

Effectiveness of Ozone Therapy in Root Canal Disinfection: Systematic Review

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Received: October 13, 2020; **Published:** November 28, 2020

Abstract

Objectives: Root canal infections are considered one of the most common dental disorders. The infection is considered very difficult to treat as the microorganisms are away from immune cells and microorganisms. The treatment depends mainly on the mechano-chemical treatment and irrigation of infected roots with disinfectants. The microorganisms should be completely eradicated for the full cure of the infected roots. Recent protocols considered new disinfectants other than sodium hypochlorite for the complete eradication of the microorganisms. Ozone therapy is widely considered however with conflicting results.

Methods: Six databases were searched using specific search terms. We included studies that assess the efficacy of ozone therapy against root canal infections. The studies were assessed for the quality of evidence-based on the type of study before being included for the review.

Results: Ten studies fulfilled our inclusion criteria and had passed the quality assessment to be included for the qualitative evidence synthesis. Three studies assess both liquid and gaseous ozone while the remaining studies assessed the efficacy of gaseous ozone. Most studies compared the efficacy of the ozone to other disinfectants like sodium hypochlorite and ultrasonication.

Conclusion: Ozone therapy did not show any better results than sodium hypochlorite, but it showed complete eradication of bacterial infection when it is combined with other disinfectants.

Keywords: Root Canal Disinfectant; Root Canal Therapy; Ozone; Endodontic Treatment; Endodontics; Sodium Hypochlorite

Introduction

The root canal infection is considered as one of the most deleterious diseases affecting not only the teeth but also the peri radicular tissues [1]. Root canal infection leads to an increase the occurrence of the peri radicular inflammatory diseases [1,2]. The microbial infection

of the root canal is considered complex and difficult to treat. The microorganisms are usually located in the most hidden positions within the root canal [1,2]. These areas usually contain necrotic pulp tissues that create the perfect anaerobic conditions for microbial proliferation, and these areas usually are away from the immune cells like phagocytes and plasma cells as well as their products like antibodies and complement. Other positions include the apical part of the root canal which is separated from the peri radicular tissues using epithelial plugs or accumulation of polymorphonuclear neutrophils so the peri radicular tissues may not be infected [3]. Another rare condition is when the microorganism is transmitted to infect the peri radicular tissues from the infected root canal [3]. As in all diseases, the balance between the host defense mechanism and the virulence of the microorganism is the determining factor for the outcome and site of root canal infection [1].

The root canal infection is either primary infection caused directly by microorganisms infesting the necrotic pulp tissues [4]. The infecting microorganisms are usually mixed but predominant by anaerobic bacteria like *Fusobacterium*, *Treponema*, *Peptostreptococcus*, *Bacteroides*, *Porphyromonas* and *Prevotella*. There was also evidence of facultative bacteria like streptococci [4]. Usually, the root canal infection is asymptomatic, nevertheless, some infections were symptomatic and were associated with acute apical periodontitis and acute periradicular abscess. Mostly, gram-negative anaerobic organisms are the ones associated with the symptomatic lesions [5]. However, this was negated as it depends on the virulence of the microorganisms and the other organisms accompanying the infection which may act in synergy or antagonism. The other type of infection is the secondary root canal infection which is usually introduced after the primary infection treatment and the microorganisms are usually introduced through the treatment [4,5].

Failure to treat the root canal infection will lead to persistent root canal infection which may be a result of either primary or secondary infection [6]. In this type of root canal infection, the predominant microorganisms are Gram-positive bacteria. Fungi are also reported to be present in higher concentrations than both primary and secondary infection [7].

The treatment of the root canal infections is considered hard as the microorganisms reside in a strategic sanctuary where the necrotic pulp has not any blood supply which hinders the presence of the immune cells and any systemically administered antibiotics [8]. That is why the treatment requires professional intervention. The treatment includes three main steps: the chemo-mechanical preparation, the intracanal medication, and the root canal obturation [8].

The first step is considered the most important step as it allows the physicians access to the root canal and necrotic tissues allowing the complete removal of the microorganisms. The mechanical factors alone did not effectively eradicate the whole bacterial colonies that is why it is combined with chemical disinfectants [8]. Stewart, *et al.* reported a negative culture in more than 70% of the root infected canal after using both mechanical and antibacterial irrigation [9,10]. Since World War I, Sodium hypochlorite is used as an irritant and it has a tissue-dissolving ability and broad-spectrum antimicrobial activity [11]. It can kill spore-forming bacteria, fungi, protozoa, and bacterial spores [12-14]. The antimicrobial effect is largely dependent on the concentration of the solution; a study found that 4 percent Sodium hypochlorite solution had eliminated more than half of *E. Faecalis* infection in the teeth. However, many studies found that there was no complete bacterial elimination urging the addition of other disinfectants as passive ultrasonic irrigation, photodynamic therapy, and ozone therapy [9,15].

Ozone therapy is a natural gas that is considered a powerful oxidant [16]. It dissociates in water into a highly reactive form of oxygen that oxidizes cells. The merit of ozone therapy that its mechanism of action does not allow any drug resistance [17,18]. It acts through oxidation of cellular proteins and unsaturated fatty acids producing toxic oxidative products. These reactive species named ozonide cause metabolic changes that cause cytotoxic microbicide effect [18,19].

Ozone therapy is used as either an alternative agent to the Sodium hypochlorite or as a complementary agent to it. However, there is still no proof of its efficacy as some studies support its antibacterial action [20,21] while others refuted its efficacy and suggested that Sodium hypochlorite had done better [16,17,22].

That is why, in this review, we try to stand on the real efficacy of ozone therapy and factors affecting its efficacy. In addition, we also did a comparison between the efficacy of Sodium hypochlorite and ozone therapy to understand whether it can be used as a standalone therapy or as adjuvant therapy.

Methods

Database search

A comprehensive search approach was used to identify research studies from PubMed, Google Scholar, SCOPUS, and ISI web of science, clinicaltrial.gov, and Cochrane collaboration. The keywords used were ('Periapical abscess' OR 'Periapical lesion' OR 'Root Canal Obturation' OR 'Dental Pulp Necrosis') AND ('Dental Pulp Devitalization' OR 'Endodontic' OR 'Ozone' OR 'Microbial Consortia' OR 'Disinfection' OR 'Bacteria' OR 'Polymerase chain reaction' OR 'Culture' OR 'Microb*' and 'Microorganism*'). We restricted our search to human studies. All types of study designs were included.

Inclusion and exclusion criteria for screening

Specific inclusion criteria were used to identify high quality and studies that fulfill the goals of this study. Papers were only included if studies discussed the efficacy of ozone therapy or compared between ozone therapy and other therapies. Experimental studies, animal studies, books, review articles, letters to the editor, editorial reports, case reports, and conference abstracts and duplicates were excluded.

Screening for studies

The retrieved studies from each database were screened based on inclusion and exclusion criteria. First, Title/abstract screening was conducted by three independent reviewers. The included studies were then screened thoroughly to make sure it fulfills the target of this review. Each study was reviewed thoroughly to extract and build a qualitative review.

Quality assessment of the included papers

Two types of studies were included in this review. For *in vitro* studies, a quality assessment was used in previous systematic reviews [23,24]. It included the following domains: (i) sample size calculation, (ii) samples with similar dimensions, (iii) control group, (iv) standardization of procedures, (v) statistical analysis, and (vi) other risks of bias. Each parameter for all included studies was judged as 'low', 'high', or 'unclear' risk of bias.

For clinical trials, the quality of included studies was evaluated by three reviewers using "The Cochrane Collaboration's tool for assessing the risk of bias" [25]. It has seven specific domains including sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective outcome reporting, and other sources of bias [25]. The answers were categorized as 'low risk,' 'high risk,' or 'unclear risk' of bias.

Domains	Yes	No	Other (CD, NR, NA)
1. Was the research question or objective in this paper clearly stated?			
2. Was the study population clearly specified and defined?			
3. Was the participation rate of eligible persons at least 50%?			
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?			
5. Was a sample size justification, power description, or variance and effect estimates provided?			
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?			
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?			
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?			

9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?			
10. Was the exposure(s) assessed more than once over time?			
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?			
12. Were the outcome assessors blinded to the exposure status of participants?			
13. Was loss to follow-up after baseline 20% or less?			

Results and Discussion

Search results

The search performed on six databases yielded 843 studies, of which, only ten studies fulfilled the inclusion criteria and were used for qualitative evidence synthesis figure 1.

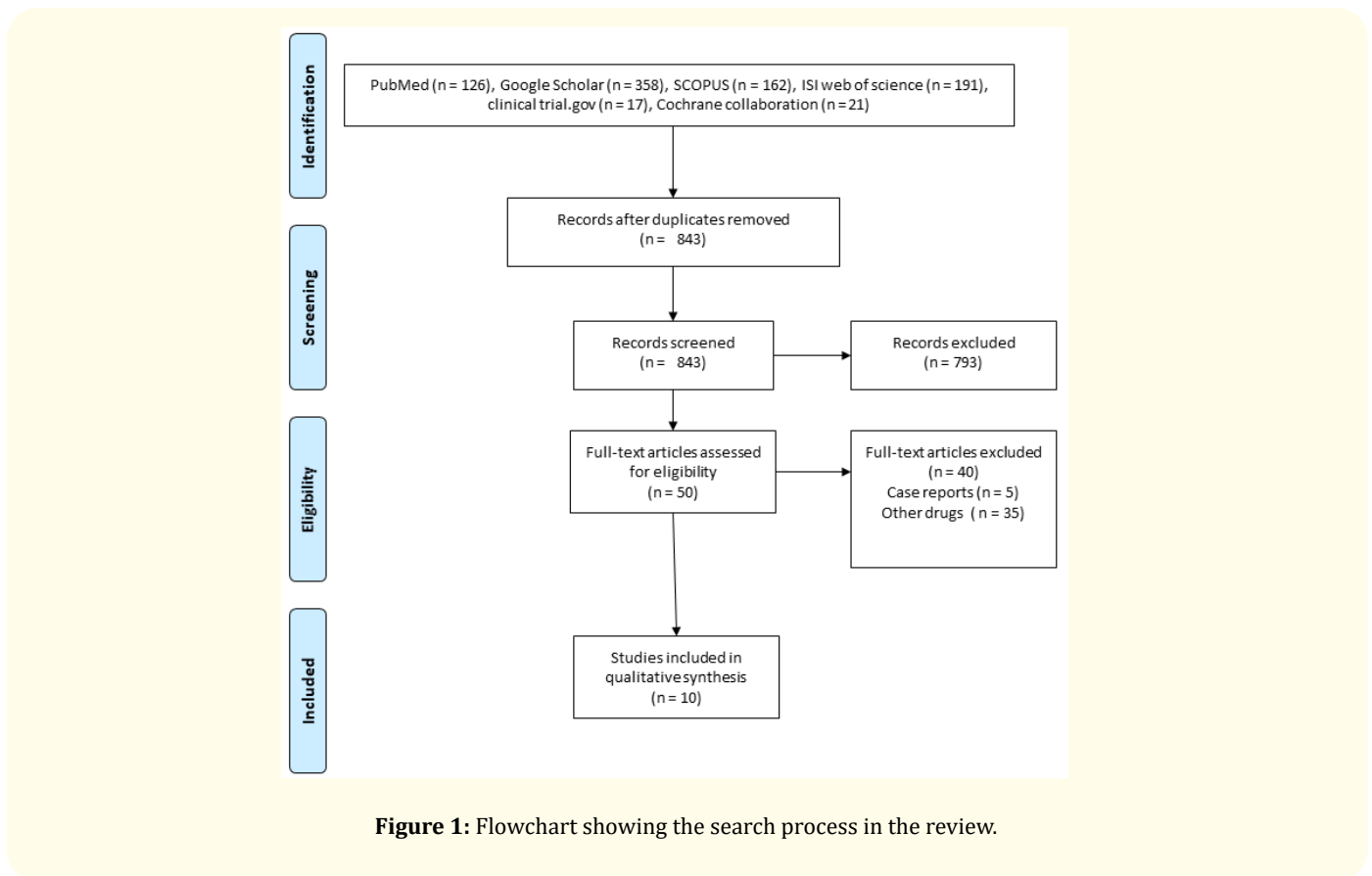


Figure 1: Flowchart showing the search process in the review.

Risk of bias

All clinical trials have a low risk of bias in figure 1; however, for laboratory studies, five studies had a high risk of bias figure 2. Other studies had a high risk in specific domains in figure 3.

Study	Risk of bias						Overall
	D1	D2	D3	D4	D5	D6	
Boch/2016	+	+	+	+	+	+	+
Case/2012	X	+	+	+	+	+	X
Hubbezoglu/2014	+	+	+	X	+	X	X
Huth/2009	X	X	X	+	+	+	+
Kaya/2014	+	X	X	X	+	+	X
Kunik/2017	+	X	X	+	+	+	X
Nagayoshi/2014	+	+	+	X	+	+	X
Noites/2014	-	X	+	+	+	+	+

D1: Sample size calculation
 D2: Samples with similar dimensions
 D3: Control group
 D4: Standardization of procedures
 D5: Statistical analysis carried out
 D6: Other risk of bias

Judgement
 X High
 - Unclear
 + Low

Figure 2: Quality assessment results of the in vitro studies.

Study characteristics

Two studies were clinical trials performed on human patients [26,27] while eight studies were experimental *in vitro*. Four studies assessed the efficacy of ozone therapy against *Enterococcus faecalis* infection [16,17,20,22]. Studies compared the efficacy of ozone therapy against the EDTA, Sodium hypochlorite, saline, H₂O₂ and Chlorhexidine digluconate (Table 1). The concentration of ozone used were 8, 12, 16 ppm. Most studies used gaseous ozone except for three studies; one study used both ozonated water and the other two used aqueous ozone [20,21].

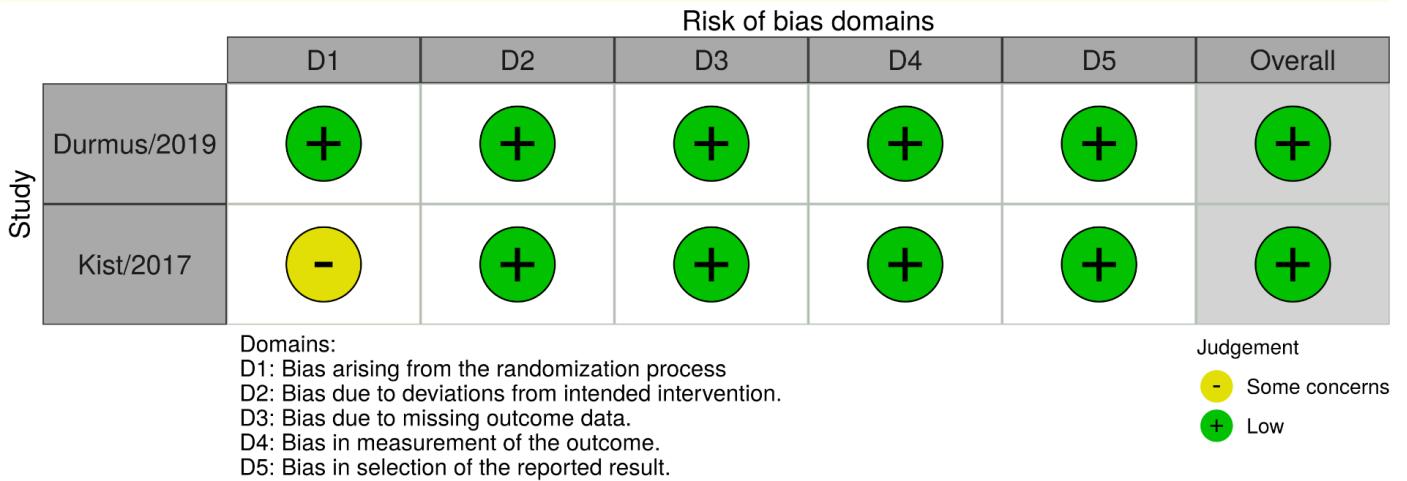


Figure 3: Quality assessment results of Cochrane risk of bias tool for randomized controlled trials in this review.

ID	Country	Type of study	Tooth type	Microorganism	Tooth number	Case	Control
Boch/2016 [16]	Germany	In vitro	Anterior teeth and premolars	<i>Enterococcus faecalis</i>	25 for each group	Ozone (n = 25) 20% EDTA-ozone (n = 25) 3% NaOCl-ozone (n = 25)	20% EDTA (n = 25) 3% NaOCl (n = 25)
Case/2012 [17]	Australia	In vitro	Single-rooted anterior teeth	<i>Enterococcus faecalis</i>	14 for each group	Ozone (n = 14) Ozone-sPUI (n = 14)	1% NaOCl (n = 14) Saline (n = 14) Saline-PUI (n = 14)
Durmus/2019 [26]	Turkey	RCT	First molar teeth	Overall bacterial load	33 for each group	Without disinfectant (n = 33)	Chlorhexidine digluconate (n = 33) Ozone (n = 33)
Hubbezoğlu/2014 [20]	Turkey	In vitro	Single-rooted mandibular premolar teeth	<i>Enterococcus faecalis</i>	10	5.25% NaOCl (n = 10) 5.25% NaOCl-PUI (n = 10)	Ozone [3, 5, 1, 2, 4, 6] (n = 10) Ozone 8 ppm-PUI (n = 10) Ozone 12 ppm (n = 10) Ozone 12 ppm-PUI (n = 10) Ozone 16 ppm (n = 10) Ozone 16 ppm-PUI (n = 10)
Huth/2009 [21]	Germany	In vitro	Single-rooted teeth	<i>Enterococcus faecalis</i> , <i>Candida albicans</i> , <i>Peptostreptococcus micros</i> , <i>Pseudomonas aeruginosa</i>	14 for each group	5.25% NaOCl (n = 14) 2.25% NaOCl (n = 14) 2% CHX (n = 14) 3% H ₂ O ₂ (n = 14) PBS (n = 14)	Ozone gas (n = 14) Ozone water (n = 14)

Kaya/2014 [22]	Turkey	<i>In vitro</i>	Mandibular premolars with straight root canals	<i>Enterococcus faecalis</i>	12 for each group	2.5% NaOCl (n = 12) Saline (n = 6) Low-temperature atmospheric pressure plasma (n = 12)	Ozone (n = 12)
Kist/2017 [27]	Germany	RCT	All groups of teeth except for maxillary molars	Overall bacterial load	20 for each group	3% NaOCl (n = 20)	Ozone (n = 21)
Kunik/2017 [29]	Bosnia and Herzegovina	Case control	Maxillary and mandibular molars and premolars	Overall bacterial load	24 for each group	Chlorohexidine (n = 24)	Ozone (n = 24)
Nagayoshi/2014 [31]	Japan	<i>In vitro</i>	Bovine incisors	<i>Enterococcus faecalis</i> and <i>Streptococcus mutans</i>	20 for each group	2.5% NaOCl (n = 20) Distilled water (n = 20) Distilled water-PUI (n = 20)	Ozone (n = 20) Ozone-PUI (n = 20)
Noites/2014 [30]	Portugal	<i>In vitro</i>	Single-rooted teeth	<i>Enterococcus faecalis</i> and <i>Candida albicans</i>	20 for each group	1% NaOCl (n = 20) 3% NaOCl (n = 20) Saline (n = 20) 5% NaOCl (n = 20) 0.2% CHX (n = 20) 2% CHX (n = 20)	Ozone 24 s (n = 20) Ozone 60 s (n = 20) Ozone 120 s (n = 20) Ozone 180 s (n = 20) Ozone 180 s- 5% NaOCl (n = 20) Ozone 24 s- 2% CHX (n = 20)

Table 1: Characteristics table of the included studies.

ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Laukkanen/2019 [36]	Yes	Yes	Yes	No	No	No	Yes	No	Yes	Yes	Yes	No	NA	No
Arya/2017 [34]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NA	Yes
Ng/2011 [38]	Yes	Yes	Yes	No	No	No	Yes	No	Yes	Yes	Yes	No	NA	No
Wang/2011 [39]	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	No	NA	No
Mindiola/2006 [37]	Yes	Yes	Yes	No	No	No	Yes	No	Yes	Yes	Yes	No	NA	No
Fouad/2003 [35]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NA	Yes

1. Was the research question or objective in this paper clearly stated?
2. Was the study population clearly specified and defined?
3. Was the participation rate of eligible persons at least 50%?
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?
5. Was a sample size justification, power description, or variance and effect estimates provided?

6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?
10. Was the exposure(s) assessed more than once over time?
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?
12. Were the outcome assessors blinded to the exposure status of participants?
13. Was loss to follow-up after baseline 20% or less?
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?

Table 2: The NIH quality assessment results.

Study	Country	Type of study	Mean age	Diabetic patients (N)	Type of teeth	Outcome
Laukkanen/2019 [36]	Finland	Prospective cohort for three years	51.5	41	Molar and non-molar	Outcome was recorded as follows: Healthy: Healthy periapical tissues (PAI score 1-2). Healing: Apical radiolucency considerably smaller in follow-up radiograph than in preoperative radiograph. No healing: Periapical radiolucency remained the same (PAI score 3-5). Teeth extracted for endodontic reasons (persisting apical infection, fistula) or for reasons not recorded in documents available after root filling. Teeth receiving periapical surgery. Periapical radiolucency not completely disappeared after 4 years. Deteriorated: Periapical radiolucency enlarged or a new periapical radiolucency emerged (PAI score 3-5)
Arya/2017 [34]	India	Prospective cohort for one year	42	21	Molar	The change in apical bone density as determined by the periapical index
Ng/2011 [38]	United Kingdom	Prospective cohort for four years	NA	22	All teeth	Tooth survival
Wang/2011 [39]	Taiwan	Prospective cohort for two years	48.6	4358	All teeth	Tooth extraction
Mindiola/2006 [37]	United States	Retrospective cohort for ten years	Range (35-44)	24	All teeth	The retention of endodontically treated teeth
Fouad/2003 [35]	United States	Cross-sectional study	Younger than 40, 40 to 60, older than 60	242	All teeth	Predefined outcome categories

Table 3: Patients characteristics in the included studies.

Antimicrobial efficacy of ozone therapy

Bach., *et al.* compared the efficacy of Ozone therapy to Sodium hypochlorite irrigation against *Enterococcus faecalis* [16]. It was revealed that Ozone therapy was ineffective against *Enterococcus faecalis* compared to Sodium hypochlorite irrigation which eradicated 99.9% of *Enterococcus faecalis* compared to 85.4% in teeth treated with Ozone. The study also compared the influence of combining both Ozone with Sodium hypochlorite and found that it had the same efficacy as Sodium hypochlorite alone, notwithstanding, the dentin chips from the teeth treated with combination therapy had a lower count of bacterial colonies [16]. The study suggested that the ozone penetrated the dental tissues and tubules allowing better diffusion of Sodium hypochlorite increasing the antibacterial effect of Sodium hypochlorite. In addition, they combined Ozone and EDTA but there was no increase in the efficacy of Ozone compared to its combination with Sodium hypochlorite [16]. The study suggested that the Sodium hypochlorite allowed better disintegration of the biofilm increasing the penetration and the efficacy of Ozone. The study had a lot of limitations as it is done *in vitro* and was conducted on a single root tooth. In clinical settings, the biofilm formation takes time than the *in vitro* studies more than 72 hours [16].

Case., *et al.* investigated the efficacy of the ozone gas against biofilms of resistant species like *Enterococcus faecalis*. The study compared the efficacy of ozone gas to other disinfectants like 1% sodium hypochlorite for 120 seconds and saline with passive ultrasonic activation and the ozone followed by ultrasonication [17]. In this study, they found that the ozone combined with ultrasonic agitation had better bacterial clearance than ozone alone or ultrasonic irrigation alone. However, 1% of sodium hypochlorite had shown greater efficacy than both of them and their combination together. However, the study found that sodium hypochlorite had a weaker penetration force and even with a high exposure time of 2 minutes, it still lacked the ability to penetrate deeper into the tissues which induce the application of ultrasonic energy combined with ozone in the endodontic treatment [17]. This combination allowed the deeper penetration and bacterial biofilm layer disruption causing a better efficacy eradicating the microbial infection. The study recommended the use of the ozone as an adjuvant treatment to the endodontic disinfectants to allow better penetration [17].

Another study found that the efficacy of Ozone therapy depended on the type of microorganisms, dose, and time of Ozone applications. For instance, ozone had higher efficacy than chlorohexidine for eradicating Streptococcus mutants while the chlorhexidine showed higher and better outcomes against other bacteria [28].

Another study also assessed the efficacy of ozone therapy on the pulp vascular superoxide dismutase, pulp vascular endothelial growth factor (VEGF), neuronal nitric oxide synthase (nNOS). The study compared gaseous ozone to the 2% chlorhexidine; the evaluation of its efficacy on bacterial infection depended on the polymerase chain reaction to detect total bacteria count and *Lactobacillus* species [29]. In this study, Ozone had significantly decreased total bacteria by 68% compared to chlorhexidine that decreased the total bacteria by 34.5%. Ozone therapy had significantly decreased the concentration of the superoxide dismutase by 6% which is lower than the control pulp. For the vascular endothelial growth factor, it was increased by 24% while the neural nitric oxide synthase was increased by 57% indicating that the ozone had the ability to diffuse through the dental tissues increasing its efficacy [29].

Periapical lesion resistant to endodontic treatment

Noites., *et al.* tested the ozone therapy against *Enterococcus faecalis* infection of the periapical lesions which were resistant to treatment. The study used 220 extracted single-rooted teeth. The study compared the efficacy of ozone therapy to sodium hypochlorite with different concentrations of 1%, 3%, 5%. It was also compared to 0.2% or 2% of Chlorhexidine solution. For gaseous ozone, it was applied to the teeth for 24, 60, 120, and 180 seconds [30].

The study found that single use of each of the fore mentioned disinfectants did not completely eliminate the bacterial infection nor candida Albicans [30]. However, ozone therapy had eliminated both resistant strains better than sodium hypochlorite and chlorhexidine in any concentrations. The study also reported better efficacy for higher ozone therapy duration. For the combined effect of ozone with other disinfectants, they reported no significant effect of the combination of both ozone and Sodium hypochlorite even with increased duration of ozone therapy [30]. However, a combination of ozone therapy for 24s and 2% chlorhexidine had completely eradicated *Candida albicans* and *Enterococcus faecalis*. It was suggested that there was a synergistic effect between 2% chlorhexidine and 24s of gas ozone. The synergistic activity is mainly due to the mode of action as both ozone and chlorhexidine depolarize the cells and the degree of action is largely dependent on the dose [30].

The use of ozonated water against root canal infection

There was only one study that tested ozonated water against *Enterococcus faecalis* and *Streptococcus mutants*. In this study, they used a model of dental tubules where they cultured the microorganism into it. Then, the infected dentin blocks were exposed to the ozonated water and Sodium hypochlorite [31]. The study also compared the cytotoxic effect of both Sodium hypochlorite and ozonated water on mouse fibroblast. The study revealed that the Sodium hypochlorite and ozonated water had significantly decreased the microbial content of the dental blocks. The study found that the antibacterial effect of the ozonated water was similar to 2.5% of sodium hypochlorite [31]. In addition, they argued that ozonated water is considered better as it does not damage the periapical regions as sodium hypochlorite is toxic to periapical tissues. Sodium hypochlorite also damages the endodontic equipment. This was proved through the damaging effect of 2.5% sodium hypochlorite on the mice fibroblasts. The study also recommended a continuous flow of ozonated water to ensure a better result. Furthermore, the combination of ozonated with sonication achieved the best results compared to 2.5% Sodium hypochlorite [31].

The use of aqueous ozone against the root canal infection

A study tested the efficacy of the aqueous ozone in mandibular premolar teeth. The study used different concentrations of aqueous ozone as follows: 8 ppm aqueous ozone group; 12 ppm aqueous ozone group; and 16 ppm aqueous ozone group [20]. Two groups were combined with manual techniques while the other was combined with ultrasonic techniques. The study revealed that 5.25% Sodium hypochlorite achieved the best and complete disinfection of the root canal. There was a significant decrease of microbial count after the use of aqueous ozone, but it was not like the sodium hypochlorite [20].

The aqueous ozone with different concentrations did not achieve similar results to the sodium hypochlorite. Nevertheless, a combination of the 16-ppm aqueous ozone with ultrasound had the same efficacy as the 5.25% Sodium hypochlorite and had completely eradicated the bacterial infection [20].

Comparison between aqueous and gaseous ozone

Huth., *et al.* assessed the disinfectant activity of both aqueous and gaseous ozone and compared the efficacy of gaseous to aqueous ozone. The study assessed their efficacy against *Enterococcus faecalis*, *Candida albicans*, *Peptostreptococcus micros* and *Pseudomonas aeruginosa* [21]. The study used planktonic culture and cultures were exposed to ozone, 5.25%, 2.25% sodium hypochlorite; chlorhexidine digluconate, hydrogen peroxide, and phosphate-buffered saline. The study authors developed two settings of canal areas either the difficult root canal or easy root canal [21]. The study assessed various concentrations of ozone ranging from 1.25 to 20 ug/ml for aqueous

ozone and 1 - 53 g/m³ for gaseous ozone. The study found that gaseous ozone (down to 1 g/m³) had higher efficacy with lower doses than aqueous ozone (down to 5 ug/mL) [23]. The gaseous ozone had completely eradicated *Enterococcus faecalis* at 32 g/m³ for one minute. The study reported the efficacy of ozone gas against *Pseudomonas aeruginosa* in a concentration down to 1g/m³ and more than 2.5 ug/mL completely eradicated the bacteria [25]. However, the study did not perform biofilm experiments. However, aqueous ozone in the highest concentration significantly decreased *Enterococcus faecalis*, *Candida albicans*, and *Pseudomonas aeruginosa*. The efficacy of gaseous or aqueous ozone is dose and time-dependent against microbial infections and it was also dependent on the infecting microorganism [21].

Results of randomized controlled trials testing the efficacy of the ozone therapy

In this review, two randomized controlled trials were included, and both tested the efficacy of ozone therapy against control groups in deeply carious teeth [26,27]. One of the studies applied the disinfectants to the first molar teeth with deep root canal infection. The study compared the efficacy of 60 seconds of 2% chlorhexidine digluconate to 60 seconds of ozone application. The treatment of root canal infection had four stages namely as initial excavation, ozone/CHX application before the temporary restoration, 4 months later immediately after removing temporary restoration, then final excavation [26]. The study assessed the outcome of the disinfectants through the examination of dentin humidity, consistency, and color properties. Dentin samples were obtained for microbial analysis and culture for streptococci mutants, and lactobacilli. In this study, they reported better efficacy of 2% chlorhexidine digluconate than ozone and had decreased total colony bacterial count [26]. Gaseous ozone therapy had eliminated 93.33% of the microorganisms compared to 2% chlorhexidine digluconate that eliminated 98.39% of the microorganisms after four months indicating the necessity to perform a two-step approach for complete eradication of the microorganisms [26].

The other clinical trial was performed by Kist., *et al.* they assessed the efficacy of 32 g/m³. Ozone gas or 3% sodium hypochlorite, and both were followed by a 1-week inter-appointment dressing (Ca(OH)₂). Then, as a final step, ozone therapy or 3% sodium hypochlorite was applied. The outcome of the treatment was tested based on the decrease in the periapical index, the size of apical lesions, and bacterial reduction through bacterial identification using mass spectrometry [27]. The study revealed that the most common bacteria causing the root canal infections were *Streptococcus species*, *Parvimonas species* and *Prevotella species*. It was found that both ozone and sodium hypochlorite had similar efficacy against root canal infections. However, there was a higher Periapical index in the ozone group compared to 3% sodium hypochlorite [27]. The study followed the patients for one year and found no significant difference in the efficacy and recommended a longer follow-up period for the accurate measurement of the efficacy of the disinfectants. The study revealed that for each type of disinfectant, there was an increase in the number of the specific type of bacteria. For instance, the ozone group, streptococci were the only bacteria that increased in number but for the Sodium hypochlorite group, the streptococci decreased in number similar to all other bacteria. The study explained that streptococci were less sensitive to ozone [27].

Side effects of ozone therapy

No study reported the side effects of ozone whatever the duration of exposure or the dosage is. The studies tested the side effects either as a report from the patients as in all randomized controlled trials or by the culture of cells like fibroblasts with ozone solution to detect the cytotoxic effect of ozone on mitochondria and induction of apoptosis [12,16,17,27,29-31]. A study assessed the effect of ozone on the oxidative stress inside the cells and found that ozone decreased the superoxide dismutase enzyme which is induced in the case of oxidative stress and cell cytotoxicity [29]. The study also measured the level of inducible nitric oxide synthase. The increase of inducible nitric oxide synthase is protective of the cells as it was found to protect the cells against the proapoptotic signals [32]. It also protects the cells from tumor necrotic factor-mediated toxicity. The inducible nitric oxide synthase inhibits apoptosis through inhibition of caspase

through nitrosylation [32]. Ozone therapy induced the vascular endothelial growth factor that is essential for cellular regeneration and angiogenesis inducing the rapid healing of the teeth [32,33].

Ozone therapy also enhances the penetrative abilities of other disinfectants increasing their efficacy against root canal infection. Furthermore, a study compared the effect of Sodium hypochlorite to ozone therapy on the dental equipment and found that Sodium hypochlorite eroded the dental equipment while ozone preserved it [31].

Conclusion

Based on the studies included in the review, ozone therapy did not show a better result to Sodium hypochlorite, however, ozone therapy combined with ultrasonication or sodium hypochlorite had completely eradicated the root canal infection. The combination showed better penetration to the dental tissues. Thus, ozone therapy should be used in combination with other disinfectants but never used alone.

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Volume 19 Issue 12 December 2020

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