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Major clinical findings on the safety and effectiveness of ozone therapy in endodontic treatment: a concise systematic review

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Abstract

Introduction: In the scenario of endodontic diseases, microorganisms, and their byproducts are the main causes of pulpal and periradicular diseases. Sodium hypochlorite (NaOCl) is the most commonly used root canal irrigant. Ozone (O_3) therapy has been investigated to reduce or eliminate the microbiota load within the root canal system, improving endodontic results. **Objective:** It was to highlight the main clinical findings of the safety and effectiveness of ozone therapy in endodontic treatment. **Methods:** The PRISMA Platform systematic review rules were followed. The search was carried out from July to September 2023 in the Scopus, PubMed, Science Direct, Scielo, and Google Scholar databases. The quality of the studies was based on the GRADE instrument and the risk of bias was analyzed according to the Cochrane instrument. **Results and Conclusion:** A total of 87 articles were found, and 23 articles were evaluated in full and 14 were included and developed in the present systematic review study. Considering the Cochrane tool for risk of bias, the overall assessment resulted in 18 studies with a high risk of bias and 21 studies that did not meet GRADE and AMSTAR-2. Most studies did not show homogeneity in their results, with X^2=47.7%<50%. It was concluded that ozone has been accumulating scientific evidence regarding its success in endodontic treatment, more specifically in the decontamination of root canals and activation of regenerative processes. Ozone therapy is a minimally invasive treatment method, without discomfort or pain. It also minimizes the patient's anxiety and stress level, as it reduces the duration of treatment. The beneficial role of ozone in treating different oral and dental conditions is still limited. There are possible side effects during intraoral application, as it can reach the upper respiratory tract.

Keywords: Ozone therapy. Endodontic treatment. Root canal. Infection.

Introduction

In the scenario of endodontic diseases, microorganisms, and their byproducts are the main causes of pulpal and periradicular diseases [1]. The mechanical removal of infected dentin favors the penetration of irrigants through the canals, enhancing the decontamination process. However, a significant percentage of the root canal surface remains intact, regardless of the instruments used [2].

In this sense, sodium hypochlorite (NaOCl) is the most commonly used root canal irrigant, as it has effective antimicrobial activity, a broad bacterial range, and a significant reduction in endotoxin levels. However, several studies have demonstrated that complete bacterial elimination cannot be achieved with any of the current disinfection protocols [3].

Given this, ozone therapy has been investigated to reduce or eliminate the microbiota load within the root canal system, improving endodontic results. Ozone is a natural gas and a very strong and selective oxidant. The therapy is based on the assumption that ozone (O_3) rapidly dissociates into water and releases a reactive substance in the form of oxygen that can oxidize cells, thus having antimicrobial efficacy without inducing drug resistance. Firstly, O_3 acts on glycolipids, glycoproteins, or certain amino acids, which are present in the cytoplasmic membrane of microorganisms [4].

Following these principles, the use of ozone therapy was tested as an alternative agent to NaOCl and as a complementary source of disinfection in chemomechanical canal preparation. Ozone acts as a strong antioxidant, leading to a disinfectant effect, and disrupting the cell membrane of the microorganism. Ozone leaves no toxic byproducts. Due to ozone's capacity, cells are not damaged and the action remains nonspecific and selective to microbial cells. All vital functions of bacteria are interrupted after a few seconds of ozone application. Gram-positive bacteria are more sensitive to ozone compared to gram-negative organisms. Ozone causes disturbances in bacterial cells, leading to the removal of acidogenic bacteria that commonly cause tooth decay [5,6].

Immunocompetent cell proliferation and immunoglobulin synthesis are stimulated by the influence of ozone on the cellular and humoral immune system. The function of macrophages is activated due to the increased sensitivity of microorganisms to phagocytosis, which leads to the production of cytokines as a consequence, other cells of the immune system are activated [7].

Furthermore, ozone results in changes in cell metabolism, increasing the partial pressure of oxygen in tissues, which improves the oxygen transport capacity in the blood. Ozone, when administered multiple times in low doses, activates enzymes such as dehydrogenase, superoxide dismutases, glutathione peroxidases, and catalases. Also, ozone allows the activation of protein synthesis in cells. Helps increase ribosomes and mitochondria that cause tissue regeneration, increasing functional activity. Ozone stimulates the production of vasodilators, such as nitric oxide, which cause dilation of arteries and veins. Nitrous oxide is used as anesthesia, improving wound healing [1-3].

Added to this, interleukins, prostaglandins, and leukotrienes are the proteins synthesized by ozone that help in cell growth and differentiation, reducing inflammation and wound healing. The lipid and protein oxidation process generates a quantitative conversion of the olefinic bonds present to reactive species (ozonide) of lipid oxidation products [3,4]. Ozonides signal and trigger metabolic changes that produce microbicidal effects [7].

Also, the action of ozone immunomodulates the patient's immune system, thus improving the body's response to the etiological agent. About all these attributions, it is understood that ozone thus has great potential to be inserted in endodontic therapy, as it requires and incorporates the two requirements necessary for any substance for endodontic use, such as antimicrobial action par excellence and biocompatibility [3,4].

Therefore, the present study aimed, through a systematic review, to highlight the main clinical findings of the safety and effectiveness of ozone therapy in endodontic treatment.

Methods

Study Design

The present study followed the international systematic review model, following the rules of PRISMA (preferred reporting items for systematic reviews and meta-analysis). Available at: http://www.prisma-statement.org/?AspxAutoDetectCookieSupport=1. Accessed on: 08/14/2023. The methodological quality standards of AMSTAR-2 (Assessing the methodological quality of systematic reviews) were also followed. Available at: https://amstar.ca/. Accessed on: 08/14/2023.

Data Sources and Research Strategy

The literary search process was carried out from July to September 2023 and was developed based on Scopus, PubMed, Lilacs, Ebsco, Scielo, and Google Scholar, covering scientific articles from various eras to the present. The descriptors (MeSH Terms) were used: *"Ozone therapy. Endodontic treatment. Root canal. Infection"*, and using the Boolean "and" between the MeSH terms and "or" between historical discoveries.

Study Quality and Risk of Bias

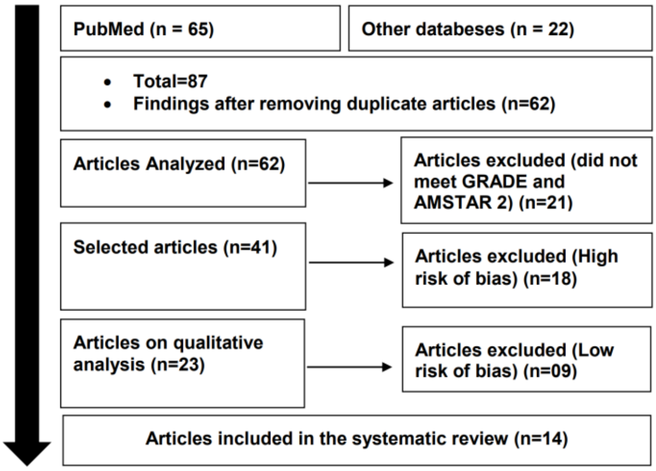
Quality was classified as high, moderate, low, or very low in terms of risk of bias, clarity of comparisons, precision, and consistency of analyses. The most evident emphasis was on systematic review articles or meta-analyses of randomized clinical trials, followed by randomized clinical trials. The low quality of evidence was attributed to case reports, editorials, and brief communications, according to the GRADE instrument. The risk of bias was analyzed according to the Cochrane instrument by analyzing the Funnel Plot graph (Sample size versus Effect size), using the Cohen test (d).

Results and Discussion

Summary of Findings

A total of 87 articles were found that were subjected to eligibility analysis, with 14 final studies being selected to compose the results of this systematic review. The studies listed were of medium to high quality (Figure 1), considering the level of scientific evidence of studies such as meta-analysis, consensus, randomized clinical, prospective, and observational. The biases did not compromise the scientific basis of the studies. According to the GRADE instrument, most studies showed homogeneity in their results, with X^2=47.7%<50%. Considering the Cochrane tool for risk of bias, the overall assessment resulted in 18 studies with a high risk of bias and 21 studies that did not meet GRADE and AMSTAR-2.

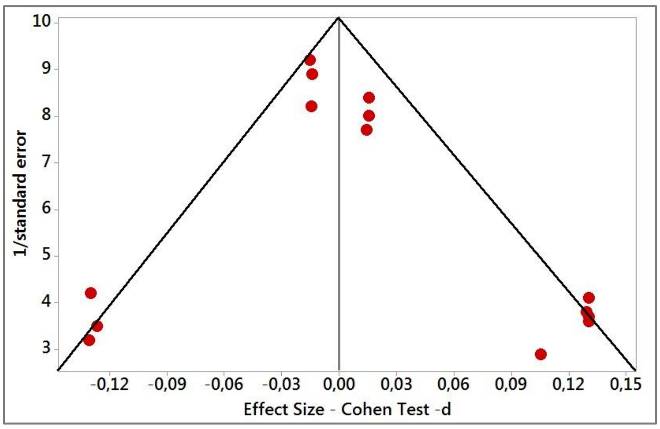
Figure 1. Article selection - exclusion process.



Source: Own authorship.

Figure 2 presents the results of the risk of bias of the studies using the Funnel Plot, showing the calculation of the Effect Size (Magnitude of the difference) using the Cohen Test (d). Precision (sample size) was determined indirectly by the inverse of the standard error (1/Standard Error). This graph had a symmetrical behavior, not suggesting a significant risk of bias, both between studies with a small sample size (lower precision) that are shown at the bottom of the graph and in studies with a large sample size that are presented at the top.

Figure 2. The symmetric funnel plot suggests no risk of bias among the small sample size studies that are shown at the bottom of the graph. High confidence and high recommendation studies are shown above the graph (n=14 studies).



Source: Own authorship.

Highlights Outcomes

Based on literary findings, a randomized clinical study compared the effect of different ozone application techniques on the prevalence of post-endodontic pain in patients undergoing endodontic treatment in a single session. A total of 108 patients with necrotic pulp in single-rooted teeth and apical periodontitis participated in this study. A standard single-visit endodontic protocol was followed with 5.25% sodium hypochlorite and nickel-titanium rotary files. After shaping and cleaning, patients were randomly allocated into the following groups: group 1 (n = 21), ozone treatment without activation, group 2 (n = 22), ozone treatment with manual dynamic activation, group 3, (n = 21), ozone treatment with passive ultrasonic activation, group 4 (n = 23), ozone treatment with sonic activation and group 5 (n = 21), without ozone treatment (control group). Ultrasonic and sonic ozone activation resulted in less pain in patients undergoing single-session endodontics compared to no ozone treatment [8].

Furthermore, a recent systematic review study carried out by the authors Meire et al. (2023) analyzed the available evidence on the effectiveness of adjuvant therapy for the treatment of apical periodontitis (AP) in permanent teeth, according to a formulated population, intervention, comparison, outcome, time and study design structure a priori by the European Society of Endodontology. A total of 14 studies (13 RCTs and one retrospective cohort) met the inclusion criteria for this review. They evaluated different types of adjuvant therapy: antimicrobial photodynamic therapy (aPDT; three studies), diode laser channel irradiation (3), Nd: YAG laser channel irradiation (2), Er; Cr laser channel irradiation: YSGG (1), ozone therapy (2), and ultrasound-activated irrigation (UAI) (4). Radiographic healing was reported in seven studies, but meta-analysis was only possible for UAI (two studies), showing no statistically significant difference in healing after 12 months. Pain after 7 days was reported in seven studies. The meta-analysis of three studies that used aPDT and two studies that used diode laser irradiation showed no significant difference in the prevalence of pain after 7 days between control and adjuvant therapy. According to the RoB2 tool, six studies had a high risk of bias, five studies had some concerns, and two studies had a low risk of bias. The GRADE assessment revealed a very low strength of evidence for the diode laser and a low strength of evidence for PDT, ozone, and UAI studies [9].

Added to this, a systematic review study developed by the author's Silva et al. (2020) proposed asking about the reduction in the load of microorganisms in patients undergoing root canal treatment, and whether the use of ozone therapy is comparable to conventional chemical-mechanical techniques that use sodium hypochlorite (NaOCl). The results demonstrated that ozone therapy provides significantly less microbial load reduction than NaOCl. As an adjuvant in chemical-mechanical preparation, ozone was ineffective in increasing the antimicrobial effect of NaOCl. Ozone performance was strongly associated with the application protocol used, being dependent on dose, time, and bacterial strain, in addition to the correlation with the use of complementary disinfection sources. Concerning reducing the load of microorganisms in patients undergoing endodontic treatment, ozone is not indicated either to replace or to complement the antimicrobial action of NaOCl [10].

A study carried out by authors Almadi et al. (2021) analyzed post-space disinfection using different irrigants and its effect on root dentin adhesion. A total of 40 single-rooted lower second premolars were collected and disinfected. All samples were decorated maintaining a root length of 12 mm and embedded vertically in acrylic resin. The post space was irrigated with 1% NaOCl before being randomly allocated into four groups (n=10) according to different canal disinfection methods. Samples from group 1 were subjected to photoactivated disinfection (PAD), group 2 was irrigated with etanol-based propolis, group 3 was disinfected with ozone, and group 4 was irrigated with 2.25% NaOCl with 17% EDTA. The highest push-out bond strengths (PBS) at all three levels were found in group 4, canal disinfected with 2.5% NaOCl with 17% EDTA and ECYL. While the lowest PBS was observed in group 1. In the intergroup comparison, the prosthetic space disinfected with propolis extract showed no significant difference concerning the canal in group 4 disinfected with 2.5% NaOCl with 17% EDTA and ECYL in all three levels ($p>0.05$). Intragroup comparison across all experimental groups showed no significant difference in the coronal and middle thirds of group 1, group 2, and group 3, respectively ($p>0.05$). 2.5% NaOCl with EDTA and ECL treatment (group 4) remains the gold standard when used as the final canal irrigant. Propolis can be used as a potential irritant for root canal disinfection [11].

Authors Sağlam and Aladağ (2023) compared the effect of ozone and low-level laser therapy (LLLT) on postoperative pain after root canal treatment in symptomatic apical periodontitis in vital teeth. A total of 80 patients were divided into four groups using a web program as follows: LLLT placebo (simulation of laser therapy), LLLT, Ozone placebo (simulation of irrigation with ozonized water), and Ozone. Postoperative pain levels for 7 days after treatment and percussion pain levels on the 7th day were recorded on the visual analog scale. A regression analysis demonstrated that the most effective variables are "group" and "jaw" ($p=0.01$). Pain in the lower jaw is higher than in the upper jaw. There was a difference between the groups regarding postoperative pain on days 1, 2, and 3; however, there was no significant difference on other days. The LLLT and ozone groups had less postoperative pain and percussion pain [12].

Furthermore, a study analyzed whether irrigation with sodium hypochlorite, chlorhexidine, and ozone gas, alone or in combination, was effective against *Enterococcus faecalis* and *Candida albicans*. A total of 220 recently extracted unimodal teeth were inoculated with *Candida albicans* and *Enterococcus faecalis*. The formulations tested were 1, 3, and 5% sodium hypochlorite, 0.2% and 2% chlorhexidine, and ozone gas applied for different periods. The combination of 5% sodium hypochlorite and 2% chlorhexidine, with gaseous ozone, was also evaluated. Sodium hypochlorite, chlorhexidine, and gaseous ozone alone were ineffective in eliminating microorganisms. The association of 2% chlorhexidine followed by ozone gas for 24 seconds promoted the complete elimination of *Candida albicans* and *Enterococcus faecalis* [13].

Finally, a randomized study looked at the effectiveness of the ozone or NaOCl/Chlorhexidine disinfection protocol compared to root canal treatment of apical periodontitis. A total of 60 permanent teeth were randomly allocated. Ozone gas (32 g m-3) or NaOCl (3%) was applied, followed by a 1-week interval dressing (Ca $(OH)_2$). There were no significant differences between the success rates between the ozone and NaOCl groups after 6/12 months. The most commonly found bacterial genera were *Streptococcus spp., Parvimonas spp.* and *Prevotella spp.* Therefore, the ozone gas and NaOCl/chlorhexidine gluconate protocols used here showed no difference in bacterial reduction in the sampled areas of the root canals [14].

Limitations

Important biases and limitations were found that can be pointed out as the variability between studies regarding methodologies, such as ozone application protocol and NaOCl concentrations, as well as the small sample size (Figure 2). Also, failure in the equivalence of parameters between control and experimental groups, and limited sample size with the absence of sample calculation. The authors also reported non-equivalent results regarding the effectiveness of argon for endodontic treatment.

Conclusion

It was concluded that ozone has been accumulating scientific evidence regarding its success in endodontic treatment, more specifically in the decontamination of root canals and activation of regenerative processes. Ozone therapy is a minimally invasive treatment method, without discomfort or pain. It also minimizes the patient's anxiety and stress level, as it reduces the duration of treatment. The beneficial role of ozone in treating different oral and dental conditions is still limited. There are possible side effects during intraoral application, as it can reach the upper respiratory tract.

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Not applicable.

Ethical Approval

Not applicable.

Informed consent

Not applicable.

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Data sharing statement

No additional data are available.

Conflict of interest

The authors declare no conflict of interest.

Similarity check

It was applied by Ithenticate@.

Peer Review Process

It was performed.

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