The Effect of Adjuvant Ozone Therapy in Nonsurgical Periodontal Treatment: A Systematic Review with Meta-analysis

Daniela Pafiadache Thomé,[1] Francisco Wilker Mustafa Gomes Muniz,[2] Alessandra Cardoso Nicolini,[1] Cassiano Kuchenbecker Rösing[1] and Juliano Cavagni[1]

[1]Department of Periodontology, Faculty of Dentistry, Federal University of Rio Grande do Sul, Brazil; [2]Department of Periodontology, Faculty of Dentistry, Federal University of Pelotas, Brazil.

Abstract

Aims: To evaluate the efficacy of ozone therapy as an adjunct to non-surgical periodontal therapy.

Materials and methods: Randomized clinical trials that used any form of ozone therapy as adjuvant to nonsurgical periodontal therapy were included. MEDLINE-PubMed, Embase and Scopus databases were searched to identify these studies. Three different meta-analyses were conducted for reduction of probing pocket depth (PPD), clinical attachment (CAL) gain and reduction of bleeding on probing (BoP).

Results: Thirteen studies were included, which utilized ozonated water, ozonated gas, ozonated olive oil and nano-bubble water. Groups that used adjunct ozone therapy demonstrated significantly higher CAL gain when compared to individuals that received scaling and root planing (SRP) alone or in association with placebo (Mean difference [MD]: -0.32; 95% Confidence interval [95%CI]: -0.52 – -0.11). Regarding PPD reduction, a significantly greater reduction was also observed in groups that used ozone therapy in comparison to control groups (MD: -0.41; 95%CI: -0.71– -0.11). However, no statistically significant differences between ozone therapy and controls were found for BoP (MD: -6.49; 95%CI: -18.74–5.75).

Conclusions: It was concluded that ozone therapy when used as an adjuvant to non-surgical periodontal therapy may provide modest additional gain in CAL and higher reduction in PPD.

Keywords: Dentistry; ozone therapy; periodontal diseases; periodontitis.

INTRODUCTION

Ozone is an instable gas made up of three oxygen atoms that is considered a potent oxidant. It was discovered in the mid-nineteenth century by the German researcher Dr. Christian Friedrich, who observed a characteristic odor when oxygen was subjected to electric discharge (Nagarakanti and Athuluru, 2011). He further suggested that ozone, besides being an oxidant, could also be exploited as a potential disinfectant. The first reports of ozone usage as a therapeutical agent are during World War I (1914-1918) when German and English doctors topically applied ozone to infected wounds. They suggested that ozone had antibiotic, hemodynamic and anti-inflammatory properties (Stoker, 1902).

The application of ozone therapy in medicine and dentistry was justified by its biological effects, with improved oxygen metabolism, increased cellular energy, immunomodulatory capacity and improvement of the antioxidant defense system (Bocci *et al.*, 1998). Therefore, ozone was proposed as a treatment alternative or an adjunct treatment measure. The interest in using ozone in dentistry is mainly due to infectious diseases associated with the oral cavity. There is some evidence showing that ozone therapy has the potential to be used as a support for conventional treatments e.g. periodontal disease (Nogales *et al.*, 2008).

Periodontitis is a multifactorial inflammatory disease which is related to dysbiotic biofilms and is characterized

Correspondence to: Francisco Wilker Mustafa Gomes Muniz (Rua Gonçalves Chaves, 457, Pelotas/RS, Brazil, 96015-560.
Email: wilkermustafa@gmail.com.

by progressive destruction of the tooth-supporting apparatus (Papapanou *et al.*, 2018). The mainstay of periodontal treatment is the mechanical removal of microbial plaque and calculus by scaling and root planing (SRP) (Kotsilkov and Popova, 2010). Due to the location within the gingival and dental tissues that are not easily accessed by periodontal instruments (Armitage *et al.*, 2003; Dayan *et al.*, 2004), the use of adjunct solutions, such as chlorhexidine, povidone–iodine, saline, and hydrogen peroxide for subgingival irrigation, has been explored (Wennström *et al.*, 1987; Hoang *et al.*, 2003; Slots, 2012). However, there is no consensus on the best method to improve the outcome of mechanical treatment (Uraz *et al.*, 2019).

The application of ozone as an adjunctive treatment represents a novel approach in the management of periodontitis and can be an alternative treatment option. Considering the great potential of use and the clinical interest for ozone therapy in dentistry, a careful evaluation of the literature is essential. Recently, a systematic review was published on this topic and did not demonstrate a positive effect of ozone therapy as adjunct to scaling and root planning (Moraschini *et al.*, 2020). In this review, even though the authors refer to the inclusion of on only randomized clinical trials, this was not the case. In addition, their search strategy was more restricted. Hence, a sensitivity analysis was not performed, which could contribute to the understanding of the source of the encountered heterogeneity.

In this sense, there is a lack of a sound body of evidence in the topic that motivated the conduction of the present study. The aim of this systematic review was to evaluate the efficacy of ozone therapy as an adjunct to non-surgical periodontal therapy.

MATERIAL AND METHODS

Focused question

The focused question supporting this study was: "In adults with periodontitis (Patients), does the use of ozone as an adjunct to nonsurgical periodontal therapy (Intervention), when compared to nonsurgical periodontal therapy alone, in association with placebo or in association with chlorhexidine (Comparison), promotes significantly improvement in the periodontal parameters (Outcome)?

Data sources

This systematic review followed the PRISMA Statement recommendations (Moher *et al.*, 2009) and the "umbrella" approach was used to identify relevant randomized clinical trials (RCT) regarding ozone therapy in dentistry. Based on a published protocol (PROSPERO- CRD42019147790) of a systematic review using ozone in dentistry, relevant RCTs were retrieved using ozone therapy in association with nonsurgical periodontal treatment. The search was performed up to January 2020. Studies were searched, retrieved and analyzed in MEDLINE-PubMed, Embase and Scopus databases. Hand search was performed in the list of references of all selected studies included at this phase and a related systematic review were also searched for eligibility (Azarpazhooh and Limeback, 2008). Articles were not restricted by language or publication data. In MEDLINE-PubMed, the following search strategy was performed:

#1 – Ozone[MeSH Terms] OR Ozone[Text word] OR ozonated[Text word] OR HealOzone[Title/abstract] OR nano-bubble[Title/abstract]

#2 –Dentistry[MeSH Terms] OR Dentistry[Text Word] OR dental[Text word] OR dentition[MeSH Terms] OR dentition[Text word] OR Biopsy[Mesh Term] OR Biopsy[Title/abstract] OR Dental Implants[Mesh Term] OR periodontal diseases[MeSH Terms] OR periodontal treatment[Title/Abstract] OR periodontitis[Title/abstract] OR DMF Index[MeSH Terms] OR dental caries[MeSH Terms] OR Dental prosthesis[MeSH Terms] OR Facial pain[MeSH Terms] OR facial pain[Title/abstract] OR Root Canal Irrigants[MeSH Terms] OR Root Canal Irrigants[Text Word] OR Mucositis[MeSH Terms] OR wound healing[MeSH Terms] OR wound healing[Text word] OR Temporomandibular Joint Disorders[MeSH Terms] OR Temporomandibular Joint Disorders[Text word] OR ulcer[MeSH Terms] OR ulcer[Text word] OR herpetic lesion[Title/abstract] OR denture[Text Word] OR oral[Text word] OR buccal[Text word]

3 - #1 and #2

In the other databases, an adaptation of the same search strategy was performed.

Studies Selection

Only RCTs were selected. The search process and study selection were carried out by two researchers independently (DPT and ACN). When a consensus was not possible, a third researcher was involved in this process (JC). The agreement between the researches resulted in a kappa of 0.94.

Studies were selected for full reading and data extraction if they fulfilled the following criteria:

1. Only RCTs (both parallel or split-mouth).
2. Patients: adults with diagnosis of periodontitis.
3. Test group: nonsurgical mechanical periodontal therapy in association with ozone therapy. Ozone of any form was accepted.
4. Control group: nonsurgical mechanical periodontal therapy alone or in association with placebo or chlorhexidine.
5. Outcomes: At least two assessment of the following periodontal parameters: probing depth, clinical attachment level or bleeding on probing.

6. Studies with at least 4-weeks of follow-up.

Studies were excluded after full reading if they presented any of the following criteria:

1. Observational or experimental animal studies.
2. Letters, case reports or literature reviews.
3. Studies without control group.

Data Extraction

The data extraction was carried out independently by two researchers (DPT and AN). When a consensus was not possible, a third researcher was involved in this process (JC). An excel spreadsheet was specifically designed for the present study, summarizing the most relevant data from each selected study (author, title, year, country, study design, number of participants, test and control group, follow-up time, mean and standard deviation of each periodontal parameter at all follow-up periods, age, gender and when ozone therapy was applied). When data was missing, the corresponding authors were contacted by e-mail.

Risk of bias assessment

The risk of bias in RCTs was assessed by two reviewers (DPT and AN) and discrepancies were resolved by a third one (JC). COCHRANE Collaboration Tool (Higgins *et al.*, 2011) was used and included randomization process, allocation concealment method, blinding of the participants, examiners and outcome evaluators, incomplete outcome data, selective outcome reports and other outcome bias. Studies were classified as low risk, unclear risk and high risk of bias.

Statistical Analyses

Different meta-analyses were conducted in the present study comparing mechanical therapy + ozone with mechanical therapy + placebo or mechanical therapy alone. Mean difference between baseline and last follow-up appointment (two or three months of follow-up) after nonsurgical periodontal therapy was computed for three outcomes: reduction of probing pocket depth, clinical attachment gain, and reduction of bleeding on probing (BoP). For all outcomes, data on mean difference and standard deviation were obtained or calculated for both test and control groups. In order to evaluate the source of heterogeneity, subgroup analyses were performed and two aspects were considered: first, particularities of the studies such as follow-up periods (3-months or other follow-up) and forms of administrating ozone therapy (gaseous ozone, ozonated nano-bubble water or ozonated olive oil). Second, methodological quality was considered, and the studies were stratified according to the risk of bias in low risk of bias (comprising all criteria) or unclear risk of bias (when risk was found in at least one criterion).

Due to the low number of studies using adjunct chlorhexidine, no meta-analysis was performed to this positive control group. Heterogeneity was assessed by Q test and quantified with I^2 statistics. All meta-analyses were performed using a random-effects model with the software RevMan 5.3. The random-effect model was used as a high statistical heterogeneity (I^2 >50%) was detected, and different forms of administrating ozone therapy were employed among the included studies in the meta-analysis. In addition, to all meta-analyses performed, publication bias was assessed by funnel plot analysis. It was used the Egger's test. The overall quality of evidence for each meta-analyses was rated using the GRADE approach (Guyatt *et al.*, 2011).

RESULTS

General characteristics of the included studies

The search strategy of this systematic review gathered 1,894 studies and 13 fulfilled the inclusion criteria (Dodwad *et al.*, 2011; Patel *et al.*, 2012; Hayakumo *et al.*, 2013; Katti and Chava, 2013; Yilmaz *et al.*, 2013; Shoukheba and Ali, 2014; Al Habashneh *et al.*, 2015; Gandhi 2019; Kaur *et al.*, 2019; Seydanur Dengizek *et al.*, 2019; Tasdemir *et al.*, 2019; Uraz *et al.*, 2019; Çalışır *et al.*, 2019). Figure 1 summarizes the flowchart of the inclusion process and the reasons for exclusion.

Risk of bias analysis

As this systematic review included only RCTs, these studies were evaluated by the Cochrane Collaboration tool, and Figure 2 summarizes this analysis. Three of the studies were classified with "low risk" of bias in all of the analyzed criteria (Hayakumo *et al.*, 2013; Seydanur Dengizek *et al.*, 2019; Tasdemir *et al.*, 2019). On the other hand, information such as random sequence generation, allocation concealment or blinding, were not adequately reported in the majority of the included studies, being classified as "unclear" risk of bias.

Qualitative results

The main methodological characteristics and results are summarized in Table 1. In the studies, different forms of ozone administration were used including water (Dodwad *et al.*, 2011; Katti and Chava, 2013; Al Habashneh *et al.*, 2015; Kaur *et al.*, 2019), gas (Yilmaz *et al.*, 2013; Seydanur Dengizek *et al.*, 2019; Tasdemir *et al.*, 2019; Uraz *et al.*, 2019; Çalışır *et al.*, 2019), ozonated olive oil (Patel *et al.*, 2012; Shoukheba and Ali, 2014; Gandhi *et al.*, 2019) and nano-bubble water (Hayakumo *et al.*, 2013). Follow-up periods were two weeks (Yilmaz *et al.*, 2013), one month (Dodwad *et al.*, 2011; Katti and Chava, 2013), six weeks (Çalışır *et al.*, 2019), two months (Patel *et al.*, 2012; Hayakumo *et al.*, 2013), three months (Al Habashneh *et al.*, 2015; Gandhi *et al.*, 2019; Kaur *et al.*, 2019; Tasdemir *et al.*, 2019; Uraz *et al.*, 2019) and six months (Shoukheba and Ali, 2014).

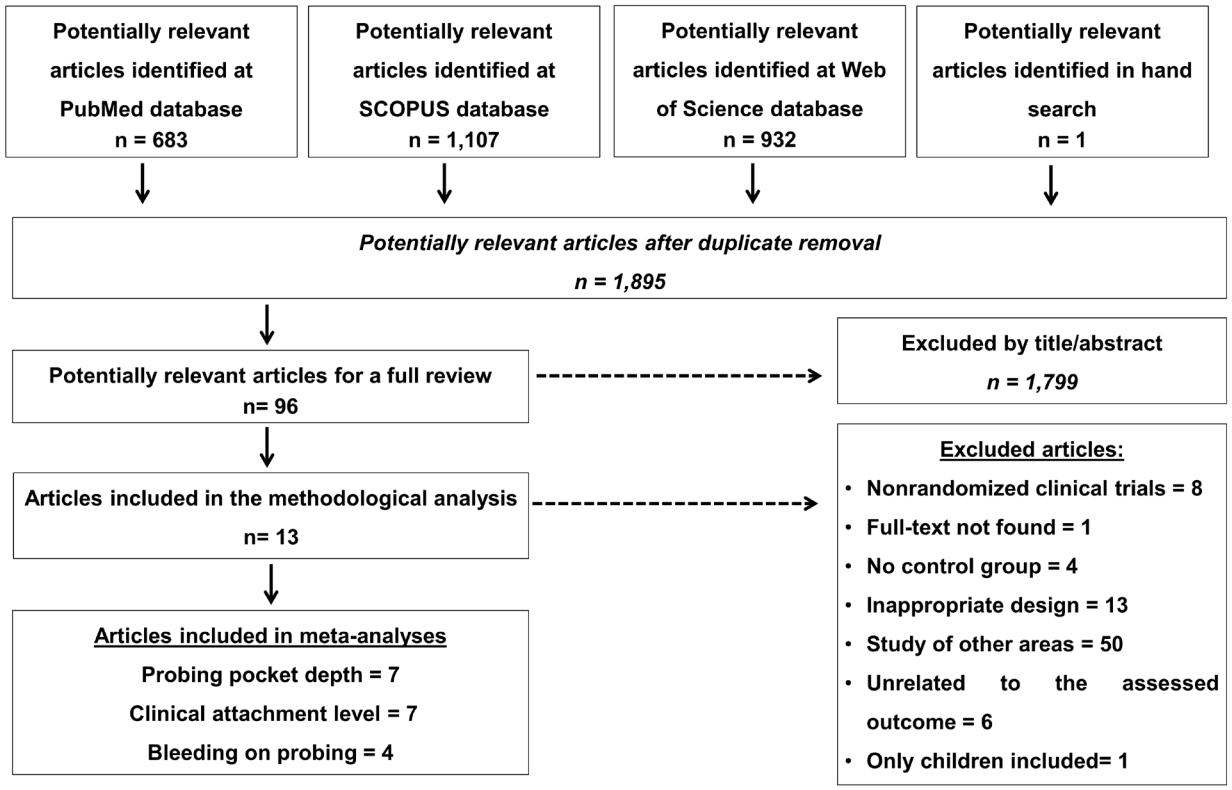

Figure 1. Study Flowchart.

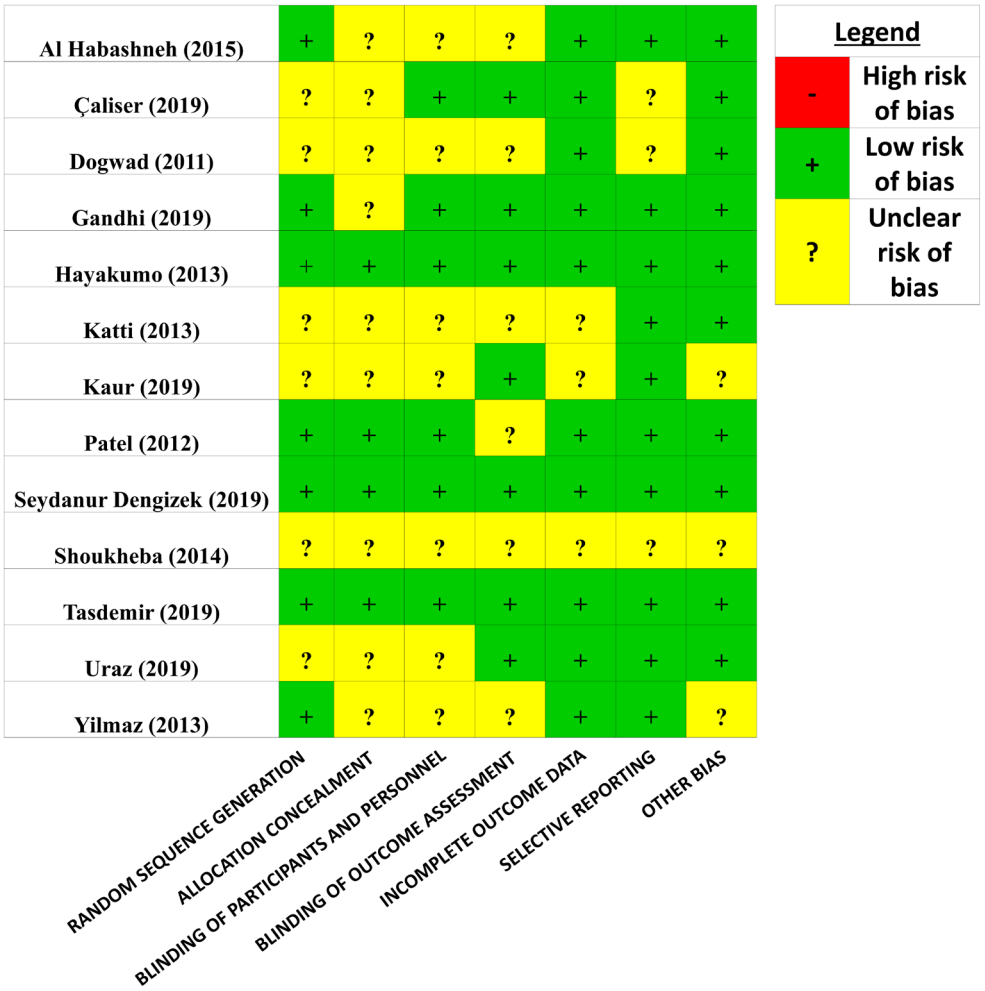

Figure 2. Risk of bias analysis.

Table 1. Objectives, main characteristics and results of the included studies.

Author, year; Country;	Study design; Follow-up	Test and control groups (sample size)	Age; male/female; Sample characteristics; Smoking exposure	Main results
Al Habashneh, 2015, Jordan	RCT (parallel); 3 months	Test: SRP + irrigation with ozonated water (n=20) Control: SRP + irrigation with distilled water (n=20)	Mean age: 39.7±13.7 (test) and 39.0±10.2 (control); 6/14 (test) and 7/14 (control); Good general health; Nonsmokers	Both groups improved after periodontal therapy. **PPD (in mm):** Baseline – Test: 2.8±0.4 – Control: 2.4±0.4 (p=0.001) 3 months – Test: 2.0±0.4 – Control: 2.0±0.3 (p=0.059) **CAL (in mm):** Baseline – Test: 1.5±1.2 – Control: 1.7±1.2 (p=0.452) 3 months – Test: 1.7±1.2 – Control: 1.1±1.0 (p=0.934) **BOP (in %):** Baseline – Test: 75.0±26.0 – Control: 72.0±28.1 (p=0.491) 3 months – Test: 23.0±20.0 – Control: 26.0±24.0 (p=0.399)
Çalisir, 2019, Turkey	RCT (split-mouth); 6 weeks	Test: SRP + ozone gas applied every two days for one week (n=27). Control: SRP + irrigation with saline solution every two days for one week (n=27).	Mean age: 25.66±4.01 (both groups); 13/14 (both groups); Good general health; Nonsmokers	Both groups improved after periodontal therapy. **PPD (in mm):** Baseline – Test: 5.67±0.92 – Control: 5.11±0.80 (p=0.127) 6 weeks – Test: 3.04±0.94 – Control: 3.19±0.79 (p=0.533) **CAL (in mm):** Baseline – Test: 8.26±1.13 – Control: 8.00±1.36 (p=0.442) 6 weeks – Test: 5.78±1.19 – Control: 6.33±1.18 (p=0.090)
Dogwad, 2011, India	RCT (parallel); 1 month	Test: SRP + ozonated water (n=10) Control: SRP + 0.2% CHX (n=10)	Range: 30 – 50 (both groups); NR/NR (both groups); Good general health; Smoking status NR	Both groups improved after periodontal therapy. **PPD (in mm):** Baseline – Test: 6.3±0.0.48 – Control: 6.2±0.42 (p=NR) 1 month – Test: 3.8±0.42 – Control: 4.6±0.70 (p=NR)
Gandhi, 2019, USA	RCT (split-mouth); 3 months	Test: SRP + ozonated olive oil (n=25). Control: SRP + 0.2% CHX (n=25).	Range: 30 – 60 (both groups); NR/NR (both groups); Good general health; Nonsmokers	Both groups improved after periodontal therapy. **PPD (in mm):** Baseline – Test: 5.47±0.32 – Control: 5.41±0.32 (p=0.96) 3 months – Test: 3.01±0.40 – Control: 2.78±0.47 (p=0.68) **CAL (in mm):** Baseline – Test: 5.02±0.37 – Control: 4.78±0.37 (p=0.96) 3 months – Test: 2.92±0.43 – Control: 2.63±0.40 (p=0.68)

Table 1 continued overleaf.....

Table 1 continued.....

Hayakumo, 2013, Japan	RCT (parallel); 2 months	Test: SRP + nano-buble water (n= 10). Control: SRP + tap water (n= 11). Mean age= 45.9±14.8 6 females and 16 males	Mean age: 45.9±13.8 (test) and 46.00±16.4 (control); 16/6 (both groups and before exclusions); Good general health; Nonsmokers	Both groups improved after periodontal therapy. **PPD (in mm):** Baseline – Test: 2.59±0.3 – Control: 2.58±0.3 (p>0.05) Change from baseline (after 2 months) – Test: 0.29±0.2 – Control: 0.14±0.2 (p<0.05) **CAL (in mm):** Baseline – Test: 2.73±0.3 – Control: 2.79±0.5 (p>0.05) Change from baseline (after 2 months) – Test: 0.27±0.2 – Control: 0.09±0.2 (p<0.05) **BOP (in %):** Baseline – Test: 32.95±15.7 – Control: 30.20±14.8 (p>0.05) Change from baseline (after 2 months) – Test: 13.47±9.2 – Control: 6.97±10.8 (p>0.05)
Katti, 2013, India	RCT (split-mouth); 1 month	Test: SRP + ozonized water (n=30). Control: SRP + saline solution (n=30).	Range: 20 – 60 (both groups); NR/NR (both groups); Good general health; Nonsmokers	Both groups improved after periodontal therapy. **PPD (in mm):** Baseline – Test: 5.4±0.92 (mesial) and 6.0±1.4 (distal) – Control: 5.4±1.2 (mesial) and 5.7±1.5 (distal) (p=0.72 – mesial and p= 0.14 - distal) 1 month – Test: 4.0±0.9 (mesial) and 4.7±1.6 (distal) – Control: 4.7±1.1 (mesial) and 5.0±1.3 (distal) (p=0.02 mesial and p=0.18 distal) **CAL (in mm):** Baseline – Test: 4.8±1.9 (mesial) and 5.4±1.5 (distal) – Control: 4.8±1.7 (mesial) and 4.9±2.0 (distal) (p=0.75 – mesial and p= 0.05 - distal) 1 month – Test: 3.5±1.5 (mesial) and 4.0±1.4 (distal) – Control: 4.2±1.4 (mesial) and 4.6±2.0 (distal) (p=0.004 mesial and p=0.14 distal)
Kaur, 2019 India	RCT (split-mouth); 3 months	Test: SRP + ozonated water (n=20). Control: SRP + 0.2% CHX (n=20).	Range: 30 – 60 (both groups); NR/NR (both groups); Good general health; Smoking status NR	Both groups improved after periodontal therapy. **PPD (in mm):** Change from baseline (after 3 months) – Test: 1.55±0.69 – Control: 1.15±0.81 (p=0.560) **CAL (in mm):** Change from baseline (after 3 months) – Test: 0.90±NR – Control: 0.75±NR (p=0.294)

Table 1 continued overleaf.....

Table 1 continued.....

Patel, 2012, India	RCT (split-mouth); 2 months	Test: SRP + ozonated olive oil (n=20). Control: SRP only (n=20).	Mean age: 42.64±7.8 (both groups); 12/8 (both groups); NR; NR	Both groups improved after periodontal therapy. **PPD (in mm):** Baseline – Test: 8.64±0.39 – Control: 9.01±0.60 (p>0.05) 2 months – Test: 3.82±0.38 – Control: 3.06±0.44 (p<0.05) **CAL (in mm):** Baseline – Test: 9.30±0.66 – Control: 9.02±0.67 (p>0.05) 2 months – Test: 3.87±0.35 – Control: 3.15±0.43 (p<0.05)
Seydanur Dengizek, 2019, Turkey	RCT (parallel); 1 months	Test: SRP + gaseous ozone in two separate applications five days apart (n=19). Control: SRP + same device used in test group without ozone (n=18).	Mean age: 44.7±5.1 (test) and 45.8±5.6 (control); 8/11 (test) and 8/10 (control); General good health; Both smokers and nonsmokers	Both groups improved after periodontal therapy. **PPD (in mm):** Baseline – Test: 3.8±0.8 – Control: 3.6±0.8 (p>0.05) 1 month – Test: 3.0±0.6 – Control: 3.0±0.8 (p>0.05) **CAL (in mm):** Baseline – Test: 4.4±1.1 – Control: 4.1±0.8 (p>0.05) 1 month – Test: 4.0±0.7 – Control: 3.8±0.8 (p>0.05)
Shoukheba, 2014 Egypt	RCT (parallel); 6 months	Test: SRP + ozonated olive oil gel (n=15). Control: SRP only (n=15).	Range: 21 – 30 (both groups); 9/21 (whole-sample); General good health; Nonsmokers	Only test group improved significantly after periodontal therapy. **PPD (in mm):** Baseline – Test: 5.85±0.29 – Control: 5.87±0.27 (p=0.799) 3 months – Test: 5.04±0.33 – Control: 5.74±0.26 (p<0.01) 6 months – Test: 5.61±0.37 – Control: 5.84±0.20 (p=0.051) **CAL (in mm):** Baseline – Test: 5.38±0.34 – Control: 5.3±0.2 (p=0.410) 3 months – Test: 4.86±0.36 – Control: 5.33±0.26 (p<0.01) 6 months – Test: 5.28±0.31 – Control: 5.26±0.24 (p=0.860) **BOP (in %):** Baseline – Test: 74.23±3.95 – Control: 74.53±3.11 (p=0.819) 3 months – Test: 54.07±6.99 – Control: 71.07±5.16 (p=<0.01) 6 months – Test: 72.66±2.71 – Control: 72.87±4.17 (p=0.877)
Tasdemir, 2019, Turkey	RCT (split-mouth); 3 months	Test: SRP + gaseous ozone (n=36). Control: SRP only (n=36).	Mean age: 43.7 ± 10.2 (both groups); 18/18 (both groups); General good health; Nonsmokers	Both groups improved after periodontal therapy. **PPD (in mm):** Baseline – Test: 3.24±0.7 – Control: 3.17±0.6 (p=0.456) 3 months – Test: 1.97±0.5 – Control: 2.01±0.5 (p=0.822) **CAL (in mm):** Baseline – Test: 3.9±0.6 – Control: 3.7±0.5 (p=0.641) 3 months – Test: 4.2±0.4 – Control: 4.3±0.3 (p=0.619) **BOP (in %):** Baseline – Test: 90±5.7 – Control: 92±6.8 (p=0.710) 3 months – Test: 32.9±12.5 – Control: 31.1±11.9 (p=0.814)

Table 1 continued overleaf.....

Table 1 continued.....

Uraz, 2019, Turkey	RCT (split-mouth); 3 months	Test: SRP + gaseous ozone (n=18). 80% oxygen 3 times for 30s (every 3rd day) for 1 week. Control: SRP only (n=18).	Mean age: 40±6.51 (both groups); 9/9 (both groups); General good health; Nonsmokers	Both groups improved after periodontal therapy. **PPD (in mm):** Baseline – Test: 5.87±1.13 – Control: 5.91±1.05 (p>0.05) 3 months – Test: 3.96±0.95 – Control: 3.98±0.92 (p>0.05) **BOP (in %):** Baseline – Test: 69.44±12.54 – Control: 67.42±18.95 (p>0.05) 3 months – Test: 15.55±18.60 – Control: 19.44±22.15 (p>0.05)
Yilmaz, 2013, Turkey	RCT (parallel); 3 months	Test: SRP + topical gaseous ozone (n=10). Control: SRP only (n=10). 18 females and 12 males	Mean age: 41.40±8.86 (test) and 41.40±4.62 (control); 3/7 (test) and 5/5 (control); General good health; Nonsmokers	Both groups improved after periodontal therapy. **PPD (in mm):** Baseline – Test: 3.91±0.24 – Control: 3.95±0.13 (p=NR) 3 months – Test: 2.83±0.27 – Control: 2.91±0.12 (p=0.365) **RAL (in mm):** Baseline – Test: 10.16±1.02 – Control: 10.06±0.81 (p=NR) 3 months – Test: 9.34±1.04 – Control: 9.26±0.87 (p=0.451)

Legend: BoP: bleeding on probing; CAL: clinical attachment loss; CHX: chlorhexidine; NR: not reported; PPD: probing pocket depth; RAL: relative attachment level; RCT: randomized clinical trial; SRP: scaling and root planing.

Ozone associated to SRP was compared to SRP alone (Patel *et al.*, 2012; Yilmaz *et al.*, 2013; Shoukheba and Ali, 2014; Uraz *et al.*, 2019) or to a placebo (Hayakumo *et al.*, 2013; Katti and Chava, 2013; Al Habashneh *et al.*, 2015; Seydanur Dengizek *et al.*, 2019; Tasdemir *et al.*, 2019; Çalışır *et al.*, 2019) in most of the included studies. The adjunct use of chlorhexidine was performed in three studies (Dodwad *et al.*, 2011; Gandhi *et al.*, 2019; Kaur *et al.*, 2019). Most of the studies presented no significant differences between test and control groups for plaque index (Patel *et al.*, 2012; Yilmaz *et al.*, 2013; Shoukheba and Ali, 2014; Çalışır *et al.*, 2019), gingival index (Shoukheba and Ali, 2014; Çalışır *et al.*, 2019), clinical attachment level (Hayakumo *et al.*, 2013; Katti and Chava, 2013; Shoukheba and Ali, 2014; Çalışır *et al.*, 2019), probing pocket depth (Hayakumo *et al.*, 2013; Yilmaz *et al.*, 2013; Shoukheba and Ali, 2014; Çalışır *et al.*, 2019). Four studies concluded that ozone therapy did not present any additional effect on almost all periodontal parameters (Al Habashneh *et al.*, 2015; Seydanur Dengizek *et al.*, 2019; Tasdemir *et al.*, 2019; Uraz *et al.*, 2019). Clinical parameters, such as clinical attachment level (Tasdemir *et al.*, 2019; Çalışır *et al.*, 2019) and probing pocket depth (Al Habashneh *et al.*, 2015; Çalışır *et al.*, 2019), could be significantly affected by ozone therapy in some studies, which means ozone group performed better than controls. In one study, the adjunctive use of ozone with SRP resulted in a significant improvement of clinical parameters, such as gingival index, sulcus bleeding index, probing pocket depth and clinical attachment level (Patel *et al.*, 2012).

A significantly lower gingival index, plaque index and probing pocket depth were observed in groups that used ozonated water irrigation and chlorhexidine for the comparison within group (Dodwad *et al.*, 2011). However, no comparison between groups is provided. Other two studies presented similar results for groups with ozone therapy and chlorhexidine for all clinical parameters, such as plaque index, gingival index, probing pocket depth and clinical attachment level (Gandhi *et al.*, 2019; Kaur *et al.*, 2019). Both studies demonstrated significant improvements in within group analyses. One of the studies suggests slightly better results for ozone compared to chlorhexidine, with a statistically significant difference in plaque index, favoring the group with ozone (Kaur *et al.*, 2019).

Safety

Five of the included studies clearly evaluated side effects (Patel *et al.*, 2012; Yilmaz *et al.*, 2013; Shoukheba and Ali, 2014; Gandhi *et al.*, 2019; Uraz *et al.*, 2019). Among those, four reported no discomfort or side effects in all treatment modalities (Yilmaz *et al.*, 2013; Shoukheba and Ali, 2014; Gandhi *et al.*, 2019; Uraz *et al.*, 2019). However, in one study, the group that used SRP and ozone therapy presented a significantly higher mean visual analogue scale score for pain/discomfort/tooth hypersensitivity when compared to group that used only SRP (Patel *et al.*, 2012).

Meta-analysis

As mentioned above, meta-analyses were performed for important periodontal parameters. The results revealed that patients who received ozone therapy as adjunct to nonsurgical SRP show a discrete but significant difference for full-mouth CAL gain (MD: -0.32; 95% CI = -0.52 to -0.11; P<0.01) (Figure 3) and full-mouth PPD reduction (MD = -0.41; 95% CI = -0.71 to -0.11; P<0.01) (Figure 4) from baseline to the end of follow-up compared to patients who received SRP alone or in association with placebo. A significant heterogeneity was found for both CAL and PPD (I^2 = 93%, P=0.003 and I^2 = 99%, P=0.008, respectively). No statistically significant differences between ozone therapy and controls were found for full-mouth BoP (MD: -6.49; 95%CI: -18.74 to 5.75, I^2 = 96%, p = 0.30) (Figure 5).

When considering the subgroup analyses, for both PPD and CAL meta-analysis no major difference in the heterogeneity were detected. For both analyses, subgroup I^2 ranged from 67% to 99% (data not shown). Similar results was detected for BoP reduction when the different follow-up periods were considered (data not shown). Conversely, when the subgroups of different forms of administrating ozone therapy was considered, a lower heterogeneity was detected (gaseous ozone: I^2=37%; ozonated nano-bubble water: I^2=0% and ozonated olive oil: I^2= not applicable). Additionally, when subgroup analysis was performed taking into consideration methodological quality of the studies, the results showed that the risk of bias is probably the only factor influencing the encountered heterogeneity (Figure 6).

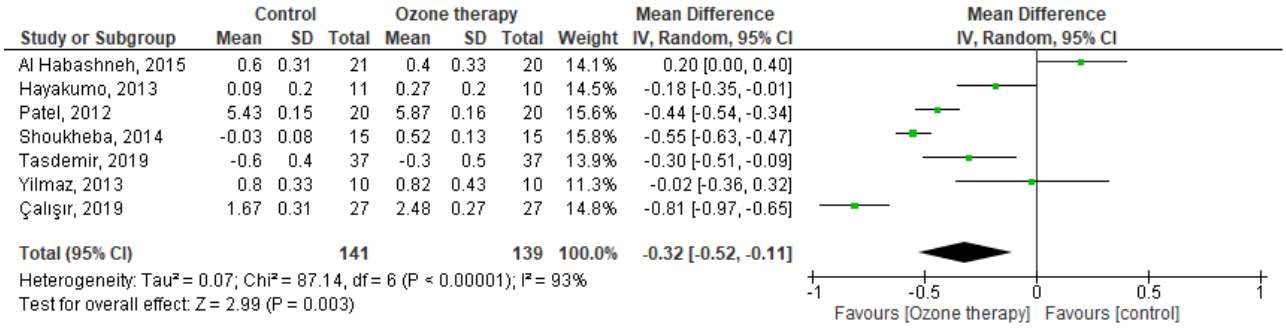

Figure 3. Forest plot for the meta-analysis for clinical attachment level gain in the studies that performed nonsurgical periodontal therapy.

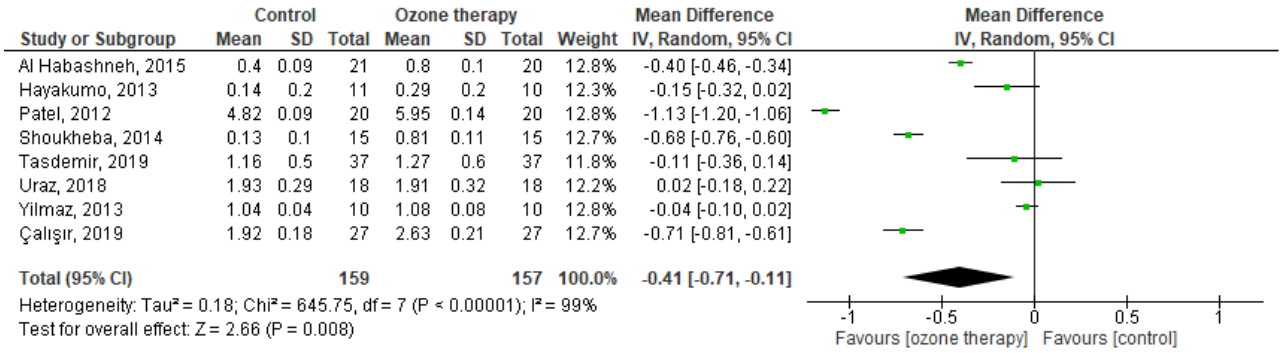

Figure 4. Forest plot for the meta-analysis for probing pocket depth reduction in the studies that performed nonsurgical periodontal therapy.

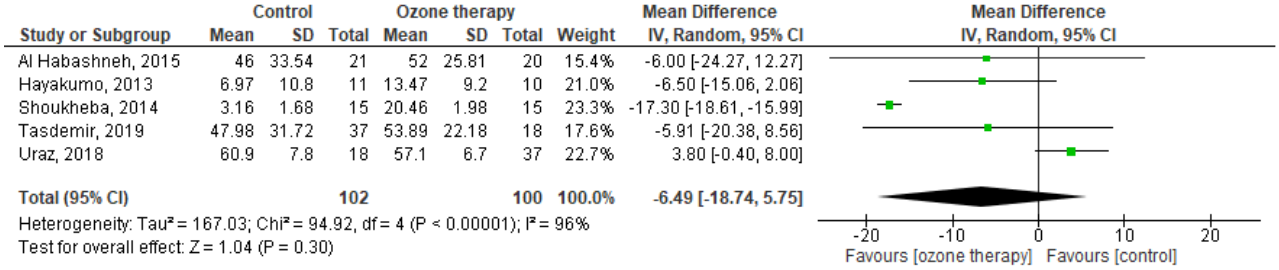

Figure 5. Forest plot for the meta-analysis for bleeding on probing reduction in the studies that performed nonsurgical periodontal therapy.

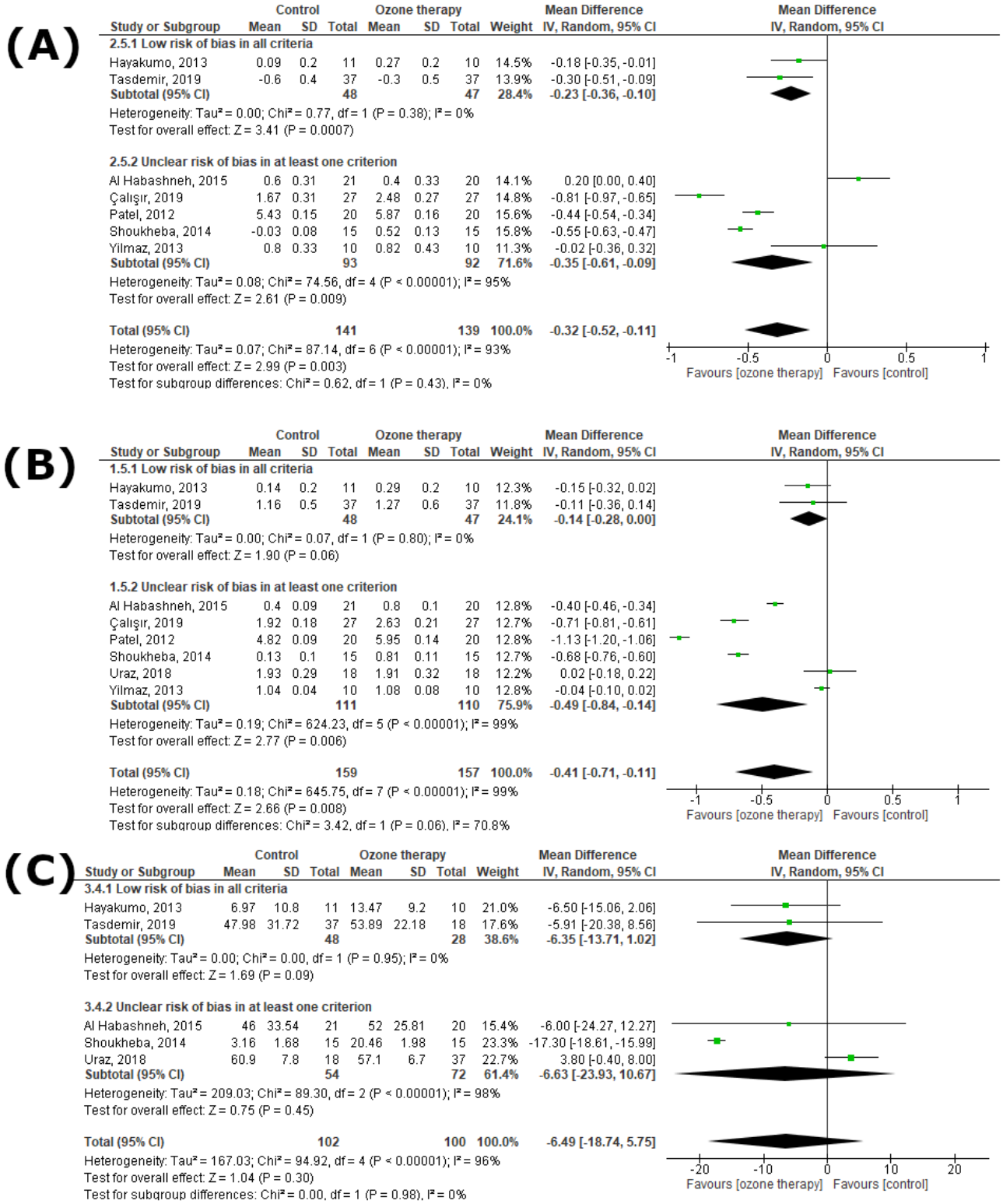

Figure 6. Forest plot for clinical attachment level gain (A), probing pocket depth reduction (B) and bleeding on probing reduction (C), using the different the assessed risk of bias as a subgroup.

Publication bias

Figure 7 displays the funnel plots illustrating the publication bias for each periodontal parameter evaluated. No publication bias were observed for all periodontal parameters CAL (Figure 7A, p=0.18), PDD (Figure 7B, p=0.98) BoP (Figure 7C, p=0.27). However, it is important to highlight that, visually, asymmetries were observed in all analyses.

Quality of the evidence at the review level

Table 2 presents the quality of evidence of all outcomes assessed in the meta-analyses. In both PPD and CAL outcomes, a very low quality of evidence was demonstrated. Conversely, for the BOP analysis, the quality of the evidence was rated as low.

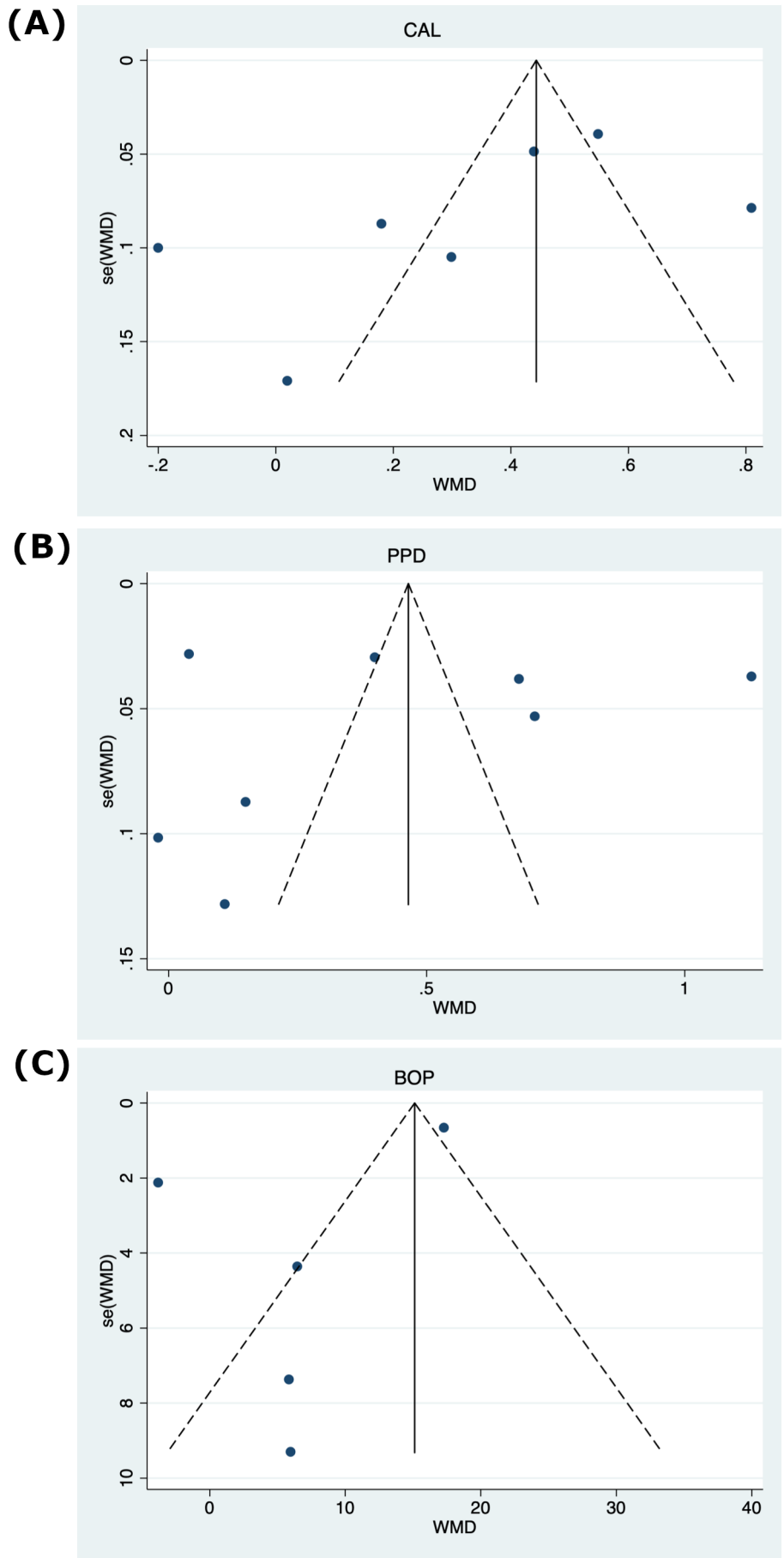

Figure 7. Funnel plot of the risk of bias analysis of clinical attachment level gain (A), probing pocket depth reduction (B) and bleeding on probing reduction (C).

Table 2. Summary of the quality assessment to all outcomes included in the meta-analyses.

Certainty assessment							Summary of findings					
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Ozone therapy group	Control group	Relative (95% CI)	Absolute (95% CI)	Certainty	Importance
Clinical attachment level gain												
7	randomized trials	Serious[a]	Very serious[b]	Not serious	Not serious	None	141	139	-	Mean **0.32 mm lower** (0.52 fewer to 0.11)	⊕OOO VERY LOW	CRITICAL
Probing pocket depth reduction												
8	randomized trials	Serious[a]	Very serious[b]	Not serious	Not serious	None	157	159	-	Mean **0.41 lower** (0.71 lower to 0.11 lower)	⊕OOO VERY LOW	CRITICAL
Bleeding on probing reduction												
4	randomized trials	Serious[a]	Serious[c]	Not serious	Not serious	None	100	102	-	Mean **6.49 lower** (18.74 lower to 5.75 higher)	⊕⊕OO LOW	CRITICAL

Legend: CI: Confidence interval; MD: Mean difference.
Explanations: [a]. At least one study presented an unclear risk of bias in at least one criteria assessed. [b]. A high heterogeneity was detected. [c]. A moderate heterogeneity was detected.

DISCUSSION

The present systematic review analyzed the effects of different ways of delivering ozone therapy as adjunct to nonsurgical periodontal treatment. Most of the available literature bases the potential of ozone therapy on its antimicrobial properties (Seidler *et al.,* 2008), since preliminary *in vitro* studies demonstrated that ozone could be effective against Gram-positive and Gram-negative bacteria, viruses and fungi (Emerson *et al.*, 1982; Greene *et al.*, 1993). To better understand those effects in periodontal treatment and to analyze the currently available information, this systematic review included only RCTs. The idea behind this criterion is to better support the decision-making process. Since periodontal diseases are inflammatory diseases triggered by oral biofilms, the antimicrobial effect is of interest.

Recently, another systematic review assessed the adjunct efficacy of ozone therapy in periodontitis treatment (Moraschini *et al.*, 2020). This study demonstrated no additional benefits, in terms of gain of clinical attachment level, reduction of probing pocket depth and gingival bleeding. However, this study included only nine RCTs and did not appraised all available evidence on the topic. Therefore, the possibility of type II error must not be ruled out. It is important to highlight that the present systematic review included 13 RCT, which may explain the contrasting results. Moreover, meta-analyses for PPD reduction and CAL gain included, respectively, a minimum of 139 and 157 individuals, which may allowed a higher study power. These characteristics may also explain the contrast between qualitative and quantitative results of the present study.

The possible effect of ozone therapy has been demonstrated by its antimicrobial action *in vitro*, which results from oxidation of microbial cellular components and altering homeostasis of oral biofilms (Issac *et al.*, 2015). Transposing such results to clinical outcomes, the meta-analysis conducted in this systematic review for studies that treated periodontitis demonstrated a significant CAL gain and PPD reduction in those that used ozone therapy. However, this was not the case for BoP. In the clinical decision-making process, not only the superiority of a therapy should be analyzed, but also the clinical relevance of the results. In the case of ozone, the effects are modest. However, the potential is clearly demonstrated in PPD and CAL, two important clinical parameters for follow-up of periodontal patients. However, bleeding on probing – that is strongly related to periodontal stability (Lang *et al.*, 1990) - was not affected by the use of ozone. Moreover, among the studies that reported side effects, most of them demonstrated that the use of ozone therapy is safe.

This systematic review focused on clinical parameters, as they are the key that guide decisions in clinical practice. Nevertheless, some results on microbial effects of ozone therapy are important to highlight. This is related to the microbial etiology of periodontitis, where a variety of antimicrobial agents are used as adjunct to periodontal therapy to suppress periodontal pathogens and affect the microbiome (Wilson, 1996). In addition to the clinical parameters evaluated, total number of bacteria (Hayakumo *et al.*, 2013; Yilmaz *et al.*, 2013) and cytokine levels (Tasdemir *et al.*, 2019; Uraz *et al.*, 2019; Çalışır *et al.*, 2019) have also been analyzed. Better results were found using irrigation with ozone nano-bubble water, in comparison with tap water, on the reduction in the mean total number of bacteria in subgingival plaque over a study period of 8 weeks (Hayakumo *et al.*, 2013). Another study presented that the percent reduction of spirochetes using ozonated water was similar as chlorhexidine and povidone iodine in chronic periodontitis patients with 4 weeks of follow-up (Dodwad *et al.*, 2011). Also, subgingival plaque samples were analyzed for assessment of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*, and a reduction could also be seen in both test and control groups, comparing ozone with chlorhexidine (Kaur *et al.*, 2019).

It is important to emphasize that the methods already consolidated and widely studied in the treatment of periodontal diseases, point to the importance of mechanical removal and debridement, to remove the residual biofilm in the periodontal pocket. Thus, adjunctive methods with the use of antimicrobial agents can potentially reduce the bacterial count within the pocket. However, the clinical relevance of such benefits might be questioned (Herrera *et al.*, 2020). If in one hand it is desirable to increase the antimicrobial effects of mechanical therapy, helping in decreasing inflammatory parameters, it should be always kept in mind that the inclusion of an additional therapy has to be looked upon cost-effectiveness. To the best of the authors' knowledge, no studies utilizing ozone therapy as adjunct to periodontal therapy have looked at cost-effectiveness. However, since the clinical benefit is modest and the use of ozone requires specific equipment and is time consuming for the oral health care personnel, this should be taken into consideration in the decision-making process.

Different substances/agents/forms of delivery have been suggested as adjunct to scaling and root planing, however without an additional benefit that could support its use as a standard protocol. In the present study, focus is given to the adjunct effect of ozone therapy subgingivally in an attempt to ameliorate the results of conventional periodontal therapy. This has been tested with chlorhexidine chips, locally-delivered antibiotics and modest results were also achieved (Herrera *et al.*, 2020). For such therapies, cost-effectiveness studies are also virtually inexistent. Ozone therapy was proposed as an alternative agent. The present study tried to compare the results of adjunct use of ozone with chlorhexidine.

In the descriptive analysis, some studies demonstrated an important potential (Dodwad *et al.*, 2011; Gandhi *et al.*, 2019; Kaur *et al.*, 2019). However, due to the high heterogeneity among studies, no meta-analysis could be performed comparing the adjunct use of ozone and chlorhexidine, which may be a considered a limitation in the present study.

In addition, the high statistical heterogeneity detected in all meta-analyses and the lack of explanation for this heterogeneity must be considered when interpreting the results of the present study. It was performed two subgroup analyses for each outcome, which included the different follow-up periods and different forms of administrating ozone therapy, but none of these variables explained the high heterogeneity detected. Conversely, the unclear risk of bias was found to be the main source of heterogeneity to all meta-analyses performed. In this sense, the results encountered in favor of the adjunct use of ozone therapy could be questioned in a clinical scenario. The body of evidence turns to be relatively weak.

The results demonstrated superiority of ozone therapy as compared to negative controls both for CAL gain and PPD reduction. However, the clinical impact of such result should be questioned. This is especially true taking into consideration the limited time of follow-up of the included studies. It has been demonstrated that the results of periodontal therapy tend to flatten overtime and are really dependent on supragingival plaque control and maintenance care (Rosling *et al.*, 2001). This could raise the possibility of using ozone therapy as an adjunct to oral hygiene. However, logistic factors prevent it to be spreadly used.

Another important argument that has to be raised is publication bias, which is very common in studies with adjunct approaches. It is possible that studies without potential benefits are not published. However, due to the low number of studies included in the meta-analyses, publication bias could not be estimated, which may be another limitation of the present study.

Conversely, the strength of the present study relies on the exclusive inclusion of RCTs, which are on the top of the evidence-generating capacity, allowing the understanding of clinical translation. However, the included studies are performed with limited number of individuals and with relatively short-term follow-up. On the other hand, the meta-analyses merge the data and the potential has been demonstrated.

Moreover, two tools were used in order to assess the methodological quality of the included studies. The first is the scale recommended by the Cochrane Collaboration and evaluates the risk of bias of the included studies. A considerable number of studies with unclear risk of bias was observed, demonstrating the poor reporting state of the papers in this subject. The second scale assessed the quality of the evidence of studies included in the meta-analyses, and detected very low degrees of certainty for CAL and PPD and low for BoP. These findings highlight caution in interpreting the findings of the present review since the real quality of the studies cannot be assessed. In addition, the need for methodologically well-conducted and well-reported studies must be reinforced before indicating ozone therapy as adjunct agent in periodontology.

The lack of consistency in the results found must be put into perspective. This may be explained due to the differences in methods used in these clinical studies, concentrations of ozone, duration of application, type of control and method of application. In this sense, further randomized, double-blind and well-controlled clinical trials are needed in order to achieve significant conclusions. Therefore, the push that the industry has continuously given to the use of ozone therapy as adjunct to periodontal therapy should be taken with caution.

CONCLUSION

It was concluded that ozone therapy when used as an adjuvant to nonsurgical periodontal therapy provides very limited additional benefits in terms of PDD reduction and CAL gain. The limited quality and number of studies need to be considered.

ACKNOWLEDGMENTS

The author would like to thank the help of professors Maísa Casarin and Alex Nogueira Haas regarding publication bias assessment. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001. All other funding was self-supported by the authors. The authors report no conflict of interest.

REFERENCES

Al Habashneh R, Alsalman W and Khader Y. Ozone as an adjunct to conventional nonsurgical therapy in chronic periodontitis: A randomized controlled clinical trial. *Journal of Periodontal Research* 2015; **50**:37-43.

Armitage GC and R Research, Science and Therapy Committee of the American Academy of Periodontology Diagnosis of periodontal diseases. *Journal of Periodontology* 2003; **74**:1237-1247.

Azarpazhooh A and Limeback H. The application of ozone in dentistry: A systematic review of literature. *Journal of Dentistry* 2008; **36**:104-116.

Bocci V, Valacchi G, Corradeschi F and Fanetti G. Studies on the biological effects of ozone: 8. Effects on the total antioxidant status and on interleukin-8 production. *Mediators of Inflammation* 1998; **7**:313-317.

Dayan S, Stashenko P, Niederman R and Kupper TS. Oral epithelial overexpression of IL-1alpha causes periodontal disease. *Journal of Dental Research* 2004; **83**:786-790.

Dodwad V, Gupta S, Kumar K, Sethi M and Masamatti S. Changing paradigm in pocket therapy-ozone vs conventional irrigation. *International Journal of Public Health Dentistry* 2011; **2**:7-12.

Emerson MA, Sproul OJ and Buck CE. Ozone inactivation of cell-associated viruses. *Applied and Environmental Microbiology* 1982; **43**:603-608.

Gandhi KK, Cappetta EG and Pavaskar R. Effectiveness of the adjunctive use of ozone and chlorhexidine in patients with chronic periodontitis. *BDJ Open* 2019; **5**(1).

Greene AK, Few BK and Serafini JC. A comparison of ozonation and chlorination for the disinfection of stainless steel surfaces. *J Dairy Sci* 1993; **76**:3617-3620.

Guyatt GH, Oxman AD, Schünemann HJ, Tugwell P and Knottnerus A. GRADE guidelines: a new series of articles in the Journal of Clinical Epidemiology. *Journal of Clinical Epidemiology* 2011; **64**:380-382.

Hayakumo S, Arakawa S, Mano Y and Izumi Y. Clinical and microbiological effects of ozone nano-bubble water irrigation as an adjunct to mechanical subgingival debridement in periodontitis patients in a randomized controlled trial. *Clinical Oral Investigations,* 2013; **17**:379-388.

Herrera D, Matesanz P, Martín C, Oud V, Feres M and Teughels W. Adjunctive effect of locally delivered antimicrobials in periodontitis therapy. A systematic review and meta-analysis. *Journal of Clinical Periodontology* 2020; doi: 10.1111/jcpe.13230.

Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 2011; **343**:d5928.

Hoang T, Jorgensen MG, Keim RG, Pattison AM and Slots J. Povidone-iodine as a periodontal pocket disinfectant. *Journal of Periodontal Research* 2003; **38**:311-317.

Issac AV, Mathew JJ, Ambooken M, Kachappilly AJ, Pk A, Johny T, Vk L, Samuel A. Management of Chronic Periodontitis Using Subgingival Irrigation of Ozonized Water: A Clinical and Microbiological Study. *Journal of Clinical Diagnosis Research* 2015 **9**:ZC29-33.

Katti SS and Chava VK. Effect of Ozonised water on Chronic Periodontitis - A Clinical Study. *Journal of International Oral Health* 2013; **5**:79-84.

Kaur A, Bhavikatti SK, Das SS, Khanna S and Jain M. Efficacy of Ozonised Water and 0.2% chlorhexidine gluconate in the management of chronic periodontitis when used as an irrigant in conjugation with Phase I Therapy. *Journal of Contemporary Dental Practice* 2019; **20**:318-323.

Kotsilkov K and Popova C. Effectivness of target antimicrobial therapy of Severe Chronic Periodontitis Part Iii: Clinical Attachment Gain. *Journal of IMAB* 2010; **16**:24-26.

Lang NP, Adler R, Joss A and Nyman S. Absence of bleeding on probing. An indicator of periodontal stability. *Journal of Clinical Periodontology* 1990; **17**:714-721.

Moher D, Liberati A, Tetzlaff J, Altman DG and Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Annals of Internal Medice* 2009; **151**:264-269, W264.

Moraschini V, Kischinhevsky ICC, Calasans-Maia MD, Shibli JA, Sartoretto SC, Figueredo CM and Granjeiro JM. Ineffectiveness of ozone therapy in non-surgical periodontal treatment: a systematic review and metaanalysis of randomized clinical trials. *Clinical Oral Investigations* 2020; **24**:1877-1888.

Nagarakanti S and Athuluru D. Ozone: a new revolution in dentistry. *WebmedCentral DENTISTRY* 2011; **2**:WMC002685

Nogales CG, Ferrari PH, Kantorovich EO and Lage-Marques JL. Ozone therapy in medicine and dentistry. *The Journal of Contemporary Dental Practice* 2008; **9**:75-84.

Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *Journal of Clinical Periodontology* 2018; **45** Suppl 20:S162-S170.

Patel PV, Patel A, Kumar S and Holmes JC. Effect of subgingival application of topical ozonated olive oil in the treatment of chronic periodontitis: a randomized, controlled, double blind, clinical and microbiological study. *Minerva Stomatologica* 2012; **61**:381-398.

Rosling B, Serino G, Hellström MK, Socransky SS and Lindhe J. Longitudinal periodontal tissue alterations during supportive therapy. Findings from subjects with normal and high susceptibility to periodontal disease. *Journal of Clinical Periodontology* 2001; **28**:241-249.

Seidler V, Linetskiy I, Hubálková H, Stanková H, Smucler R and Mazánek J. Ozone and its usage in general medicine and dentistry. A review article. *Prague Medical Report* 2008; **109**:5-13.

Seydanur Dengizek E, Serkan D, Abubekir E, Bay KA, Onder O and Arife C. Evaluating clinical and laboratory effects of ozone in non-surgical periodontal treatment: A randomized controlled trial. *Journal of Applied Oral Science* 2019; **27**:e20180108.

Shoukheba M and Ali SA. The effects of subgingival application of ozonated olive oil gel in patient with localized aggressive periodontitis. A clinical and bacteriological study. *Tanta Dental Journal* 2014; **11**:63-73.

Slots J. Low-cost periodontal therapy. *Periodontol 2000* 2012; **60**:110-137.

Stoker G. Ozone in chronic middle-ear deafness. *The Lancet* 1902; **160**:1187-1188.

Tasdemir Z, Oskaybas MN, Alkan AB and Cakmak O. The effects of ozone therapy on periodontal therapy: A randomized placebo-controlled clinical trial. *Oral Diseases* 2019; **25**:1195-1202.

Uraz A, Karaduman B, Isler S, Gönen S and Çetiner D. Ozone application as adjunctive therapy in chronic periodontitis: Clinical, microbiological and biochemical aspects. *Journal of Dental Science* 2019; **14**:27-37.

Wennström JL, Heijl L, Dahlén G and Gröndahl K. Periodic subgingival antimicrobial irrigation of periodontal pockets (I). Clinical observations. *Journal of Clinical Periodontology* 1987; **14**:541-550.

Wilson M. Susceptibility of oral bacterial biofilms to antimicrobial agents. *Journal of Medical Microbiology* 1996; **44**:79-87.

Yilmaz S, Algan S, Gursoy H, Noyan U, Kuru BE and Kadir T. Evaluation of the clinical and antimicrobial effects of the ER: YAG laser or topical gaseous ozone as adjuncts to initial periodontal therapy. *Photomedicine and Laser Surgery* 2013; **31**:293-298.

Çalışır M, Talmac AC, Ege B, Poyraz Ö and Dumlupınar E. The Effect of Ozone Gas on IL-1β and IL-10 Levels of Gingival Crevicular Fluid in Aggressive Periodontitis Patients. *Ozone: Science and Engineering* 2019; **41**:571-578.