REVIEW ARTICLE

Antimicrobial Effect of Ozone Therapy in Deep Dentinal Carious Lesion: A Systematic Review

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Abstract

Aim: This systematic review aimed to answer the following focused question: Is ozone therapy more effective in reducing microbial count as compared to conventional methods in deep dentinal carious lesion?

Objective: The purpose of this systematic review was to perform a review on the effectiveness of ozone therapy in reduction of microbial count in deep dentinal carious lesion.

Study eligibility criteria, participants, and interventions: The inclusion criteria comprised studies that compared effect on microbial count in deep dentinal carious lesion after treatments with ozone and other disinfectants in primary or permanent teeth in randomized clinical trials.

Materials and methods: This review was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRIMSA) guidelines. The searched databases included Medline (via PubMed), Cochrane, and Google scholar. Articles published until 29 February 2020 without year restriction but only in English language were included.

Results: The search resulted in 359 published studies. After removal of duplicate studies and full-text analysis, seven studies were selected. Overall, the results demonstrated the promising effects of ozone therapy in reduction of microbial count as compared to other disinfectant.

Conclusions: Within the limitations of this review, it can be asserted that the ozone therapy is effective in reduction of microbial count in deep dentinal carious lesion.

Clinical significance: Ozone therapy can be a useful tool to reduce the microorganisms in deep dentinal carious lesion.

Keywords: Deep carious lesion, Microbial count, Ozone therapy, Systematic review.

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Introduction

Despite the numerous advances in dentistry, it is a well-known fact that, dental caries continues to be the most common disease affecting people from all age groups. It is a multifactorial disease which is characterized by local destruction of the hard tissue of tooth that involves interplay of four factors: tooth, saliva, microflora, and diet.[1] Due to bacterial fermentation of dietary carbohydrates to acids, particularly lactic acid, there is decrease in the pH that causes demineralization of hard tissue of tooth structure with loss of minerals[1] which result in cavitation, discomfort, pain, and finally loss of tooth.[2] These organic acids are produced by various bacteria predominantly *Mutans streptococci* and *lactobacilli* which have the ability to colonize the tooth surface.[3]

The integral component of caries management is to ensure that the caries process does not continue and is achieved through complete debridement and elimination of bacteria which is the main etiology of dental caries, followed by subsequent restoration of tooth.[4] However, the success of restorative treatment is influenced by bacterial remnant in the cavity. It has been documented that bacteria remaining after restorative procedure may survive and multiply which may lead to pulpal irritation,[5,6] risk of recurrent caries[7] and therefore failure of dental restoration.[8,9]

For elimination of bacteria, different methods have been recommended, such as cavity disinfectants, use of laser, ozone therapy, and antibacterial adhesive system with restorative material. Currently, disinfectant such as chlorhexidine digluconate, sodium hypochlorite, ethylene diamine tetra-acetic acid, hydrogen peroxide, iodine, and calcium hydroxide are used to eliminate or reduce the residual bacteria left in the cavity.[10]

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Recently, ozone has been extensively studied as a cavity disinfectant owing to its powerful bactericidal, virucidal, and fungicidal action.[11,12] Ozone is an allotropic form of oxygen, one of the most powerful oxidants whose disinfecting properties are known and used in the field of medicine since 19th century.

Thus, in accordance with the research question, "Is ozone therapy more effective in reducing microbial count as compared to conventional methods in deep dentinal carious lesion?", this systematic review was aimed to assess the antimicrobial efficacy of ozone in case of deep dentinal carious lesion.

Materials and Methods

Protocol and Registration

The current systematic review was abided by the recommendations of Preferred Reporting Items for Systematic Review and

Meta-Analysis (PRISMA) guidelines and was duly registered in PROSPERO (CRD42020164505).

Search Strategy

Two examiners independently performed the search strategy. Articles published before 29 February 2020 were searched using electronic database Medline (via PubMed), Cochrane, and Google Scholar. The articles were restricted to English language.

Commonly cited descriptions used in already published articles aided in developing the electronic search strategy. This was done by using the combination of Medical Subject Heading (MeSH) terms and text word (tw).

Following terms were combined for each database: Dental Caries, Caries, Carious lesion, Dentinal lesion, Root caries, Ozone, Ozon*, Antimicrobial, Antibacterial, Microbi*, Culture. Application of Boolean operators such as "AND" and "OR" was carried out for combining the terms to create appropriate search strategy. The search strategies performed for individual database are mentioned in Table 1.

Eligibility Criteria

According to the PICOS strategy, following eligibility criteria was devised.

- P (Population)— teeth with deep carious lesion involving dentin in asymptomatic primary or permanent dentition
- I (Intervention)— ozone therapy
- C (Comparison)— calcium hydroxide, chlorhexidine, sodium hypochlorite laser therapy or any other disinfectant
- O (Outcome)— reduction in microbial load/count
- S (Study design)— Randomized clinical trials.

Nonrandomized clinical trials, review articles, case series, case reports, interviews, replies to editor or author, conference abstracts, commentaries, opinion articles, letters, and studies performed in animals or those including artificial teeth were excluded.

Table 1: Search strategy in the database

Database	*Search strategy*	*Findings*
PubMed	1 ((((Dental caries[MeSH Terms]) OR Caries[Title/Abstract]) OR Carious lesion[Title/Abstract]) OR Dentinal lesion[Title/Abstract]) OR Root caries[Title/Abstract]	58,131
	2 ((Ozone[MeSH Terms]) OR Ozone [Title/Abstract]) OR Ozon*[Title/Abstract]	24,142
	3 (((((Antimicrobial[MeSH Terms]) OR Antimicrobial[Title/Abstract]) OR Antibacterial[Title/Abstract]) OR Microbi*[Title/Abstract]) OR Culture[MeSH Terms]) OR Culture[Title/Abstract]	167,9816
	1 AND 2 AND 3	38
Cochrane	1 (Caries):ab OR (Dental caries):ab OR (Carious lesion):ab OR (Dentinal lesion):ab OR (Root caries):ab	4,083
	2 (Ozone):ab OR (Ozon*):ab	648
	3 (Antimicrobial):ab OR (Antibacterial):ab OR (Microb*):ab OR (Culture):ab	23,203
	4 (Incomplete caries removal):ti,ab,kw OR (Stepwise caries excavation):ti,ab,kw OR (Indirect pulp therapy):ti,ab,kw	80
	1 AND 2 AND 3 AND 4	3
Google Scholar	ozone therapy, deep dentinal lesion, dental caries, deep dental caries, microbial effect, antimicrobial effect, antibacterial effect, deep carious lesion	318

Selection of Studies

Selection and retrieval of the studies was independently performed by two authors. This was preferably done by examining the titles and abstracts. In cases where the accessed information from the title and abstract were considered inadequate, full texts were read and studied by the authors. Opinion of a third author was considered in case disagreements related to the eligibility aroused. All the duplicate studies were considered only once.

Data Extraction

All the data from the included studies were independently collected by two authors. A third author was consulted to solve any evoked disagreement. Data extraction comprising of authors, country, year, study type, micro-organisms, tooth type, sample size, disinfectants, and micro-organism reduction values was performed. If the data was found to be missing, authors were duly contacted through e-mail.

Quality Assessment

Cochrane risk of bias tool (Bias Risk Assessment of Randomized Controlled Studies- Cochrane Handbook) was used to assess the qualitative analysis of the studies.[14]

Due to the type of intervention in the studies, blinding of the operator was technically not possible. The general judgment of the risk of bias was stated as follows: 1) High risk of bias (Red)— Negative domain, 2) Low risk of bias (Green)— Positive domain, and 3) Uncertain risk of bias (Yellow)— Unclear response.

Results

Study Selection

Flowchart 1 shows the flow diagram depicting the search strategy conducted for the review. Initially, 359 published studies were extracted through the search, out of which seven studies were excluded due to duplication. Then, out of the 352 studies, 15 studies were considered after "titles and abstracts" analysis.

After reading full text, eight studies[15–22] were excluded because they did not fulfill the inclusion criteria. The reasons for exclusion of these studies are mentioned in Table 2.

Thus finally, seven studies[23–29] were considered for this systematic review.

Data Collection

Table 3 gives the summary for collected data from the seven included studies.[23–29]

Risk of Bias Assessment

All the included studies [23–29] were considered "high" risk of bias. Figures 1 and 2; and Table 4 show the results for the risk of bias of the studies.

Discussion

Pertaining to the current scientific evidence available, this assessment, revealed the clinical effectiveness of ozone therapy in management of dentinal carious lesion in primary and permanent

Flowchart 1: PRISMA flow diagram of the literature search and selection process

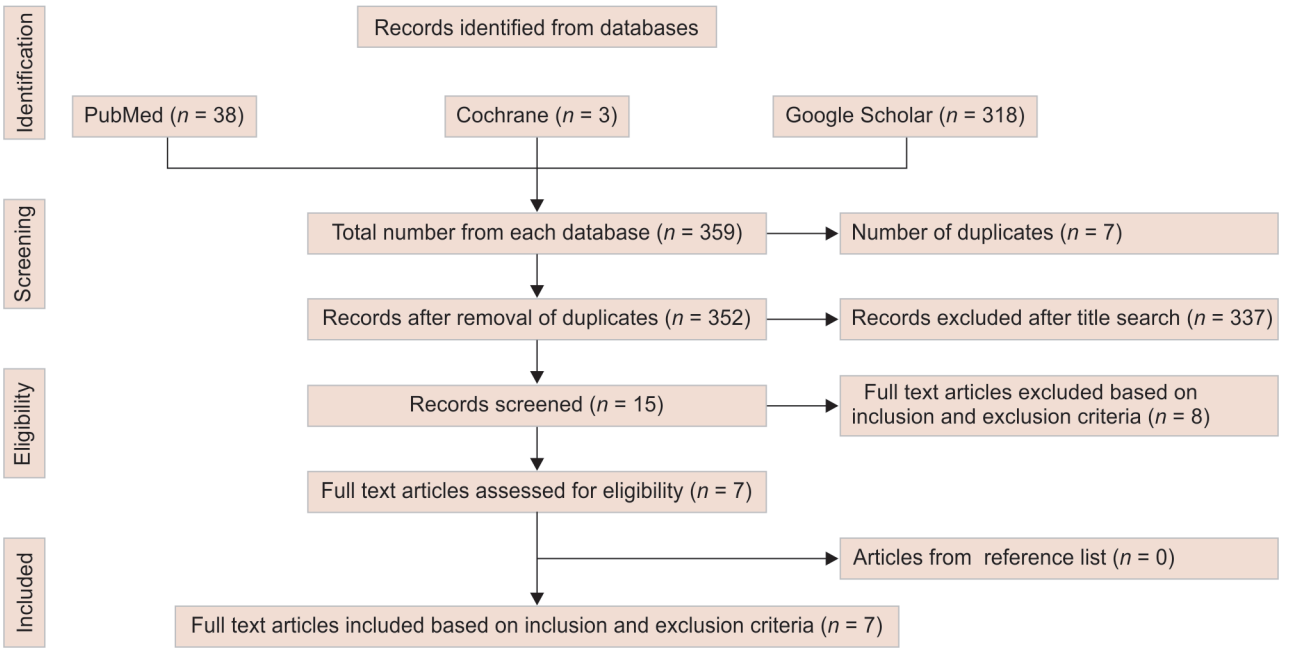

Table 2: Characteristics of excluded articles

Sr. No.	*Author and year*	*Reason for exclusion*
1	Polydorou et al. 2006	*In vitro* study
2	Almaz et al. 2013	Review article
3	Kapdan et al. 2013	*In vitro* study
4	Dukić et al. 2013	*In vitro* study
5	Kalnina et al. 2016	No microbiological assessment
6	Ximenes et al. 2017	*In vitro* study
7	Kirilova et al. 2019	Nonrandomized clinical trial
8	Rickard et al. 2019	Review article

teeth. In all included studies,[23–29] ozone therapy in gas form was used via a special delivery system.

The quality of all included studies was evaluated. One of the criteria for this assessment was randomization which aimed to ensure that each lesion has an equal chance of receiving any of the treatments under experiment. Only four of the included studies[24,26,27,29] reported adequate methods for randomization. Another aspect for quality assessment was performance bias, which should be avoided by appropriate blinding. Blinding of the participants or the investigator performing the treatment was not mentioned in any of the study, as it was not possible to blind participants and main investigator to the interventions because of the nature of intervention. The risk of performance bias was considered high, since the operators as well as the participants were aware of the treatment procedure and it might be because of the special device used for application of ozone. No study mentioned about the mock treatment using the ozone delivery device in control group. Random sequence generation was adequately reported in four studies[24,26,27,29] (low risk) whereas unclear random sequence generation was seen in the study of Safawat et al.[25] Allocation concealment as well as blinding of the outcome assessment were not adequately reported. Two studies[26,27] reported

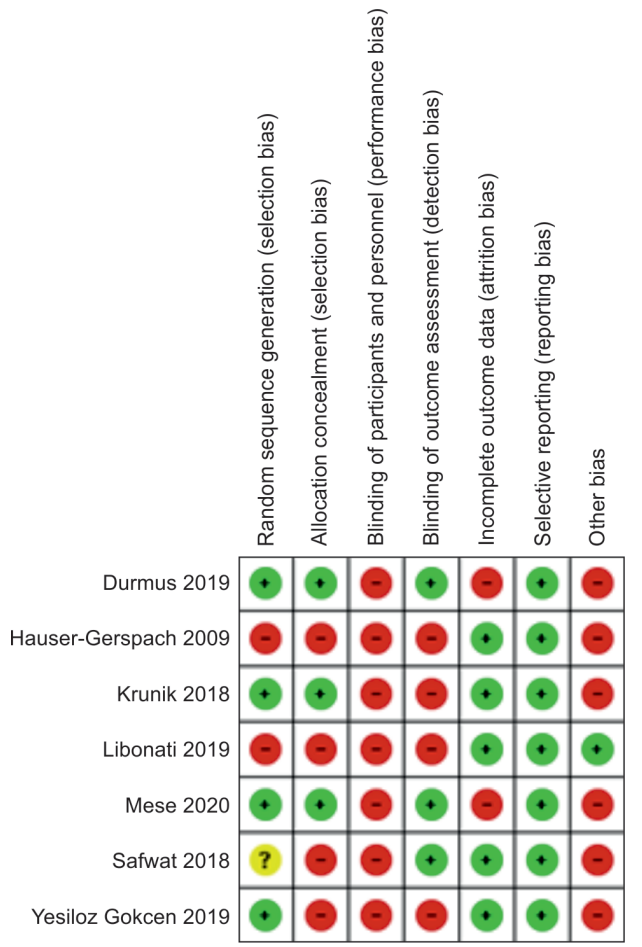

Fig. 1: Risk of bias summery

about missing participants but did not mention about measures taken to compensate for missing data. Selective reporting was

Table 3: Characteristics of included studies

Sr. no.	*Authors, country, year*	*Micro-organism*	*Tooth type*	*Group, sample size*	*Ozone protocol*	*Control protocol*	*Outcome*
1	Hauser-Gerspach et al. Switzerland, 2009.	Overall bacterial load	Primary molars	*Control-* 1% Chlorhexidine (without excavation) *n* = 20. 1% Chlorhexidine (with excavation) *n* = 20. *Experimental-* Ozone gas (without excavation) *n* = 20. Ozone gas (with excavation) *n* = 20.	*Physical state-* Gas *Time of application–* 30 sec *Concentration-* 2100 ± 200 ppm *Application form-* via a novel ozone delivery system, HealOzone, KaVo, Germany with silicon cup	1% Chlorhexidine (Corsodyl gel, GSK, Weybridge GB) *Physical state-* Gel *Time of application–* 30 sec *Concentration-* 1% *Application form-* with a binangle Swiss-made stainless steel spatula HCB 203 4	***Result-*** The total reduction of bacteria in the group without excavation was 7% after ozone treatment and 36% after chlorhexidine treatment. With excavation it was, 19% after ozone treatment and 41% after chlorhexidine treatment. There was no statistical difference comparing sample before and after ozone and chlorhexidine treatment and comparing sample from excavated and non-excavated lesion *Conclusion-*Ozone application as well as 1% chlorhexidine application for 30 seconds was not effective in reducing micro-organisms
2	Kurnic et al. Bosnia and Herzegovina, 2018.	Total bacteria and lactobacilli species.	Posterior permanent teeth	*Control-* 2% chlorhexidine *n* = 24 E*xperimental-* ozone gas *n* = 24	*Physical state-* Gas *Time of application–* 40 sec *Concentration-* 525 ppm *Application form-* via Ozonytron X-Bioozonix, Munich, Germany with special disposable silicon cup.	2% chlorhexidine *Physical state-* Solution *Time of application–* 60 sec *Concentration-* 2% *Application form-* with brush tip (Black Mini® Brush, Ultradent, South Jorden, UT, USA) and then gently dried	*Result-* Ozone and chlorhexidine application significantly decreased the number of total bacteria for 68% and 34.5%, respectively. Ozone and chlorhexidine application significantly decreased counts of Lactobacilli species for 30% and 66%, respectively. *Conclusion-* Application of ozone in deep carious lesion after incomplete caries removal provides significant antibacterial effect measured by total number of bacteria and Lactobacilli species.
3	Safawat et al. Saudi Arabia, 2018.	Mutans streptococci, lactobacilli, and *Candida albicans*	Immature permanent first molar	*Control-* Calcium hydroxide *n* = 40 *Experimental-* Ozone gas *n* = 40	*Physical state-* Gas *Time of application–* 40 sec *Concentration-* Not given *Application form-* via HealOzone device (KaVo Co. GmbH, D- 88,400 biberach/ Riss-Germany) with silicon cup	Dycal (Dentsply Co. Rua Alice Herve, 86-25,665-010-Petropolis-RJ.Brasile) *Physical state-* Paste *Time of application–* 6 month/ 12 month *Concentration-* Not applicable *Application form-* two paste are mixed and placed in cavity with dental instrument	*Result-* Mutans streptococci (MS) Lactobacilli and *Candida* counts were significantly reduced immediately after ozone application. Also, after 6 and 12 month there is significant reduction in count of Mutans streptococci, Lactobacilli and *Candida*. *Conclusion-* Ozone gas application for 40 sec has significant antimicrobial effect especially against Mutans streptococci in deep class I carious lesion.

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Sr. no.	*Authors, country, year*	*Microorganism*	*Tooth type*	*Group, sample size*	*Ozone protocol*	*Control protocol*	*Outcome*
4	Durmus et al. Turkey, 2019.	Mutans streptococci, lactobacilli, and total bacteria	Mandibular first permanent molar	*Control*-without disinfectant *n* = 35 *Positive control*- 2% chlorhexidine *n* = 35 *Experimental*- Ozone *n* = 35	*Physical state*-Gas *Time of application*- 60 sec *Concentration*- 2100 ppm *Application form*- via HealOzone device (KaVo Dental GmbH, Germany)	*Control group*- No disinfectant used for first two samples. *Positive control group*- Chlorhexidine digluconate *Physical state*- Solution *Concentration*-2% *Time of application*- 60 sec *Application form*- applied with brush and excess was removed with new brush without drying to leave site moist	*Result*- The total bacterial reduction between the samples, which were taken after initial excavation and after 4 months, were 79.11% in no disinfectant group, 98.39 % in 2% chlorhexidine group and 93.33% in ozone group. (2% Chlorhexidine > ozone > no disinfectant). Between samples taken after initial excavation and immediately after ozone or chlorhexidine application total bacterial reduction in 2% chlorhexidine was 90.32% and in ozone was 53.42%. When samples taken after initial excavation and after final excavation, no statistically significant difference observed in all group. *Conclusion*- Both 2% chlorhexidine and ozone gas were effective as an antibacterial agent.
5	Mese et al. Turkey, 2020.	Mutans streptococci, lactobacilli, and total bacteria	Primary molars	*Control*- No disinfectant *n* = 35 *Positive control*- 2% chlorhexidine *n* = 35 *Experimental*- Ozone *n* = 35	*Physical state*- Gas *Time of application*- 60 sec *Concentration*- 2100 ppm *Application form*- via HealOzone device (KaVo Dental GmbH, Germany)	*Control group*- No disinfectant used for first two samples. *Positive control group*- Chlorhexidine digluconate *Physical state*- Solution *Concentration*- 2% *Time of application*- 60 sec *Application form*- applied with brush and excess was removed with new brush without drying to leave site moist	*Result*- The total bacterial reduction between the samples, which were taken after initial excavation and after 4 months, were 74.18 % in no disinfectant group, 93.25% in 2% chlorhexidine group and 82.29% in ozone group. (2% Chlorhexidine > ozone > no disinfectant). Between samples taken after initial excavation and immediately after ozone or chlorhexidine application total bacterial reduction in 2% chlorhexidine was 77.67% and in ozone was 47.39%. When samples taken after initial excavation and after final excavation, no statistically significant difference observed in all group.(No disinfectant 98.43%, chlorhexidine 99.78% and ozone 98.66%) *Conclusion*- Usage of cavity disinfectants in the stepwise excavation procedure contributes to the reduction of bacterial population in the cavity. Both chlorhexidine and ozone application were effective in bacterial reduction in the stepwise excavation procedure, with the chlorhexidine application found to be superior to ozone gas.

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Sr. no.	*Authors, country, year*	*Microorganism*	*Tooth type*	*Group, sample size*	*Ozone protocol*	*Control protocol*	*Outcome*
6	Libonati et al. Albania, 2019.	Streptococcus mutans and lactobacilli	Permanent first and second molars	*Control*- No disinfectant *n* = 75 *Experimental*- Ozone gas *n* = 75	*Physical state*- Gas *Time of application*- 60 sec *Concentration*- 329/m^3 *Application form*- via HealOzone X4 device with silicon cup.	No disinfectant was used	*Result*- After ozone application the CFU count decreased for *S. mutans* by 17.90 % and for lactobacilli by 25.32% than the control group. *Conclusion*- The application of ozone in deep caries, with partial excavation of dentin has an antibacterial effect against *S. mutans.* ozone is more effective on lactobacilli than *S. mutans*
7	Yesiloz Gokcen et al. Turkey, 2019.	Streptococcus mutans	Deciduous second molar	*Control*- Clearfil Protect Bond (CPB) *n* = 10 *Positive control*- Dycal *n* = 10 *Negative control*- Physiologic saline (PS) *n* = 10 *Experimental*- Ozone *n* = 10	*Physical state*- Gas *Time of application*– 30 sec *Concentration*- Not given *Application form*- via Ozonytron X with CA probe.	*Control*- Clearfil Protect Bond (CPB) *Physical state*- Solution *Time of application*– 20 sec *Concentration*- Not applicable *Application form*- applied with brush and then light cured *Positive control*-Dycal *Physical state*- Paste *Time of application*– Not given *Concentration*- Not applicable *Application form*- two paste are mixed and placed in cavity with dental instrument *Negative control*- Physiologic saline (PS) *Physical state*- solution *Time of application*– Not given *Concentration*- Not given *Application form*- Not given	*Conclusion*- Except physiological saline (PS) there was reduction in bacterial count in all group. Ozone was most effective in bacterial reduction followed by Clearfil protect bond (CPB), dycal. (Ozone > CPB > Dycal > PS)

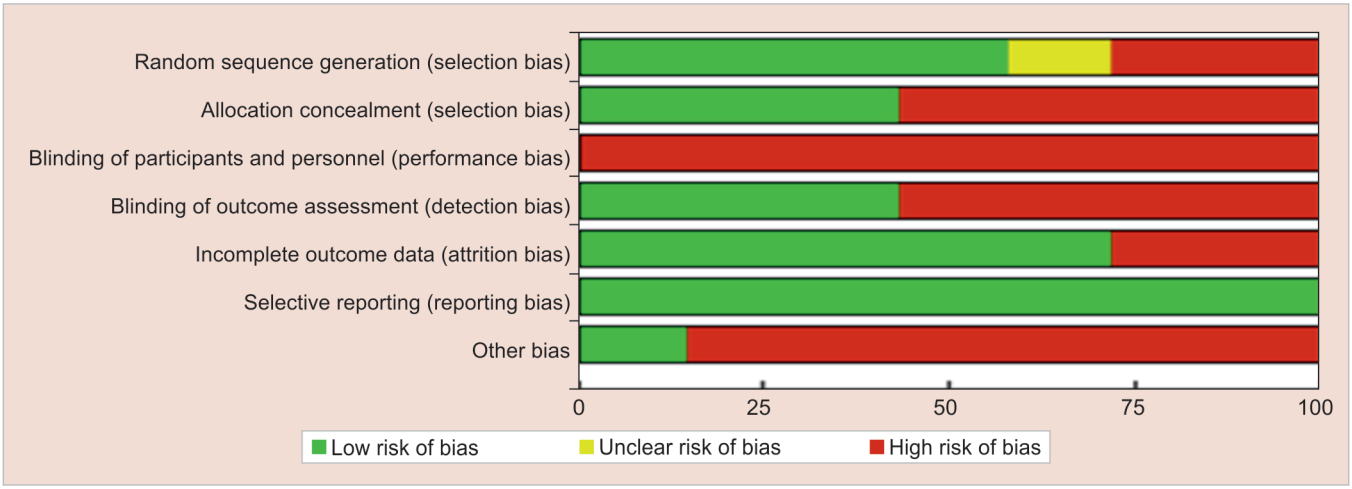

Fig. 2: Risk of bias graph

Table 4: Quality of assessment of the included studies

Sr. No.	*Study*	*Random sequence generation*	*Allocation concealment*	*Blinding of participants and personnel*	*Blinding of outcome assessment*	*Incomplete outcome data assessment*	*Selective reporting ofoutcome*	*Other source of bias*	*Risk of bias*
1	Durmus et al. 2019	Low risk	Low risk	High risk	Low risk	High risk	Low risk	High risk	High risk
2	Hauser-Gerspach et al. 2009	High risk	High risk	High risk	High risk	Low risk	Low risk	High risk	High risk
3	Kurnic et al. 2018	Low risk	Low risk	High risk	High risk	Low risk	Low risk	High risk	High risk
4	Libonati et al. 2019	High risk	High risk	High risk	High risk	Low risk	Low risk	Low risk	High risk
5	Mese et al. 2020.	Low risk	Low risk	High risk	Low risk	High risk	Low risk	High risk	High risk
6	Safawat et al. 2018	Unclear risk	High risk	High risk	Low risk	Low risk	Low risk	High risk	High risk
7	Yesiloz Gokcen et al. 2019	Low risk	High risk	High risk	High risk	Low risk	Low risk	High risk	High risk

avoided in all the studies.[23–29] Other unspecified types of bias were also considered as associated with the lack of information on sample size estimation and no mention of baseline demographic and clinical variables.

Two studies[23,28] included two carious lesions per subject while in three studies[24,26,27] only one carious lesion per subject was incorporated. Also, only two studies[25,29] designed the study according to split-mouth design,[30] in which each of the subjects acted as their own control to decrease the intersubject variability.

For caries detection and selection, most of the studies[24,26–29] did not mentioned the method, Hauser-Gerspach et al.[23] used bitewing radiography method while Safawat et al.[25] used Diagnodent device. Three studies[23,25,28] incorporated teeth with occlusal lesion, two studies[24,29] involved teeth with occlusal and/or proximal lesion while two studies[26,27] did not mentioned the surface for caries detection. In concern with teeth involved, three studies[23,27,29] involved primary posterior teeth and four studies[24–26,28] involved permanent posterior teeth.

Considering the method of caries excavation, few studies[25–27,29] involved stepwise caries excavation method, Kurnic et al.[24] involved incomplete caries removal, Libonati et al.[28] involved complete caries removal method while Hauser-Gerspach et al.[23] didn't mentioned any method.

Five studies[23,24,26,27,29] used chlorhexidine while two studies[25,28] used calcium hydroxide as a disinfectant in control group. Considering disinfection property of material used, Hauser-Gerspach et al.[23] showed no effect of chlorhexidine as well as ozone on bacterial reduction, two studies[24,25] concluded significant antimicrobial effect of ozone, two studies[28,29] evaluated ozone as more effective while two studies[26,27] showed chlorhexidine more effective. Apart from chlorhexidine and calcium hydroxide, Yesiloz Gokcen et al.[29] evaluated antimicrobial efficiency of Clearfil Protect Bond (CPB) which showed more effective than calcium hydroxide but less than ozone.

Further, regarding the microbial evaluation, studies evaluated effect of ozone gas on total bacteria,[23,24,26,27] mutans streptococci,[25–29] and lactobacilii.[24–28] In majority of studies, there were reduction in microbial count. Immediate and long term antimicrobial effect of ozone was assessed in five studies[25–28] while two studies[23,24] assessed only immediate antimicrobial effect. Regarding follow-up, four studies[25–28] had average follow-up of 4 to 12 months while Yesiloz Gokcen et al.[29] had four week of follow-up and two studies[23,24] had no follow-up.

Despite the diversity in methodology, the outcome of these studies were similar and revealed the promising effect of ozone in reduction of microbial count, except for Hauser Gerspach

et al.[23] which showed no additional benefit of this technique. Though all included studies used ozone as a gas, none of the study evaluated the adverse effects of the technique used on hard or soft tissue in oral cavity.

One of the limitations of included studies was that the variability in the methods used such as time of application, ozone concentration, and different ozone generating equipments. In three studies,[23,24,28] no sample size calculation was performed and in two studies[23,29] sample size in experimental group was inadequate. Also, there was no standardization for caries detection methods, caries excavation methods, selection of tooth, and tooth surface. Another limitation is the scarcity of *in vivo* studies with split mouth design,[30] only two studies[25,29] incorporated this study design.

Strength of this systematic review is that the authors conducted the studies according to PRISMA guidelines. Also, to the best of our knowledge, this systematic review is one of a kind that depicted the effect of ozone therapy on deep dentinal carious lesion. But due to the heterogeneity in the methodology and difficulty in comparison between the treatment protocols of the included studies, meta-analysis was not recommended. Another limitation of this study is limited data base search.

As future research implications, it is important to have a rational comparison between the experimental and control groups. Most of the studies except Hauser-Gerspach et al.[23] were conducted recently in the years 2018 to 2020. Therefore, more studies evaluating the long term antimicrobial efficacy of ozone against bacterial load in deep dentinal carious lesion using split mouth design[30] and incorporation of more data bases for electronic search are required. To reduce the overall risk of bias, future studies should incorporate homogenous methodology such as same concentration, physical state, application form, application time of ozone, tooth type, involved tooth surface, caries detection method, caries excavation method, microbial evaluation, duration of clinical, and radiological follow-up and use of split-mouth study design. In all included studies,[23–29] ozone therapy was used but only in gaseous form which required a special device for its application. However, the ease of application, practicality, and affordability should be considered. So we recommend the use of ozone therapy in gel, oil, or aqueous form which does not require any additional special device or equipment for application of ozone.

Conclusion

After conducting this profound study, we can conclude that, before final restoration, ozone therapy can be used to reduce microbial count in deep dentinal carious lesion. However, adequate information regarding the clinical evidence of ozone application is currently not available. Therefore, additional evidences are required before considering ozone therapy as an alternative method for the management of deep dentinal carious lesion.

Clinical Significance

Ozone therapy can be effectively used to reduce microorganisms in deep dentinal carious lesion in pediatric as well as adult patients.

Orcids

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