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Antimicrobial efficacy of ozone therapy in endodontic treatment: A systematic review

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Abstract
AIM. The objective was to assess whether endodontic treatment using ozone therapy improves the antimicrobial efficacy in patients undergoing root canal treatment.
MATERIALS AND METHODS. Electronic search on various data sources like pubmed, Scopus, Web of science and Google scholar along with hand searching of the articles in Institutional library was done. Studies comparing the results of ozone used as irrigant and intracanal medicament with conventional root canal irrigants in improving the disinfection and antimicrobial effect were eligible for this review. The study designs published were comparative in-vitro studies, randomized controlled trials, controlled clinical trials, experimental studies, and cohort studies between 1st January 2018 and 31st March 2023 Studies in English language or those possible of getting translated into English language were included.
The studies including intervention using ozone therapy as irrigant and intracanal medicament and comparison with conventional root canal irrigant were included. The primary search yielded a total of 144 studies from various search engines mentioned above,74 studies were excluded based on screening titles. After screening the remaining 70 articles for abstract and full text, final 6 studies were selected for the review and remaining 64 duplicated articles were excluded.
RESULTS. The ozone therapy has a significant impact in reducing colony forming units and thus can be used as an adjunct to root canal irrigant and thus has better antibacterial properties over conventional root canal irrigating solutions.

Keywords: antibacterial property, intracanal medicament, irrigants, ozone therapy, endodontic therapy

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Антимикробная эффективность озонотерапии в эндодонтическом лечении: систематический обзор

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Резюме
ЦЕЛЬ. Оценить, улучшает ли озонотерапия антимикробную эффективность у пациентов, проходящих эндодонтическое лечение.
МАТЕРИАЛЫ И МЕТОДЫ. Для проведения обзора был осуществлен электронный поиск в различных базах данных, таких как PubMed, Scopus, Web of Science и Google Scholar, а также ручной поиск статей в библиотеке учебного заведения. В обзор включались исследования, сравнивающие результаты использования озона в качестве ирриганта и внутреканального медикамента с традиционными эндодонтическими ирригантами в повышении дезинфекции и антимикробного эффекта.
Включались исследования следующих дизайнов: сравнительные in vitro исследования, рандомизированные контролируемые исследования, контролируемые клинические испытания, экспериментальные исследования и когортные исследования, опубликованные в период с 1 января 2018 г. по 31 марта 2023 г. Включались статьи на английском языке или те, которые возможно было перевести на английский язык. В обзор были включены исследования, в которых проводилась интервенция с использованием озонотерапии в качестве ирриганта и внутреканального медикамента и проводилось сравнение с традиционными ирригантами для корневых каналов. Первичный поиск выявил 144 исследования из вышеуказанных поисковых систем, из которых 74 исследования были исключены на основании анализа заголовков. После проверки оставшихся 70 статей по рефератам и полным текстам, для обзора было отобрано 6 исследований, а оставшиеся 64 дублирующиеся статьи были исключены.

РЕЗУЛЬТАТЫ. Озонотерапия оказывает значительное влияние на снижение количества колониеобразующих единиц, что позволяет использовать её в качестве вспомогательного средства к традиционным ирригантам для корневых каналов, обеспечивая лучшие антибактериальные свойства по сравнению с обычными растворами для ирригации.

Ключевые слова: антибактериальные свойства, внутреканальные медикаменты, ирриганты, озонотерапия, эндодонтическая терапия

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INTRODUCTION

A successful root canal therapy involves removing the infected or inflamed pulp, cleaning and disinfecting the root canals followed by filling with biocompatible material to create a coronal and apical seal [1]. Additionally, a mix of mechanical and chemical techniques must be used to completely eradicate or significantly reduce the bacteria burden within the root canal system [2]. The common causes of endodontic failure include bacterial persistence in the apex and canals, poor obturation quality, coronal leakage, under- and over-extension of the root canal filling, and improper mechanical debridement [3]. Certain microorganisms may persist even after a treatment that complies with the many steps that have been documented to lower the number of microorganisms in the root canal system, such as the use of different instrumentation techniques and irrigation regimes. Instruments mostly work on the main canal, whereas irrigants and intracanal medications primarily work chemically to clean and disinfect isthmuses [4]. The most common endodontic irrigant, with a concentration range of 0.5% to 5.25%, is sodium hypochlorite which has good antibacterial properties and is very effective in removing vital or necrotic tissues. When used in contrast to EDTA, which is employed as an adjuvant to dissolve the smear layer [5; 6]. Similarly, a meta-analysis reported chlorhexidine in the concentration of 2% is as effective disinfection as NaOCl [7].

The other irrigating solution comprise; mixture of tetracycline, acid and detergent (MTAD), tetraclean, electrochemically activated solutions, photon-activated disinfection, herbal irrigants, and ozonated water [8]. The recently introduced ozone has been used in endodontics either in aqueous form or a gaseous form. It is cheap, effective, and easy to use root canal disinfectant. The literature reports ozone to have significantly stronger antimicrobial action than other irrigating solutions and acts faster without causing any cytotoxic effects [9].

However, there is no updated evidence on the superiority of ozone as antimicrobial therapy over other irrigating solutions. Though one systematic review reported ozone therapy to be less effective than NaOCl in terms of microbial load reduction, the inference was limited with a small number of randomized controlled trials [10]. Considering this, the present systematic review was undertaken with an aim to assess whether irrigation using ozone therapy improve the antimicrobial efficacy in patients undergoing endodontic treatment when compared to other irrigating solutions and intracanal medicaments.

AIM

Thus, the purpose of this systematic review was to answer the focused question “Does irrigation using ozone therapy improve the antimicrobial efficacy in patients undergoing endodontic treatment”.

MATERIAL AND METHODS

Protocol and registration the current systematic review was conducted and written according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA Statement) checklist Recommendations and was registered in PROSPERO under ID CRD42023396307

Search strategy

The search process was carried out by two examiners independently. The electronic databases pubmed-Medline, Scopus, Web of science and Google scholar were searched for articles published from 1st January 2018 to 31st March 2023. The articles published in English and those translated into English were searched.

Table 1. Keywords representing PICO

Таблица 1. Ключевые слова, представляющие PICO

Primary keywords	Secondary keywords
Root canal therapy (P)	Root canal therapies Endodontic treatment Root canal treatment Dental pulp cavity Endodontics
Ozone therapy (I)	Ozone therapy Ozonation Ozonizer
Conventional irrigation solution (C)	Therapeutic irrigation Medicaments Intracanal medication Irrigants
Antibacterial property (O)	Antimicrobials Anti-infective
In-vitro (S)	In-vitro Extracted teeth Human teeth Ex-vivo

Table 2. Search strategy developed for Pubmed-Medline database

Таблица 2. Стратегия поиска, разработанная для базы данных PubMed-Medline

Sr. No.	Search strategy	Articles found
1	(“ozonated”[All Fields] OR “ozonating”[All Fields] OR “ozonation”[All Fields] OR “ozonations”[All Fields] OR “ozone”[mesh Terms] OR “ozone”[All Fields] OR “ozone s”[All Fields] OR “ozonization”[All Fields] OR “ozonized”[All Fields] OR “ozonizer”[All Fields]) AND ((“intracanal”[All Fields] AND (“medica”[All Fields] OR “medicament”[All Fields] OR “medicaments”[All Fields] OR “medicas”[All Fields])) OR (“irrigant”[All Fields] OR “irrigants”[All Fields] OR “irrigate”[All Fields] OR “irrigated”[All Fields] OR “irrigates”[All Fields] OR “irrigating”[All Fields] OR “irrigational”[All Fields] OR “irrigator”[All Fields] OR “irrigators”[All Fields] OR “therapeutic irrigation”[mesh Terms] OR (“therapeutic”[All Fields] AND “irrigation”[All Fields]) OR “therapeutic irrigation”[All Fields] OR “irrigation”[All Fields] OR “irrigations”[All Fields]) OR (“rot”[All Fields] AND (“canal s”[All Fields] OR “canaled”[All Fields] OR “canals”[All Fields] OR “dental pulp cavity”[mesh Terms] OR (“dental”[All Fields] AND “pulp”[All Fields] AND “cavity”[All Fields]) OR “dental pulp cavity”[All Fields] OR “canal”[All Fields])) OR (“endodontal”[All Fields] OR “endodontic”[All Fields] OR “endodontical”[All Fields] OR “endodontically”[All Fields] OR “endodontics”[mesh Terms] OR “endodontics”[All Fields])) AND (“anti-infective agents”[Pharmacological Action] OR “anti-infective agents”[mesh Terms] OR (“anti-infective”[All Fields] AND “agents”[All Fields]) OR “anti-infective agents”[All Fields] OR “antimicrobial”[All Fields] OR “antimicrobials”[All Fields] OR “antimicrobially”[All Fields])	128

Table 3. Search strategy developed for Scopus database

Таблица 3. Стратегия поиска, разработанная для базы данных Scopus

Sr. No.	Search strategy	Articles found
1	TITLE-ABS-KEY (ozone AND intracanal AND medicament OR irrigant)	7

Table 4. Search strategy developed for Web of Science database

Таблица 4. Стратегия поиска, разработанная для базы данных Web of Science

Sr. No.	Search strategy	Articles found
1	Ozone intracanal medicament or irrigant antimicrobial (All Fields) [Search within all fields: Clinical Trial]	9

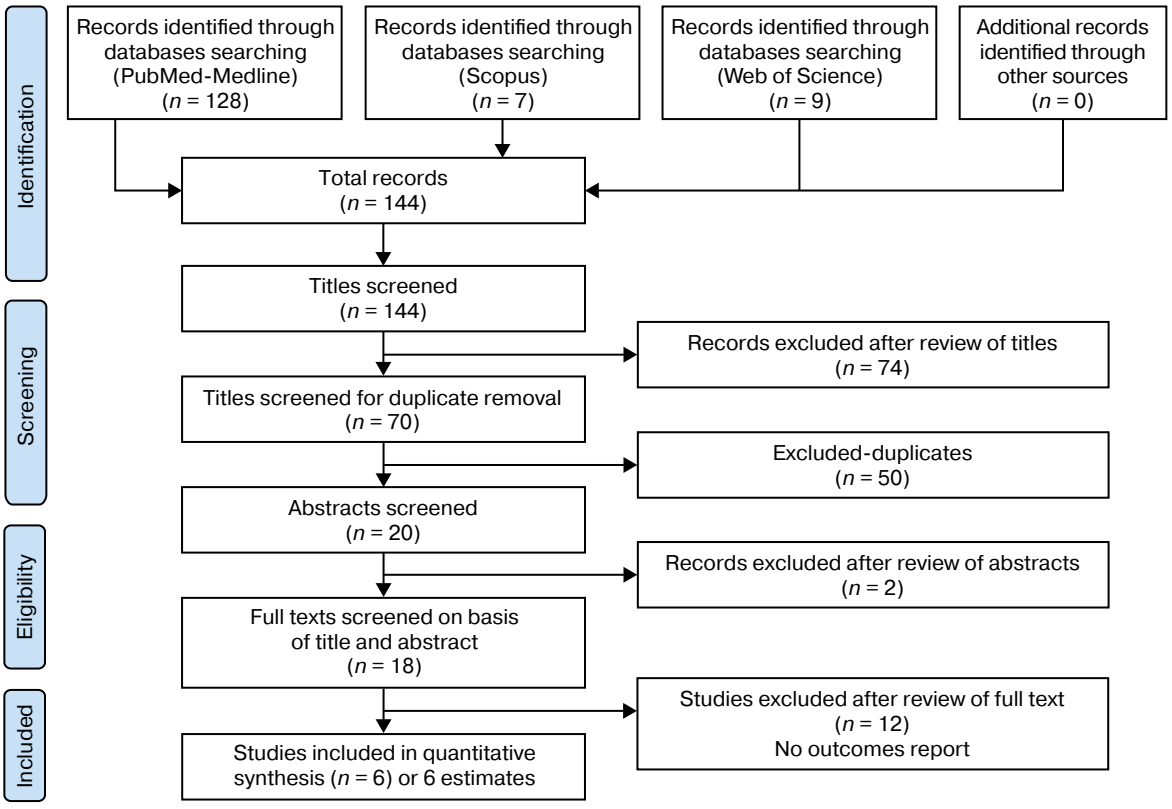

Fig. 1. Prisma flowchart

Рис. 1. Блок-схема Prisma

Eligibility criteria

Inclusion:

1. Comparative studies in which the disinfection during cleaning and shaping in one group was done using ozone therapy and other group (s) with any other irrigation solution were included.

2. Studies assessing efficacy of the irrigating solutions and ozone used in root canal treatment were included.

3. Research conducted in English or in other languages for which translation into English was feasible.

4. Studies published from the year 1st January 2018 and 31st March 2023 were included.

5. All in-vitro studies or ex-vivo studies done of human teeth.

Exclusion:

1. Studies assessing outcomes other than antibacterial property for the irrigating solutions used during root canal treatment.

2. Studies done on animals or their extracted teeth were excluded.

3. Review articles.

4. Case reports.

5. Letters to the editor.

6. Short communications.

7. Conference proceedings.

8. Patents.

PICO can be referred as:

1. Patient Population: Extracted human teeth.

2. Intervention: Ozone therapy.

3. Comparison: Conventional irrigation solutions.

4. Outcome: Antibacterial property (colony forming unit, pH variation).

5. Study design: In-vitro or ex-vivo comparative studies.

Study selection

The titles and abstracts acquired through the search strategy were independently reviewed by one review author, who included them if they satisfied the inclusion criteria. All of the included papers' full texts were later retrieved. After obtaining the full text of the articles, they were read full text and were included if they met the requirements for inclusion. When there was doubt about a study's eligibility for inclusion, that uncertainty was resolved by referring to the second author. Finally, the search yielded 144 studies in initial search which were subjected for the further screening in systematic review process.

Data collection process

A standardized data extraction form was prepared in Microsoft Excel; called as pilot form. Initially 2–3 entries of the data extraction from the articles were made in the Excel spreadsheet. The entries were reviewed by an expert. Any disagreement between the authors was resolved by discussion.

Data items included for extracting the data were:

1. Study Id: Number given to each included study.

2. Study title: The title of the publication.

3. Author's name: Name of the authors.

4. Year of publication: Year in which the study was published.

5. Country of research: The country in which the study was performed.

6. Study design (groups): Whether the study was invitro or ex-vivo study.

7. Sample size: Sample size for that particular study.

8. Groups: Number of groups in the study.

9. Intervention: The details regarding the ozone therapy used as irrigant during root canal therapy.

10. Control: The details regarding the conventional irrigating solution used during root canal therapy.

11. Results.

a. Colony forming units after the use of ozone therapy and conventional irrigating solution.

b. Reduction in bacterial count units after the use of ozone therapy and conventional irrigating solution.

RESULTS

The results are presented in Table 5.

The risk of bias was assessed using OHAT tool developed for assessing risk of bias for human and animal invitro studies (randomized and non-randomized studies). For selection domain, two studies had probably high risk of bias and four studies had probably low risk of bias. For performance domain, all studies had probably low risk of bias. For attrition bias, detection bias, and selective reporting bias domains, all studies had definitely low risk of bias. For other potential bias domain, one study had definitely high risk of bias while five studies had definitely low risk of bias.

DISCUSSION

The irrigation of the root canal, one of the three crucial phases in root canal treatment, has the greatest impact on the periapical tissues ability to heal [1]. By washing away debris, disintegrating tissue, and cleaning the root canal system, irrigations can support mechanical debridement. In teeth with complex internal architecture, such as fins or other irregularities that instruments could fail, chemical debridement is particularly important [11]. For more than ten years, ozone has been utilised successfully to treat an array of diseases [12] It has been widely used in the treatment of pit and fissure caries, restorative treatment, oral lesions, root caries, endodontics, hypersensitive teeth, periodontics for periodontal diseases, denture stomatitis, post-extraction for enhanced wound healing, and reducing demineralization [12].

The gold standard for antibacterial root canal irrigants is sodium hypochlorite, which is often utilised in endodontic procedures. Despite its many benefits, NaOCl lacks the essential characteristics of smear layer removal and substantivity [13]. To overcome this, the use of NaOCl has been reported in combination with many other irrigating solutions. NaOCl's pH has been demonstrated to fall when EDTA is added in a time-dependent way [14]. It has been shown that combining CHX and NaOCl can strengthen their antibacterial properties. Additionally, this precipitate exhibits cytotoxicity and has the potential to alter tooth colour [14].

Table 5. The characteristics of the articles included in this systematic review

Таблица 5. Характеристики статей, включенных в данный систематический обзор

Title	Authors	Year of study	Country of research	Type of study	Sample size	Study design (groups)	Intervention	Control	Outcome assessment (methodology / test)	Results	Inferences	Notes
Antimicrobial Effect of Calcium Hydroxide Combined with Electrolyzed Superoxidized Solution at Neutral pH on Enterococcus faecalis Growth	H.A. Jimenez-Gonzalez et al.	09.11.2021	Mexico, USA	Ex vivo	60 maxillary 2nd premolar	Group 1, 0.9% nacl; group 2, 0.9% nacl plus Ca(OH)2 paste; group 3, oxoral®; and group 4, oxoral® plus Ca(OH)2 paste	Caoh and superoxidized oxoral	Caoh, Nacl	Cfu and pH variation	A permanent reduction in bacterial growth was observed at days 1, 6, 12, and 18 after oxoral® plus Ca(OH)2 treatment from 4:4±0:074 log10 CFU/ml to 0:0±0:001 log10 CFU/ml	The combination of oxoral® and Ca(OH)2 provides an alkaline pH and inhibits E. Faecalis growth into the root canals.	–
In Vitro Comparison of Antibacterial Effect of Ozonated Water and Ozonated Gas	F. Agostini et al.	14.08.2020	Brazil	Ex vivo	15	3 groups – positive control – bacteria, negative control – chx and experimental group	Ozonated water and ozonated gas	CHX	CFU	For the E. Faecalis, ozonated gas was significantly more effective than ozonated water.	When compared to controls, all ozone concentrations were effective in reducing bacteria.	–
Effectiveness of Ozone against E.Faecalis in a root canal suspension – an in-vitro study	V.V. Kumar et al.	20.06.2019	India	In vitro	Not mentioned	3 groups ozone gas ozone water, control – NaOCl	Ozonated gas and ozonated water	NaOCl	CFU	Concentrations of gaseous ozone for I min/53ug almost and aqueous ozone 1min/20ug completely eliminated the suspended microorganisms as did 0.5% NaOCl	High-concentrated gaseous and aqueous ozone was dose-, strain- and time-dependently effective against the tested microorganisms in suspension	–
Antimicrobial action of ozonated water and photodynamic therapy with sonic activation in root canals infected with Enterococcus faecalis	I.A. Nunes et al.	20.04.2022	Brazil	Ex vivo	70	6 – ozonized water without (O + S-) and with sonic activation (O + S +); PDT without (PDT + S-) and with sonic activation (PDT + S +); PDT + ozonized water without (PDT + O + S-) and with sonic activation (PDT + O + S +); and two control groups, one positive (n = 5) and one negative (n = 5). ozonated water and pdt with sonic activation	Ozonated water and pdt with sonic activation	NaOCl	CFU	Here was a significant microbial reduction (p = 0.025), whereas in the groups in which sonic activation was used, the microbial reduction was significantly greater (p = 0.001)	The treatments significantly reduced the number of microorganisms in the root canals. Sonic activation helped to increase the microbial reduction in infected root canals	–
The antimicrobial effect of different ozone protocols applied in severe curved canals contaminated with Enterococcus faecalis: ex vivo study	M.M. Moraes et al.	15.01.2021	Brazil	Ex vivo	50	3 – experimental – 15 1 – positive control	Ozone in gaseous and water and ultrasonic	NaOCl	Cfu and pcr	NaOCl positive control group presented total elimination of CFU/ml bacterial counting	Based on the results of the present study, it can be concluded that the 3 ozone protocols assessed are similar in regard to E. Faecalis reduction	NaOCl presented better antimicrobial effect than ozone
Evaluation of antimicrobial efficacy of gaseous ozone and ozonized water against enterococcus faecalis biofilm	A. Dawood	21.01.2021	Egypt	In vitro	40	4 – NaOCl, ozone, ozonated water, gaseous ozone, normal saline	Ozonated water gaseous ozone and NaOCl	Saline	CFU	Antimicrobial reduction was observed more with NaOCl	It can be concluded that the gaseous ozone reduced E. Faecalis number significantly but was not able to eradicate it completely	Gaseous ozone could be used as a synergistic disinfectant but not an alternative to sodium hypochlorite

The present systematic review focuses on the use of ozone therapy in endodontics for root canal irrigation. Based on the eligibility criteria of the present systematic review, six studies were considered for qualitative synthesis. The search criteria for this review was considered from the studies published between 1st January 2018 and 31st March 2023. From the six studies included, one study was reported in the year 2019, one study in 2020, three studies were reported in 2021 and one study was reported in 2022. All these studies compared the antibacterial property of ozone therapy with that of other irrigating solutions used in root canal treatment. All the studies were done on the extracted human teeth. With respect to the study design, two studies had in-vitro study design and four studies had ex-vivo study design.

A total of 235 teeth were involved in the included studies and the sample size ranged from the lowest of 15 extracted teeth to the highest of 70 extracted human teeth. With respect to the characteristics of the included teeth, cases of re-treatment were chosen as test subjects in the study by Kumar et al. [20] In the study by Nunes et al. [15] teeth with straight channels, no internal or external resorption, fracture, or endodontic filling were included. Teeth with severe curved canals of 20° to 40° were included in the study by Moraes et al. [16] A study by Dawood A. [17] used single-canalled permanent teeth with mature closed apices. Thus, the type of teeth and the characteristics of teeth included across studies differed with few being straight canals while few being canals with severe curvature. Only in one of the studies, the strains of the microorganisms like, Staphylococcus aureus were obtained from the laboratories. The bacteria were then incubated and inoculated in the broth. 1 ml of each bacterial suspension was then added to the tube, and the mixture was incubated for an additional 24 hours. The bacteria were then exposed to ozonated water or ozonated gas to evaluate CFUs [18]. In all studies, the extracted human teeth were obtained which were sectioned to have standardized root lengths. The canals were then irrigated after instrumentation with conventional irrigating solutions like NaOCl followed by EDTA. The bacterial suspensions obtained from the laboratories were suspended into the root canals and inoculated and subjected to assess CFUs.

In the included studies of this review, ozone was used as both, aqueous form and gaseous form across the studies. A study by Jimenez-Gonzalez et al. [17] assessed superoxidized oxoral and oxoral paste as root canal disinfectant. Agostini et al. [19] demonstrated the use of both, ozonated water and ozonated gas as root canal irrigant. Likewise, studies by Kumar et al. [2] and Dawood [18] also presented with similar intervention groups. A study by Nunes et al. [15] focused on ozonated water without (O + S-) and with sonic activation. In four of the study, NaOCl a conventional root canal irrigant was used as a positive control while one study compared ozone with chlorhexidine and other with a normal saline.

All the included studies assessed the antibacterial property via CFU count. Of the six studies, four studies supported the use of ozone therapy over other irrigating solutions while two studies favoured the use of NaOCl over ozone therapy in root canal treatment. A study by Jimenez-Gonzalez et al. [17] demonstrated a permanent reduction in the bacterial growth during 1–18 days of using oxoral® plus $Ca(OH)_2$. The combination produced an alkaline pH that prevented E. Faecalis from growing into the root canals, which made the action noteworthy. According to a study by Agostini et al. [19], ozonated gas was noticeably more effective against E. faecalis than ozonated water.[2] A significant reduction in microbes was reported with sonic activation ozone therapy by Nunes et al. [15] In contrast, Moraes et al. [16] stated that ozone therapy did show effect against root canal microorganisms, however, when compared with NaOCl, the later presented with higher effects against E.faecalis reduction. On the same line, Dawood A [18] gave supporting data demonstrating that while gaseous ozone was able to drastically lower the amount of E. faecalis, it was unable to remove it entirely. Furthermore, it was mentioned that ozone therapy was to be used in conjunction with sodium hypochlorite, not as a substitute for it.

The possible reason for the poor performance of ozone over NaOCl was longer application time with lesser concentration. Studies also report ozone-generating machines contributing to the variation in the obtained results. It should be noted that there is still no standard for application frequency to be effective against canal microbes. According to Hems et al. [20] ozone exhibited a substantial antibacterial impact after being administered for 4 minutes [20] while another study reported that using ozonized water and gaseous ozone for more than 20 minutes did not completely eradicate E. faecalis [21].

Majority of the included studies in this review investigated the action of ozone therapy on E. faecalis bacterial samples. The possible reason for this could be the linkage of pathogen with chronic apical periodontitis and failed root canal filled teeth. Moreover, the culture of E. faecalis is non-fastidious and easy to obtain [22]. Differences in specimen size and type, bacterial count, incubation duration, depth of bacterial invasion, irrigant concentrations, and irrigant quality are among methods variations that could account for the discrepancy in CFU values between studies.

The included studies in this review have some limitations when considered the methodology and canal characteristics considered with respect to curvature. Also, the sample size of included studies was small.the ozone therapy used in studies varied with respect to the form of ozone used.

The randomized controlled trials were not considered while collection of data. Thus, future reviews can include studies that have large sample sizes, severly curved canals and in vivo study designs.

CONCLUSION

Given the study's constraints, it may be said that ozone therapy outperforms traditional root canal irrigating solutions in terms of antibacterial effects. The use of ozone therapy has a significant impact in

reducing colony forming units and thus can be used as a modest root canal irrigant. However, considering the data coming from the in-vitro and ex-vivo studies, clinicians should cautiously take decision on the use of ozone therapy as an adjuvant or as an alternative to conventional root canal irrigants.

Limitations: Studies included had methodological variations, canal characteristics presented dissimilarity with respect to curvature, small sample size, variation in the mode of use of ozone therapy (few with aqueous: few with gaseous form), only ex-vivo or in-vitro studies were included in the review

Future recommendations: Studies with large sample size should be conducted. The studies on severely curved canals with in vivo randomized controlled study design should be considered.

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Purva Doshi – conducted a comprehensive literature review, contributed to manuscript preparation, including writing, and was responsible for editing the text, ensuring academic precision and stylistic excellence.

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Surya R. Srinidhi – performed the review of the article, providing valuable recommendations for improving its content.

Mayuresh Bhujbal – ensured data collection, forming the basis for the empirical part of the study.

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