan P et al., (2019) have demonstrated beneficial effects of 2.5%-5.25% of NaOCl solution for effectively reducing *Enterococcus faecalis* (*E. faecalis*), *Candida albicans* (*C. albicans*) and *Staphylococcus aureus* (*S. aureus*) [8-10]. Despite these benefits, it also imparted various disadvantages, further restricting its use, thereby demanding for investigating substitute or complementary irrigants [6,11].

Recently, ozone therapy discovered by Dutch physicist Martin Van Vrun in 1785 is used for both medical and dental purposes in the form of gas, water and gel solutions. Derived from the Greek word "ozein," which means odour, it has anti-bactericidal qualities, minimal cytotoxicity and accelerated healing potential, ozone is now gaining attention as a multipurpose bio-oxidative therapy in dentistry [12-14]. Hems RS et al., (2005), Huth KC et al., (2005), Huth KC et al., (2009) demonstrated that ozone therapy was effective against planktonic *E. faecalis* biofilm and was beneficial in reversing caries process in high-risk patients [15-17]. Comparative metanalytical studies conducted by Nogales CG et al., (2016), Nagayoshi M et al., (2004), Estrela C et al., (2007), Case PD et al., (2012), Hubbezoglu I et al., (2014),

Zan R et al., (2016), Boch T et al., (2016), and Pinheiro SL et al., (2018) reported that ozone in gaseous or aqueous form, when combined with ultrasonic agitation at various concentrations could effectively impart antibiofilm and antimicrobial activity with minimal or zero cytotoxic effect [13,14,18-23]. Furthermore, Cardoso MG et al., (2008) reported that ozone's instability in aqueous form, limited biofilm activity, poor tubule penetration, and lack of residual effect, inability to neutralise endotoxins against *E. faecalis*, *Streptococcus mutans (S. mutans)* and *Candida albicans* raises questions about its ability as a potent endodontic irrigant [24]. It may serve as an effective alternative irrigation solution in clinical endodontic treatments. These controversial findings further raised a curiosity on ozone's impact on post-operative pain management. Additionally, the threshold of pain and post obturation flare up, after Multiple Visit Endodontics (MVE-RCT) Single Visit Endodontics- Root Canal Treatment (SVE-RCT) and Single Visit Non-surgical Endodontics (SVN-RCT) demonstrated varied results in apical periodontitis [25]. Hence, the precise role of ozone therapy in management of post-operative pain in patients with apical periodontitis undergoing SVE-RCT needs to be evaluated.

Based on the identification of this research gap, the aim of the present study was to assess postoperative pain in patients receiving SVE following apical periodontitis treated with aqueous ozone and NaOCl at six hour, 12 hour, 24 hour, 48 hour, 72 hour as well as after seven days. The null hypothesis was set stating that "There is no difference in pain following SVE treatment with ozonated water and 5.25% NaOCl at various time period in apical periodontitis patients".

MATERIALS AND METHODS

The present single-centre, prospective, double-blinded, parallel-group, equivalency, split-mouth, randomised clinical trial was conducted during the period of June 2022-May 2025 at Department of Conservative Dentistry and Endodontics, SDS, KVV, Karad, Maharashtra, India. The study was initiated after due approval from the Institutional Ethical Committee bearing the IEC clearance No: KIMSDU/IEC/06/2023. CTRI registration CTRI/2024/09/074489 was done (https://ctri.nic.in/Clinicaltrials/pubview2.php). The present study was carried out as per the "World Medical Association Declaration of Helsinki (Carlson, Boyd, and Webb, 2004) and the PRIRATE 2020 principles" [26].

Inclusion and Exclusion criteria: The inclusion criteria included individuals with symptomatic apical periodontitis on bilateral posterior teeth, aged between 18-65 years, good general health available for follow-up and readiness to participate in the present study. The exclusion criteria included individuals with periapical lesions, abscess, cellulitis, complicating systemic diseases, history of allergic reactions and mobile teeth.

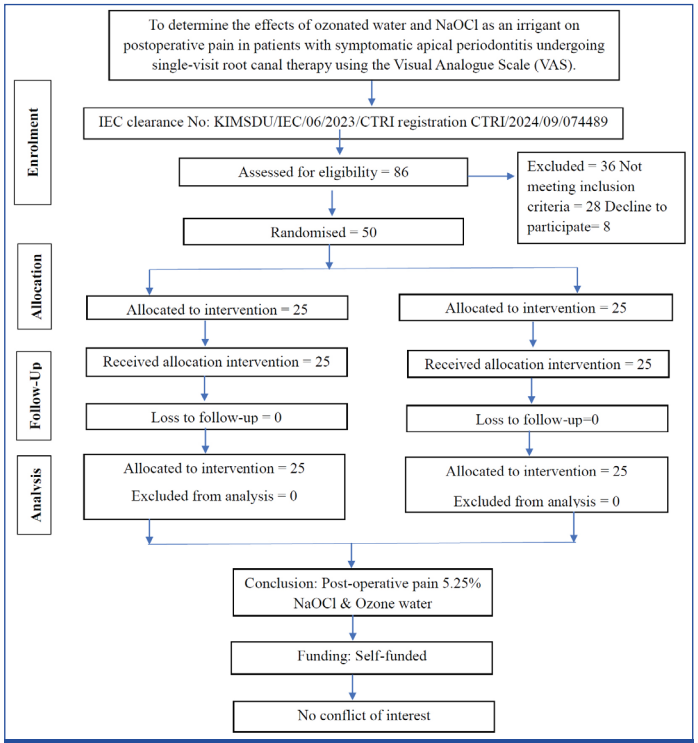
Sample size calculation: A study conducted by Pinheiro SL et al., (2018) that evaluated the antimicrobial activity of 2.5% NaOCl, 2% chlorhexidine (CHX), and ozonated water (O_3) on biofilms of *E. faecalis*, *S. mutans*, and *C. albicans* in mesiobuccal root canals of severely curved 60 mandibular molars (n=60) was considered as a reference/parent article for choosing the appropriate sample size [23]. G*Power version 3.0.1 (Franz Faul universitat, Kiel, Germany) was used to create a power analysis. The split-mouth study's total anticipated sample size of 23 participants and 46 teeth was rounded to 25 individuals and 50 teeth (25 in Group A and 25 in Group B), which would give 80% power to detect significant changes at a significance level of 0.05 and an effect size of 0.85.

Thus, a total of 25 patients (n=25) and 50 teeth (n=50) were recruited for the study and randomised into two groups. Group A (n=25) was irrigated with 5.25% NaOCl and Group B (n=25) was irrigated with ozone water and then subjected to 20 seconds of UA.

Study Procedure

Demographic profile and detailed anamnesis were documented. The vitality status of the tooth that needed treatment was assessed. All patients were given thorough information regarding the present study, prior to its initiation, and any queries related to the current study were answered. Verbal informed consent was taken from all the study participants. Because we used a split-mouth trial design, each patient could serve as their own control. This method works especially well at reducing the impact of factors like age, gender, and other patient characteristics that could otherwise skew the study's findings.

Randomisation and allocation concealment: The endodontist began the root canal treatment by opening SNOSE that the staff nurse had filled with the randomised order of instrumentation techniques based on the created sequence of random numbers. The same certified operator, an endodontist, performed every step of this study. [Table/Fig-1] describes the randomisation, group assignment, and analysis in accordance with the Preferred Reporting Items for Randomised Trials in Endodontics (PRIRATE) 2020.



[Table/Fig-1]: Standards for reporting randomized studies in endodontics (PRIRATE 2020).

Biomechanical tooth preparation for SVE: Before the anaesthetic was given, a skin test was performed to rule out any allergic conditions by injecting 1:10 dilution of 2% LA (Xicaine, ICPA health products, Mumbai) on the formarm. This was subsequently followed by injecting a mixture of 2% lignocaine and 1:100,000 epinephrine and isolating the tooth with a rubber dam (Neelkanth Healthcare Pvt., Ltd.,). All the steps as per the principles for biomechanical tooth preparation for root canal were judciously followed. The working length was estimated by keeping the instrument 0.5 mm short of the radiography apex. After determining the working length, a hand file was used to create the first glide route. For the preparation and instrumentation sequence, each group adhered to the manufacturer's instructions. It was set up to rotate 30° clockwise and 150° counterclockwise using the wave one gold file system (Dentsply Sirona, IN). Every time an instrument was changed, debris was removed using gauze soaked in alcohol.

Preparation of ozonated water: Ozonated water was prepared using the Medical Grade Ozone Generator (Waterhouse, IN). The medical oxygen cylinder and the machine's inlet nozzle were connected by a rubber pipe. The machine's outlet nozzle was connected by another pipe which emitted the transformed oxygen as ozone gas. As directed by the manufacturer, this output pipe was submerged in a bowl of distilled water that had been autoclaved

for seven minutes. A 35 µg/mL concentration of ozone gas was set-up to be released by the ozone generating unit. After seven minutes of flow, this gas saturated the distilled water. Since ozone has a short shelf life, it was prepared prior to biomechanical tooth preparation and was used within immediately by filling a sterile single-use syringe [27].

Irrigations treatment with 5.25% NaOCl and Ozonated water: One arch was irrigated with 20 milliliters of 5.25% NaOCl per canal during the treatment was designated as "Group A" while the other arch irrigated with ozone water was designated as "Group B". Each canal was then ultrasonically activated for 20 seconds using the Ultra-X Tip-Silver/20/2/21MM. Ethylenediaminetetraacetic Acid (EDTA), ozone water, and 5.25% NaOCl were deliberately excluded as flushing agents in order to assess the relative efficacy of each irrigant.

Obturation of root canals: After drying the canals with paper points, the teeth were obturated using a gutta-percha master cone and a resin-based sealer (AH Plus, Dentsply, Konstanz, Germany) applying the single cone heated vertical compaction method. The teeth were later sealed and filled with a temporary restorative material. Patient was recalled within seven days and a periapical radiograph was taken following the procedure to evaluate the accuracy of the root canal obturation.

Assessment of postoperative pain outcome: Patients were guided on using VAS (MeDoc, Ltd.,) to measure their postoperative discomfort. The operational definition set for this study was "the level of postoperative discomfort measured using a VAS at six, twelve, twenty-four, forty-eight, seventy-two hours, and seven days". After the postoperative questionnaire, patients applied VAS to evaluate their level of discomfort. Mild pain (1-4), moderate pain (5-6), severe pain (7–10), and no pain (0) were the four categories of pain intensity (primary outcome) that were identified and applied [28]. Postoperative assessment was done on telephonic conversation during follow-up.

Postoperative follow-up protocol: A 400 mg of Ibuprofen (one tablet every six hours) was prescribed. If in case of severe discomfort/emergency, patients were instructed to visit the department or the treating physician.

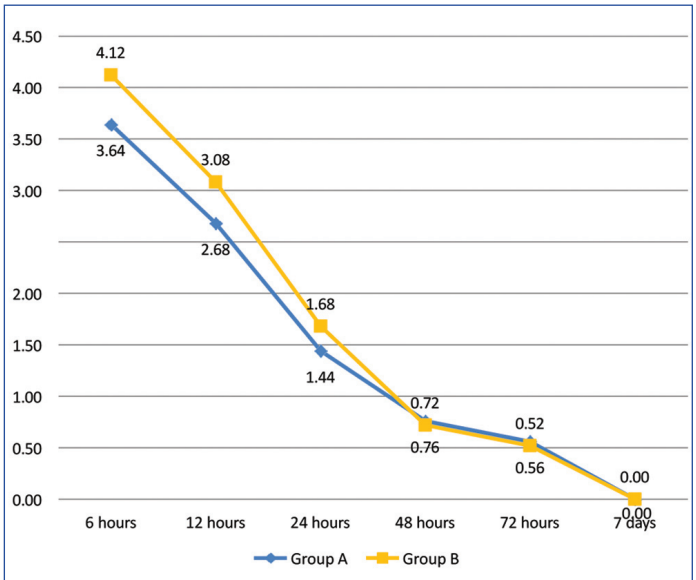
STATISTICAL ANALYSIS

Data was tabulated in MS Excel worksheet (Microsoft, USA) and subjected to IBM's SPSS, Version 21.0, Armonk, NY: IBM Corp. software. With 95% confidence intervals, the mean and Standard Deviation (SD) was used to describe continuous data, whereas frequencies and percentages were used to illustrate discrete data. The data was verified to have a regular distribution using the Kolmogorov-Smirnov test. A paired t-test was used for means, and Pearson's chi-square test was used for categorical data.

RESULTS

Among all the study participants, the age range was 25-38 years with the mean of 27.32±6.15. A 72% of the study cases (n=18) were females and 28% (n=7) were males. Regarding the distribution of type of teeth {as per Fédération Dentaire Internationale (FDI) classification} among both the groups, Group A involved 28% (n=7) permanent mandibular left first molar followed by 20% cases (n=5) in permanent mandibular right first molar, 20% cases (n=5) in permanent maxillary left first molar, 12% cases (n=3) in permanent maxillary right first molar, 8% cases (n=2) in permanent maxillary left second premolar, 8% cases (n=2) in permanent maxillary right first premolar and 4% cases (n=1) in permanent maxillary right second premolar tooth. Group B involved 24% of cases (n=6) in permanent maxillary left first molar, 20% cases (n=5) in permanent mandibular right first molar, 20% cases (n=5) in permanent mandibular left first molar, 20% of cases (n=5) in permanent maxillary left first premolar, 8% of cases (n=2) in permanent maxillary right first premolar and 8% of cases (n=2) in permanent maxillary left second premolar. The distribution of teeth did not provide evidence of any group differences that was statistically significant (p=0.998). The preoperative pain levels were also assessed for both the groups and majority of the cases reported level 7 to level 10 indicating severe pain.

Comparison of mean VAS scores between Group A and Group B at 6 hours: Group B had a marginally higher mean score of 4.12 with an SD of 0.97 than Group A, which had a mean score of 3.64 with an SD of 0.95. The comparison's p-value of 0.084 showed that there was no statistically significant difference between the two groups [Table/Fig-2,3].



[Table/Fig-2]: Comparison of mean VAS scores between two groups at various intervals.

Study group	Time interval	Mean	SD	p-value
Group A	6 h	3.64	0.95	0.084
Group B		4.12	0.97	
Group A	12 h	2.68	0.90	0.106
Group B		3.08	0.81	
Group A	24 h	1.44	0.58	0.190
Group B		1.68	0.69	
Group A	48 h	0.76	0.52	0.792
Group B		0.72	0.54	
Group A	72 h	0.56	0.51	0.782
Group B		0.52	0.51	
Group A	7 days	0	0	Cannot be computed
Group B		0	0	

[Table/Fig-3]: Depicts the mean VAS scores for Group A and Group B at various time intervals.

Comparison of mean VAS scores between Group A and Group B at 12 hours: Group A had a mean VAS score of 2.68 with a SD of 0.90, whereas Group B had a mean score of 3.08 with an SD of 0.81. The comparison's p-value of 0.106 showed that there was no statistically significant difference between the two groups [Table/Fig-2,3].

Comparison of mean VAS scores between Group A and Group B at 24 hours: Group B had a mean score of 1.68 with an SD of 0.69, while Group A had a mean score of 1.44 with an SD of 0.58. There was no statistically significant difference between the groups, as indicated by the p-value of 0.190 [Table/Fig-2,3].

Comparison of mean VAS scores between Group A and Group B at 48 hours: Group B had a mean score of 0.72 with an SD of 0.54 while Group A had a mean score of 0.76 with an SD of 0.52 at 48 hours. The p-value was 0.792, indicating that there was no statistically significant variation [Table/Fig-2,3].

Comparison of mean VAS scores between Group A and Group B at 72 hours: Group B had a mean score of 0.52 with an SD of 0.51 at 72 hours, while Group A had a mean score of 0.56 with an SD of 0.51. There was no discernible difference between the two groups, according to the p-value of 0.782 [Table/Fig-2,3].

Comparison of mean VAS scores between Group A and Group B at seven Days: After seven days, the mean VAS score for both Group A and Group B was 0.

DISCUSSION

In the current study, the postoperative pain in patients receiving SVE following apical periodontitis treated with 5.25% NaOCl and ozonated water at different time intervals (6 hour, 12 hour, 24 hour, 48 hour, 72 hour as well as after seven days) were evaluated and compared. The inclusion criteria included multirooted teeth as they are bestowed with complex anatomical features such fins, isthmuses, bifurcations, trifurcations, and auxiliary canals, thereby making it more challenging to irrigate and obturate than the single rooted teeth. Additionally, they typically have greater levels of postoperative discomfort following root canal surgery [4]. Therefore, to avoid any potential bias, the current study investigated postoperative pain using two different irrigants in subjects with symptomatic apical periodontitis affected in posterior teeth with multiple roots having almost similar root morphologies.

The split-mouth method was applied by irrigating with 5.25% NaOCl and ozonated water to different areas within the same patient, thereby ensuring that inter-patient differences would not affect comparisons of postoperative pain between treatments. This methodological rigor lessened the influence of external influences and increased the reliability of the current study findings.

The study results documented that the postoperative pain as measured qualitatively using VAS scores peaked after six hours and then progressively decreased at 12, 24, 48, 72 hours, respectively for both the groups. Hence, there was no discernible difference between the groups after seven days interval supporting the null hypothesis. These findings were consistent with a study conducted by Sinha N et al., (2024) who found that the use of 5.25% NaOCl, ozonised CHX, and ozonised octenidine dihydrochloride gradually reduced post-therapy discomfort [29]. Both groups reported no pain after seven days, which is in alignment with the systematic review conducted by Pak JG and White SN (2011) who found that the prevalence of postoperative pain following root canal therapy decreased significantly within the first 48 hours and then decreased to 10% or less after the first week [30]. The hypothetical reason for early pain after six hours as observed in this study could be affirmated to pre-existing inflammatory conditions like pulpitis or apical periodontitis. Moreover, a slight procedural irritation might also have momentarily exacerbated discomfort in these situations since inflammation is already developed as a result of infection or tissue injury.

In the present study, higher concentration of NaOCl (5.25%) and aqueous ozone was utilised demonstrating beneficial effects on post operative pain reduction. The results were in accordance to study conducted by Huth KC et al., (2009) who found that bacterial load and postoperative pain were significantly reduced at higher concentration of NaOCl and aqueous ozone (5 and 20 µg/mL) [17]. Since, there is a strong correlation of postoperative pain with bacterial pathogens such as *E. faecalis*, *C. albicans*, *Peptostreptococcus micros*, and *Pseudomonas aeruginosa* in the root canal system, decreased bacterial load results reduced frequency of postoperative pain. Supporting these evidences, Gomes BP et al., (2001), Fidalgo TK et al., (2010), Neelakantan P et al., (2019), Zan R et al., (2016), demonstrated reduced endotoxins levels of bacterial pathogens using NaOCl at higher concentrations against multispecies endodontic pathogens [8-10,21]. Pinheiro SL et al., (2018) discovered similar results with 2.5% NaOCl and ozonated water [23]. The present study reported decrease in inflammatory component with both the irrigants might have resulted in gradual reduction in postoperative pain (VAS Scores) especially after seven day interval, natural healing trajectory and tissue repair mechanism in the affected area.

In the present study, ultrasonic irrigation was applied rather than conventional syringe irrigation. An in-vitro investigation conducted on human root canals polluted with *E. faecalis* by Hubbezoglu I et al., (2014) inferred that the total bacterial eradication was accomplished, when 5.25% NaOCl and 16 ppm aqueous ozone were activated by ultrasonic application [20]. These findings were in alignment with the present study wherein 5.25% NaOCl or aqueous ozone, ultrasonically triggered, for intra-canal irrigation did not significantly change postoperative discomfort during a 7-day period. The present finding suggests that the UA of both the irrigants was comparable, as was their effect on tissue healing and inflammation following surgery.

In case of ozone therapy, few studies conducted by Bocci VA (2006), Nogales CG et al., (2016), Nagayoshi M et al., (2004), Hems RS et al., (2005), Case PD et al., (2012) Boch T et al., (2016), and Rojas-Valencia MN et al., (2011) [12-15,19,22,31], inferred high proficiency of ozone therapy, individually or in combination with other irrigants, against pathogenic and non-pathogenic bacteria in root canal, thereby supporting the present study. However, in contrast, Estrela C et al., (2007) assessed the antibacterial effectiveness of aqueous ozone and NaOCl in root canals infected with *E. faecalis* and discovered that aqueous ozone was unable to completely eradicate *E. faecalis* after 20 minutes [18]. However, all the available data in the present study appear to recommend ozone therapy as an effective irrigant for treatment of apical periodontitis in RCT. The positive influence of ozone therapy on post operative pain reduction with potential tissue healing phenomena is encouraging and beneficial. It could be hypothesized that the generation of Reactive Oxygen Species (ROS) and Lipid Oxidation Products (LOPs) with ozone therapy could function as messengers to stimulate neovascularisation and reduce the postoperative pain. Moreover, the release of growth factors and immune cell activation are amongst the other the biological processes that underpin ozone's effectiveness [12].

Overall, the present study data presented herein demonstrates that both groups reduced postoperative pain (VAS) at different time intervals with comparable differences. However, there was no significant difference in pain reduction between the two groups. The currrent study finding significantly proved the efficacy of ozone therapy as a root canal irrigant with better outcomes after one week period postoperatively, thus proving the hypothesis that it can be used in root canal therapy effectively.

Limitation(s)

The present study has certain drawbacks. Despite the current study's goal of uniformity, a number of inevitable factors could have affected the results because of its in vivo design. These include intrinsic differences in root canal size, intricate pain mechanisms, reactions to local adaptation, and microbial interactions. Additionally, because of the unique smell of NaOCl, it was not possible to blind the principal investigator.

CONCLUSION(S)

The findings suggest reduced postoperative pain with in patients with symptomatic apical periodontitis undergoing single-visit root canal therapy was observed when ozonated water and NaOCl were used as irrigants. However, there was no significant difference in pain reduction between the two groups. Both the irrigants were successful in decreasing the postoperative pain during a 7-day period endodontic procedure. Further research should focus on

evaluating these irrigants in diverse clinical scenarios. Investigating optimal treatment protocols and long-term outcomes would enhance the understanding and application of these agents in endodontics.

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