

An investigation into the efficacy of intra-articular ozone (O_2-O_3) injection in patients with knee osteoarthritis: a systematic review and meta-analysis

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Purpose: This study aimed to review and pool the current literature on intra-articular ozone injection in knee osteoarthritis (OA) patients.

Methods: A systematic review of three big databases was performed to identify all English-language randomized clinical trials (RCTs) that evaluated the efficacy of intra-articular ozone injection vs a control injection for knee OA sufferers, using the following two measuring tools: pain VAS and Western Ontario and McMaster Universities Arthritis Index (WOMAC).

Results: A total of 428 patients in five RCTs were included, from which 53% (n=225) were in the ozone group and 47% in the control (hyaluronic acid [HA], dextrose, and air injection) group (n=203). The mean age of the patients in both groups was 64 years. Females were the majority. All studies had at least 2 months of follow-up (F/U). Mean difference (MD) between the groups for VAS in the first month was -0.23 with a *P*-value of 0.71 (negative value was in favor of ozone), whereas this difference in the third and sixth months reached 1.04 and 1.31, respectively, favoring the control group. These data demonstrated that control injection had a more prolonged pain relief period. A similar trend was seen regarding WOMAC scores; pooled results showed that ozone was slightly better than the control injections during the first month (MD = -7.84 [*P*=0.15]), but it declined to MD=2.55 and 8.23 at 2- to 3- and 4- to 6-month F/U, respectively, again in favor of control injections. Also, adverse events occurred homogeneously in both ozone (6/150 cases, 4%) and control groups (7/129 cases, 5.4%; *P*-value=0.31).

Conclusion: Based on the current meta-analysis, intra-articular ozone injection efficacy was significantly superior to placebo and slightly lower to other control injections with non-significant difference. Therefore, ozone could be recommended as an efficient non-surgical treatment, durable for at least 3–6 months, in mild or moderate knee OA management.

Keywords: ozone, hyaluronic acid, knee osteoarthritis, systematic review, meta-analysis

Introduction

Knee osteoarthritis (OA) is a prevalent degenerative condition in which functional impairment is caused by mechanical and chemical stress against the joint, resulting in pain and decreased range of motion (ROM). Obese females above the age of 50 years are the most vulnerable group.¹ OA is the fourth most common cause of hospital admission in 2009 in the United States with an annual cost of 42.3 billion dollars.² The prevalence of disease highly varies among different populations: from 19.3% in some rural areas of Iran to 2.8% in the Philippines. About 19% of the Framingham adult population aged 50–60 years showed radiographic signs of knee OA.³ Patients

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with knee OA usually present with pain, stiffness, swelling and crepitus inside their joints, and it can occasionally result in severe limb deformity. Diagnosis is mainly based on this clinical picture, accompanied by more specific radiological features.^{4,5}

There is no specific cure for knee OA; however, several pharmacological options are available including acetaminophen, oral selective and non-selective NSAIDs, glucosamine, chondroitin sulfate, topical products, and so on, which can alleviate pain and improve function. Although these drugs have proved to be beneficial over a short period of time, there is no evidence showing that such interventions could modify the underlying condition.⁶⁻⁸ Also, there are many nonpharmacological treatments including exercise, orthotics or assistive devices, and physical agent modalities.^{9,10} The only definite therapeutic option is total knee replacement (arthroplasty), which is reserved for the last stages.⁵ For nonresponder patients to conservative treatment who are simultaneously not a candidate for arthroplasty, different intra-articular injections could be considered, such as corticosteroids, saline, dextrose, hyaluronic acid (HA), autologous blood, platelet-rich plasma (PRP), Botulinum toxin, and ozone (O_2-O_3) injection.^{7,11-14}

HA has been approved by the US Food and Drug Administration (FDA) in knee OA treatment since 1997.¹⁵ However, other injections are all still under evaluation in order to be accepted as the minimally invasive method of choice. Ozone is a well-known product, which has been used in many fields of dentistry and medicine worldwide. A large number of studies have confirmed the efficacy and safety of ozone therapy in the treatment of herniated lumbar disc, plantar fasciitis, meniscal injuries and other musculoskeletal disorders.¹⁶⁻²² In the recent decade, many orthopedic centers in Europe have begun to treat knee OA patients with intra-articular ozone insufflation.²³ Ozone is now available as a solution of O_2-O_3 .^{5,24}

Although the precise biochemical mechanism of ozone intra-articular injection is still unclear, there is increasing evidence confirming ozone efficacy in the treatment of knee OA sufferers.^{13,25-33} An O_2-O_3 solution can suppress acute reactive mediators by downregulation of tumor necrosis factor (TNF) α and TNF α _{R2}.^{21,24,33,34} Ozone could also be analgesic mediated by phosphodiesterase-A2 blockage.^{5,16} The current data suggest that ozone leads to neither acute nor chronic toxicity.^{21,23} In regard to the high costs and increasing controversy, the aim of this systematic review was to pool current data in order to compare the safety and efficacy of intra-articular ozone vs other similar injections in improving pain and functional status among knee OA sufferers.

Methods

Inclusion and exclusion criteria

Two authors independently (SR-D and ET) searched the following three databases: PubMed, Cochrane Central Register of Controlled Trials and Google Scholar; also, www.clinicaltrials.gov was reviewed for any ongoing registered randomized clinical trials (RCTs). The search was performed with the help of an expert librarian and was limited to English language human studies published up to the end of February 2018. Two reviewers screened all study titles to identify relevant RCTs. After removing duplication, 183 records remained and among them only 26 studies were eligible. Any disagreement was resolved by discussion, and if no consensus was achieved, one of the senior authors (SAR or SMR) made the final decision. After reviewing all eligible full texts, 19 trials were excluded due to non-randomized design or using combination therapy; eventually seven RCTs with a total number of 544 patients were included in the qualitative review (Figure 1). From these, two studies^{5,34} were not eligible for meta-analysis, thereby five trials with 428 participants were considered for the quantitative review.

Primary and secondary outcome measures

Studies reporting one of our primary outcome measures were eligible for meta-analysis: pain (based on VAS or Western Ontario and McMaster Universities Osteoarthritis Index [WOMAC] subscale of pain) and other WOMAC subscales including joint stiffness and patient's function. Moreover, for the assessment of adverse events, post-injection flare reactions, septic arthritis and withdrawal because of complications were considered.

Data extraction

Data of all seven included studies were extracted using a standardized form, which include the following: study title, first author and publication year; study design and setting; sample size and demographics of participants; details of interventions in both groups including dose, duration, frequency and number of injections; outcome measures and times of follow-up.

Quality assessment

Two separate reviewers independently evaluated the quality of each study using the PEDro (Physiotherapy Evidence Database) score.³⁵ This scoring system contains 11 domains (Table 1); we assessed risk of bias for each domain of the five included studies using a table. For each domain, a score

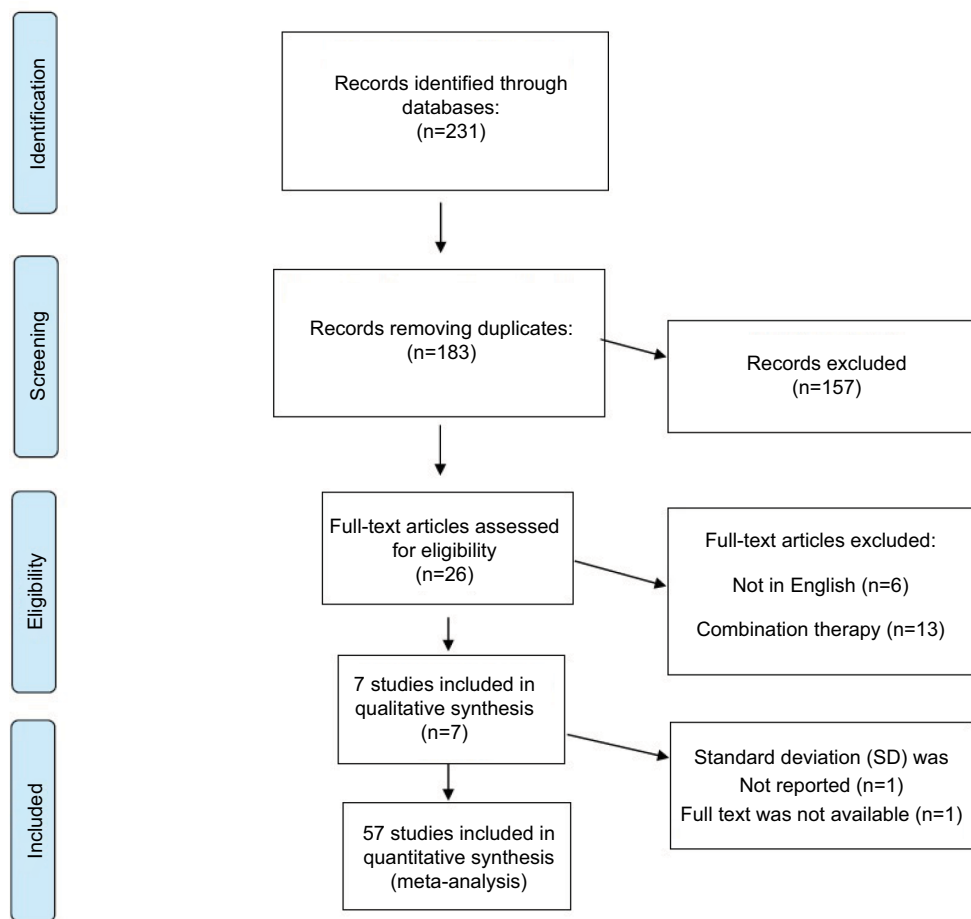


Figure 1 Standard PRISMA study flow diagram.

Abbreviation: PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis.

Table 1 Quality assessment of the included studies using PEDro score

| Study | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | PEDro score |
|---------------------------------------|---|---|---|---|---|---|---|---|---|----|----|-------------|
| Hashemi et al (2015) ¹⁹ | + | + | ? | + | ? | ? | ? | + | - | + | ? | 5 |
| Duymus et al (2016) ²⁶ | + | + | ? | + | ? | ? | ? | + | - | + | + | 6 |
| Lopes de Jesus (2017) ³⁷ | + | + | + | + | + | + | - | + | + | + | ? | 9 |
| Invernizzi et al (2017) ³⁸ | + | + | ? | + | - | - | + | + | - | + | ? | 6 |
| Raeissadat et al (2018) ²⁰ | + | + | + | + | + | ? | ? | + | - | + | + | 8 |

Notes: Numbers 1–11 follow PEDro format (https://www.pedro.org.au/wp-content/uploads/PEDro_scale.pdf); PEDro score is calculated from the following different sets of criteria: 1=eligibility criteria specified; 2=patients randomized to groups; 3=concealment of allocation; 4=groups similar at baseline; 5=patients blinded; 6=practitioners administering intervention blinded; 7=assessors blinded; 8=measurements of key outcomes obtained from >85% of the patients; 9=intention to treat analysis; 10=statistical comparisons between groups; and 11=point measures and measures of variability provided. Interpretation: scores 4–5= fair quality; scores >6= high quality; +: criterion clearly satisfied; -: criterion not clearly satisfied; ?: unclear whether criterion was satisfied.

Abbreviation: PEDro: Physiotherapy Evidence Database.

of “+” or “-/?” indicates a low risk or high risk of bias, respectively. Four of our included trials achieved high quality in the assessment (score>5) and another one was the fair quality (score =5).

Data analysis

Along with the registration process of this systematic review in the PROSPERO database (with ID No CRD42018088858 available at: www.crd.york.ac.uk/PROSPERO/display

record.php?RecordID=88858), the data collected were extracted and analyzed in RevMan 5.3 software (Cochrane Collaboration, Oxford, UK) using calculated pooled effect sizes including RR for dichotomous variables such as presence or absence of adverse events and weighted mean difference (MD) (both raw and standardized MDs [RMD and SMD]), with 95% CI for continuous data. Regarding interpretation, SMD was considered as “large effect size” if the SMD was above 0.8, “moderate” if it was larger than

0.5, and “small” if it was greater than 0.2.³⁶ Also, heterogeneity was assessed using I^2 (a value of less than 25% was considered as low heterogeneity and a value of more than 75% as high heterogeneity). The significance level for all tests was 0.05. Based on the I^2 value, random and fixed-effect models were used to pool results accordingly. Moreover, a sensitivity analysis was performed after the removal of one placebo study³⁷ to assess the pure comparison of ozone and comparable control interventions and not placebo.

Results

All seven included articles were in English, and all had been published after 2015. Three of them belonged to the Middle East (Iran and Turkey), one Indian study, two Italian, and another trial was from Brazil. Four of them had 4–6 months of follow-up (F/U) and one study continued until 1 year. VAS and WOMAC were the main outcome measuring tools. The Indian study⁵ did not report exact values of mean and SD and one of the Italian trials³⁴ had no full text available, despite contacting the authors several times. We therefore continued with five RCTs in the quantitative review.

Baseline demographics

Demographics of the trials reviewed in our review have been demonstrated in Table 2. A total of 428 patients in five RCTs were included, of whom, 225 patients (53%) were included in the ozone group and 203 patients (47%) in the control group. The number of participants in each study ranged from 42 to 141 patients. The mean age of the ozone group was 64.5 years, and 80% of whom were females, whereas in the control group the mean age was 64.4 years, and 77% of the participants were women. There was no significant difference between groups in their demographic characteristics.

Summary of studies

Four studies compared ozone vs control interventions (HA and dextrose) and another one³⁷ assessed ozone against placebo (air injection). Except for one trial,³⁷ almost all studies performed 3–4 weekly ozone injections. The mean value and concentration of ozone used varied between 7 and 15 cc of a solution with 15–30 $\mu\text{g/mL}$ ozone. According to three studies^{13,26,38} that reported Kellgren–Lawrence score (KLS) for severity, ~39% of the participants in both ozone and control groups had grade III knee OA and the rest (61%) had grade II. Another two studies^{19,37} did not report the exact number of patients in each KLS grade.

One of the two studies not included in our meta-analysis compared three monthly intra-articular injections of 10 mL

ozone with 30 $\mu\text{g/mL}$ vs 40 mg methylprednisolone, in two groups (23 knee OA patients of grade I and II KLS in each group, with a mean age of 42 years); after 3 months of F/U, the authors detected 80% success rate for participants in the ozone group against 60% in the corticosteroid group.⁵

Another study³⁴ excluded from the quantitative review compared the short-term efficacy of five weekly intra-articular injections of HA ($n=23$), oxygen ozone ($n=23$), and the combination of both ($n=24$) among 70 middle-aged knee OA patients based on the Knee injury and Osteoarthritis Outcome Score (KOOS) questionnaire and VAS for pain. Two months of F/U revealed a significant efficacy in all three groups for pain, symptoms, activities of daily living, and quality of life. They finally concluded that the scores in the combination therapy group were higher compared to the HA and ozone groups.³⁴

Hashemi et al (2015).¹⁹ As the first included RCT, in the pain clinic of anesthesiology department, the authors divided 80 knee OA patients with grade I and II KLS (mean age = 58.2 years and mean BMI = 27.5 kg/m^2) into two equal groups; participants of one of the two groups received three weekly intra-articular injections of 7 cc ozone at 15 $\mu\text{g/mL}$. Another group's intervention was three injections of hypertonic dextrose (12.5% concentration) with 10-day intervals. Within 3 months of F/U, significant improvement was seen in three subscales of WOMAC and VAS for pain among both groups. The authors eventually stated that prolotherapy with dextrose and ozone injection resulted in the same pain relief and functional improvement.¹⁹

Lopes de Jesus et al (2017).²⁶ Among the included RCTs, this is the only trial that compared ozone intra-articular injection vs placebo (air injection), which was carried out in Brazil. They divided 98 symptomatic knee OA patients with a mean age of 70 years (grade II and III KLS) into two unequal groups. Ninety-six participants finished the study; among them 61 patients received eight weekly intra-articular injection of 10 cc ozone at 20 $\mu\text{g/mL}$ vs eight weekly intra-articular injections of 10 cc sterile air in another 35 participants. The authors evaluated patients' improvement based on VAS, WOMAC, and Lequesne index at 1, 2, and 4 months after the last injections. They eventually concluded that although both groups showed relatively similar improvement in short-term F/U, ozone injection was significantly superior to placebo and was still effective by the fourth month after injections.³⁷

Invernizzi et al (2017).³⁸ In another RCT that had the smallest sample size ($n=42$), Italian researchers compared four weekly intra-articular injections of 10 cc ozone at 20

Table 2 Summary of main characteristics of the included studies

| Study | Country (setting) | Age Mean (SD) | Gender F:M% (no) | Severity grade (KLS) Grades% (no) | Body mass index (SD) | Injection protocol (ozone) | Follow-up | Outcome measure |
|-------------------------------------------|----------------------------------|------------------|---------------------|--------------------------------------|-------------------------|----------------------------------------------------|------------------------|--------------------|
| Hashemi et al (2015) ¹⁹ | Iran (Anesthesiology) | 59.1 (±12.3) | 57:43% (n=23:17) | I and II (NR in detail) | 31.2±1.1 | 3× weekly ozone (7 cc, 15 µg/mL) | 3 months | VAS |
| • Ozone (n=40) | | | | | | | | |
| • Dextrose (n=40) | | 57.3 (±15.1) | 65:35% (n=26:14) | | 31.8±0.9 | 3× dextrose _{1.5%} with 10 days intervals | | WOMAC |
| Duymus et al (2016) ²⁶ | Turkey (Orthopedics- PM&R) | 59.4 (±5.7) | 88:12% (n=31:4) | II:III =66:34% (n=23:12) | 27.6±4.4 | 4× weekly ozone (15 cc, 30 µg/mL) | 1–3–6– 12 months | WOMAC |
| • Ozone (n=35) | | 60.3 (±9.1) | 97:3% (n=33:1) | II:III =62:38% (n=24:10) | 28.4±3.6 | 1× HA (HMMW), stat | | VAS |
| • HA (n=34) | | | | | | | | |
| Lopes de Jesus et al (2017) ³⁷ | Brazil (Geriatrics) | 70.5 (±7.2) | 91:9% (n=56:5) | II and III (NR in detail) | NR | 8× weekly ozone (10 cc, 20 µg/mL) | 1–2–4 months | WOMAC |
| • Ozone (n=61) | | 69.5 (±7.6) | 85:5% (n=30:5) | | | 8× weekly air injection | | VAS |
| • Air (n=35) | | | | | | | | |
| Invernizzi et al (2017) ³⁸ | Italy (PM&R) | 70.3 (±6.5) | 73:27% (n=16:6) | II:III =68:32% (n=15:7) | 27.1±1.9 | 4× weekly ozone (10 cc, 20 µg/mL) | 1–2–3–8 weeks | VAS |
| • Ozone (n=22) | | 70.7 (±5.4) | 65:35% (n=13:7) | II:III =70:30% (n=14:6) | 26.8±1.7 | 4× HA (LMW), weekly | | |
| • HA (n=20) | | | | | | | | |
| Raeissadat et al (2018) ¹³ | Iran (PM&R) | 58.1 (±6.4) | 75:25% (50:17) | II:III =56:44% (n=37:30) | 26.6±1.9 | 3× weekly (10 cc, 30 µg/mL) | 6 months | WOMAC |
| • Ozone (n=67) | | 61.1 (±6.2) | 77:23% (56:18) | II:III =55:45% (n=40:34) | 28.5±1.6 | 3× HA (LMW), weekly | | VAS |
| • HA (n=74) | | | | | | | | |
| Total (n=508) | | | | | | | | |
| • Ozone (n=225) | | 64.52 | 80:20% (173:42) | II:III =60:40% (n=75:49) | 28.00 | – | 1 week to 12 months | WOMAC |
| • Control (n=203) | | 64.47 | 77:23% (150:43) | II:III =61:39% (n=78:50) | 29.06 | | | VAS |

Abbreviations: HA, hyaluronic acid; HMMW, high molecular weight; LMW, low molecular weight; NR, not reported; PM&R, Physical Medicine and Rehabilitation; WOMAC, Western Ontario and McMaster Universities Arthritis Index.

µg/mL vs four weekly HA injections. They evaluated clinical improvement in pain and other symptoms based on VAS and Oxford knee questionnaire (OKQ) at 1, 2, 3 and 8 weeks after injections. Results showed that although both ozone and HA injections were effective and comparable treatments in knee OA patients, the latter showed longer duration of pain reduction.³⁸

Duymus et al (2016).²⁶ The second RCT among those three trials, which compared HA vs ozone, was performed in Turkey. The authors evaluated the efficacy of four weekly intra-articular injections of 15 cc ozone at 30 µg/mL (n=35) vs a single HA injection (n=34) among moderate knee OA patients with a mean age of 59.9 and BMI =28.0. They followed participants at 1, 3, 6 and 12 months using the WOMAC index and VAS for pain. Researchers found that despite the similar efficacy of both methods within the first month, this trend in the ozone group was not so long-lasting in the following months. Finally, at the sixth month while the clinical efficacy of HA was continued, the ozone effectiveness had disappeared. Thereby, they concluded that HA was more successful than ozone injection.²⁶

Raeissadat et al (2018).²⁰ This RCT as the third comparative trial of HA vs ozone included 141 patients with a mean age =59.6 and BMI =27.5. The authors performed three weekly injections in both groups, 10 cc ozone at 30 µg/mL (n=67) vs 2 cc low-molecular-weight (LMW) HA (n=74). They reassessed patients after 6 months using WOMAC and VAS. The results revealed that both ozone and HA were still effective by the sixth month, and there was no significant difference between clinical effects of HA and ozone.¹³

Summary of pooled results

VAS pain

Pooled data on pain reduction (based on VAS) at different moments of F/U in comparison to baseline revealed some remarkable results; at the first month after injection, the MD for VAS between ozone and control groups favored the ozone group (three studies and 193 patients, MD =-0.23 [95% CI: -1.46 to 1.00], $I^2=87\%$, $P=0.7$), whereas at 2–3 months, based on four studies and 287 patients, this superiority evidently disappeared and reached MD =0.28 (95% CI: -1.46 to 2.02), favoring the control group. Longer F/U times at 4–6 and 12 months, with sensitivity analysis to remove Lopes de Jesus's study effect, showed MD =1.31 (95% CI: -2.02 to 4.64) and MD =0.80 (95% CI: 0.43–1.17), respectively. Although this declining trend appears to be definite, only the last meta-analysis revealed a significant difference between two groups (P -value <0.0001). Therefore, it could be summarized that pain reduction (on VAS) in the control

group was significantly more effective than that in the ozone group, at long-time F/U periods (Figure 2). Furthermore, as Figure 2E revealed, based on the only placebo study,³⁷ comparison between the VAS MDs of intra-articular ozone vs air injection at 4-month F/U yielded a large³⁶ SMD of 1.49 (95% CI: 1.02–1.96, $P=0.00001$). Therefore, regarding pain reduction, intra-articular ozone was significantly superior to placebo until 4 months of F/U.

WOMAC pain

Pain reduction on the WOMAC pain subscale also showed similar findings; at the first month, MD for WOMAC pain favored the ozone group (MD =-1.45), but at the second time moment, it reached MD =0.98 favoring the control group. After removing Lopes de Jesus's study effect, this declining trend became more obvious and at 6-month F/U MD reached 3.28 in favor of the HA/dextrose group. Therefore, after 3 months of F/U, neither VAS nor WOMAC scales did not reveal any significant superiority to ozone injection compared with other intra-articular interventions (Figure 3).

WOMAC joint stiffness

Joint stiffness was the next subscale. At the first month, MD was -0.29 and finally it decreased to 0.17 and 0.76 at 2–3- and 4–6-month F/U, respectively. In summary, the MD for joint stiffness differences between groups showed no remarkable preference for ozone injection even at the first month (Figure 4).

WOMAC function

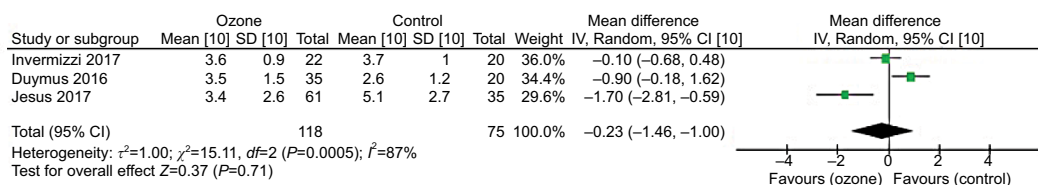
Patients' reported function is often the most practical scale to be improved by various treatments. Our findings assume that in comparison to control injections, ozone can be more beneficial in improving the function at the first month. With and without considering the Lopes de Jesus placebo study, MD was -6.14 and -2.6 at the first month after injection in favor of ozone. These MDs at 2–3 and 4–6 months decreased to some values in favor of the control group (MD =3.26 and 6.7 for the whole control group and MD =13.6 and 13.45 for the control group without considering placebo study). Thus, we can conclude that comparative function improvement at longer F/U periods was in favor of control interventions (HA or dextrose), rather than ozone injection (Figure 5).

WOMAC total

Altogether, three subscales revealed a slightly higher efficacy in the ozone group compared to the control group in short-term F/U (within the first month) and significantly lower

A

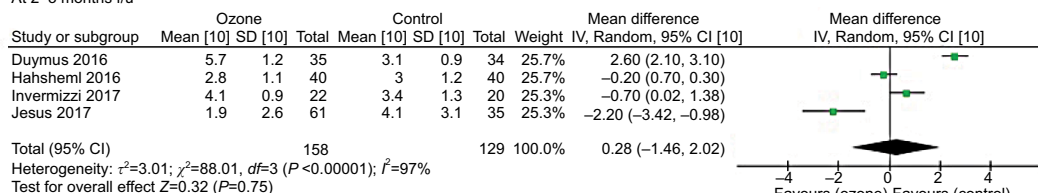
At 3–4 weeks f/u



Results after sensitivity analysis performed for removing data of Jesus et al.: MD=0.38 (-0.60, 1.35), $I^2=78\%$, P -value=0.45

B

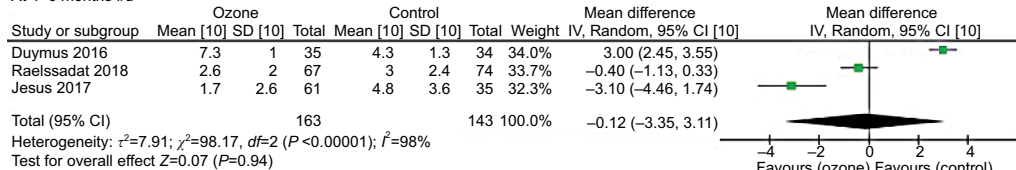
At 2–3 months f/u



Results after sensitivity analysis performed for removing data of Jesus et al.: MD=1.04 (-0.74, 2.81), $I^2=97\%$, P -value=0.25

C

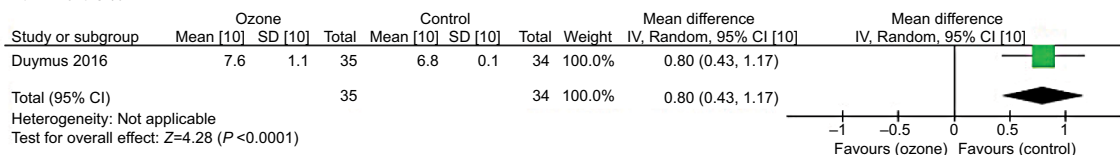
At 4–6 months f/u



Results after sensitivity analysis performed for removing data of Jesus et al.: MD=1.31 (-2.02, 4.64), $I^2=98\%$, P -value=0.44

D

At 12 months f/u



E

At 4 months f/u, comparison to placebo group (SMD)

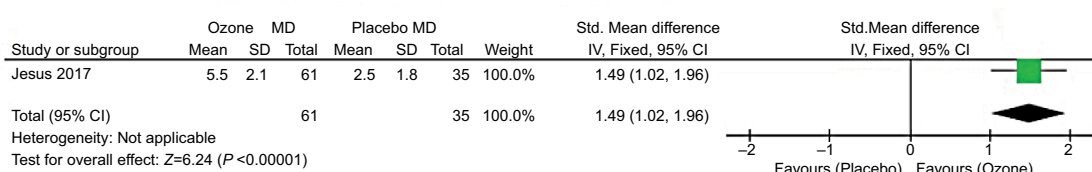


Figure 2 Comparison of VAS-based pain improvement between ozone and control groups.

Abbreviations: F/U, follow-up; MD, mean difference; SMD, standardized MD.

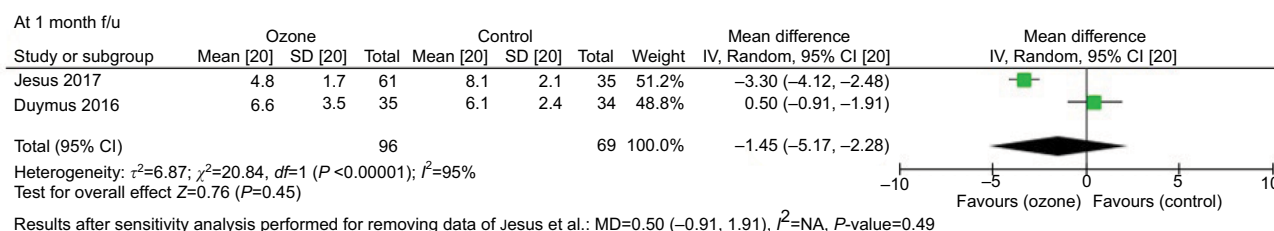
efficacy in long-term F/U (after 2–3 months and later). MD for the total WOMAC score decreased from -7.84 at the first month to MD=2.55 and 8.23 at 2- to 3- and 4- to 6-month F/U. Finally, this comparison was significantly decreased to MD=-7.70 in favor of the control group (HA) at the 12th month (Figure 6). Of note, as demonstrated in Figure 6E, based on the only placebo study,³⁷ comparison between the overall WOMAC MDs of intra-articular ozone vs air injection found

a large³⁶ SMD of 0.81 (95% CI: 0.38–1.24, $P=0.0002$) at the fourth month. So, in regard to overall WOMAC improvement, ozone was a more successful treatment than placebo injection.

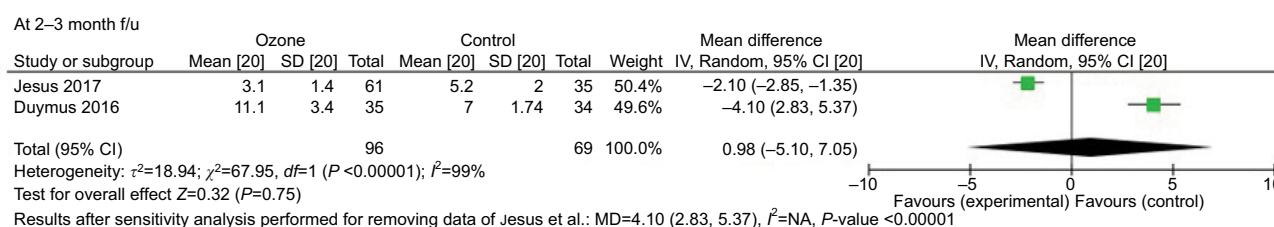
Intra-group comparison

We finally calculated changes in VAS and three WOMAC subscales (MD) between baseline level and 3–6 months after injections in the ozone group. The mentioned time was cho-

A



B



C

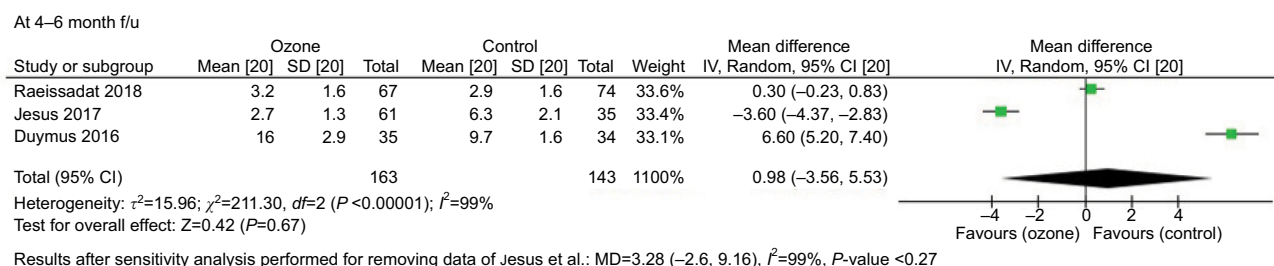


Figure 3 Comparison of WOMAC pain subscale improvement between ozone and control groups.

Abbreviations: F/U, follow-up; MD, mean difference; SMD, standardized MD; WOMAC, Western Ontario and McMaster Universities Arthritis Index.

sen since our aim was to evaluate the long-term efficacy of intra-articular ozone injection, as well as the highest number of participants at that moment (four studies with 203 patients, with this consideration that one of them¹⁹ just reported the total WOMAC score, not each subscale's details). Findings demonstrated that after 3–6 months of F/U, ozone injection would still have a significant effect on pain relief, based on both VAS and WOMAC (MD =3.79 with $P=0.01$, and MD =4.74 with $P=0.04$, respectively); however, patients at sixth month had no longer better ROM (MD =1.35 with $P=0.12$, for joint stiffness) and physical function (MD =11.17 with $P=0.06$). Of note, in order to compare these subscale changes, we also calculated SMD between baseline and the sixth month scores, showing that ozone injection had a large effect in pain reduction on both VAS and WOMAC (SMD =2.03 with 40% changes, and SMD =1.38 with 24% improvement,

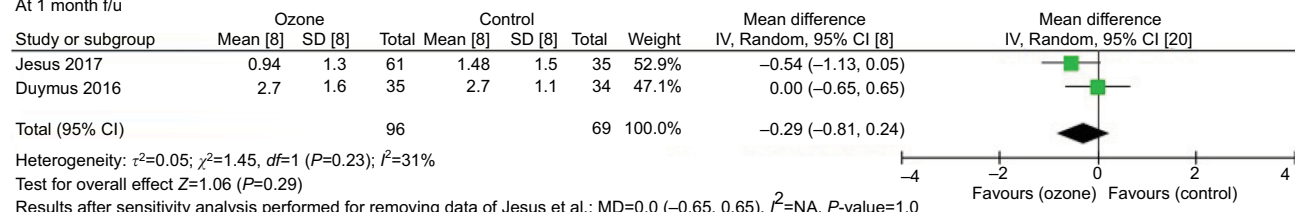
respectively), whereas it was associated with lower improvement in ROM and physical functionality (SMD =0.85 and 1.70, respectively, with 17% changes for each subscale). Ozone could therefore be considered as an intervention with at least 6 months of pain relief, among mild-to-moderate knee OA patients, who had no response to other conservative therapies (Figure 7).

Adverse events

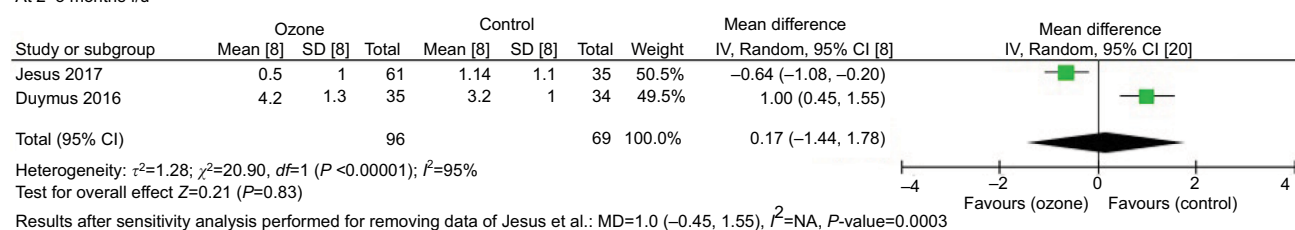
The adverse events were also similar between two groups (Table 3). None of the seven RCTs have reported any major complications. Three studies^{13,37,38} that reported the exact number of complications revealed no significant difference between safety of ozone and control intra-articular injections (occurrence rate =4.0% and 5.4%, respectively, with P -value=0.31), and according to the findings of the study by

A

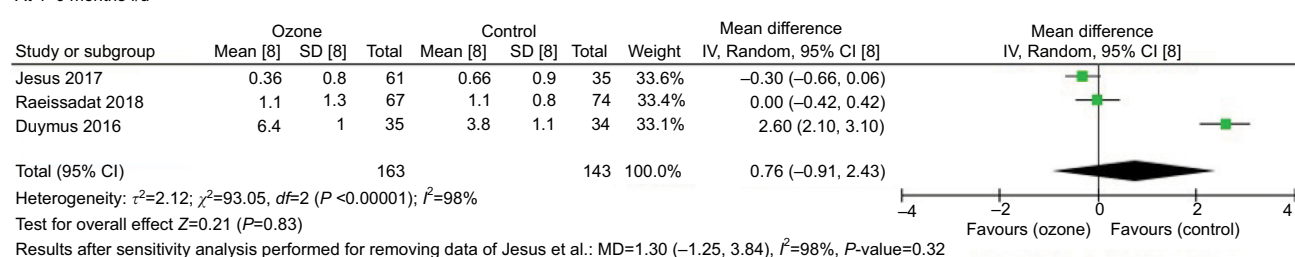
At 1 month f/u

**B**

At 2–3 months f/u

**C**

At 4–6 months f/u

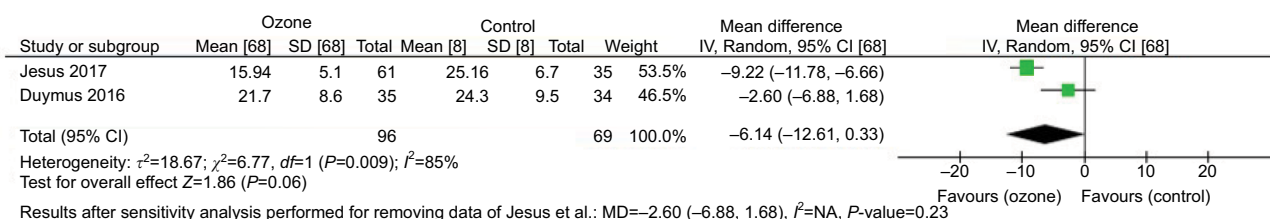
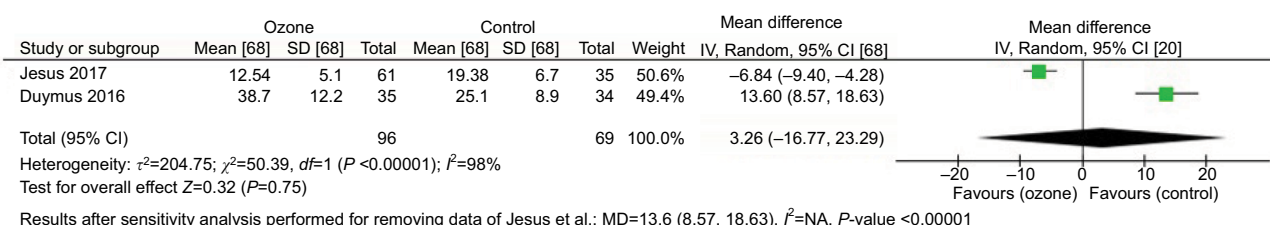
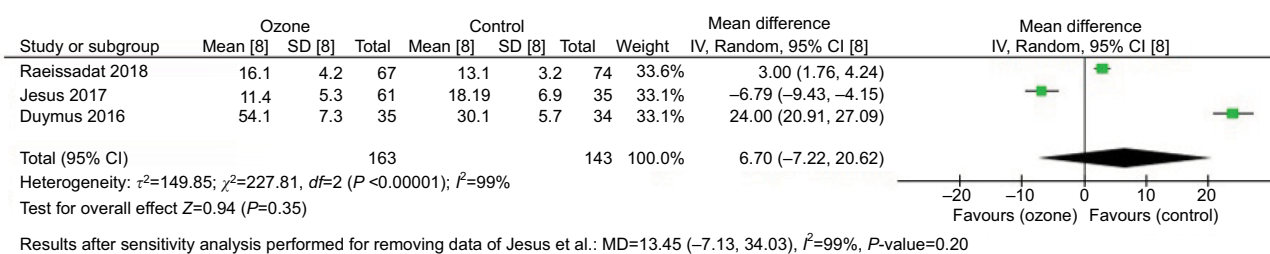
**Figure 4** Comparison of WOMAC joint stiffness subscale improvement between ozone and control groups.**Abbreviations:** F/U, follow-up; MD, mean difference; SMD, standardized MD; WOMAC, Western Ontario and McMaster Universities Arthritis Index.

Lopes de Jesus et al.³⁷ ozone had minor reactions no more than placebo (sterile air) injections. Other two studies did not mention the number of patients with minor complications.^{19,26}

Discussion

This review aimed to investigate the efficacy and safety of intra-articular ozone injection through comparing with other similar interventions, such as HA or dextrose injection as the control group. The existing body of evidence had well demonstrated that ozone injection was evidently effective for short-term management (1–3 months) of mild-to-moderate knee OA patients (grade I–III KLS). But the main challenge was on longer periods of time in which different studies had declared heterogeneous results. As depicted in Figures 2–6, the present pooled data gathered from seven included RCTs

confirm the short-term efficacy of intra-articular ozone, better than placebo (air)³⁷ and corticosteroids,⁵ but equal to other well-documented control injections such as dextrose¹⁹ or HA.^{13,26,34,38} However, at 3–6 months after injections, the therapeutic efficacy of ozone decreased to a level, slightly lower than that of other injections. The declining trend of ozone efficacy, in comparison to the control group, appears to be definite in all WOMAC subscales and VAS scores. Evidently, the therapeutic trajectory of comparative ozone efficacy vs the control group has gradually changed from negative values (favoring ozone) toward positive amounts (in favor of the control group), but only two of the comparative meta-analyses revealed a significant difference between two groups at the 12th month of F/U, MD =0.80 and 7.70 with P -values <0.0001 for VAS and total WOMAC, respec-

A At 1 month f/u**B** At 2–3 months f/u**C** At 4–6 months f/u**Figure 5** Comparison of WOMAC function subscale improvement between ozone and control groups.**Abbreviations:** F/U, follow-up; MD, mean difference; SMD, standardized MD; WOMAC, Western Ontario and McMaster Universities Arthritis Index.

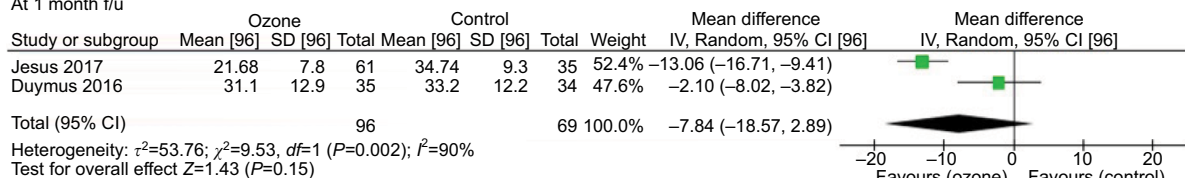
tively; it means that, before the mentioned time, there was no statistically significant superiority for none of these two groups (Figures 3–5); exactly in contrast to the conclusion of the study by Duymus et al, which had declared that at 3-month F/U ozone had significantly lower efficacy compared to HA, and at the sixth month after injection while the clinical efficacy of HA continued, ozone effectiveness had disappeared.²⁶

As mentioned earlier, comparing three WOMAC subscales revealed that the maximum changes (expressed as MD between baseline and 3–6 months post-injection values) were seen for pain subscale (MD =4.74, SMD =1.38 with 24% reduction), approximately similar to VAS improvement (MD =3.79, SMD =2.03 with 40% reduction), whereas the improvement was remarkably lower for joint stiffness section

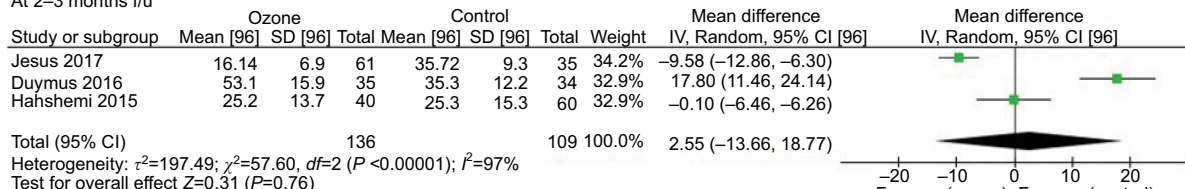
(MD =1.35, SMD =0.85, 17% change) and physical function (MD =11.17, SMD =1.7, 16% change). Therefore, it could be concluded that after 3–6 months of F/U, ozone injection would still provide significant efficacy in pain relief. However, after 6 months, ozone therapy was not associated with significant improvement of ROM and functionality (Figure 7). Also, as Figures 2E and 6E reveal, it is noteworthy that intra-articular ozone efficacy on all symptoms relief was significantly higher than placebo injection up to 4 months of F/U (SMD =1.49 and 0.81 for VAS and overall WOMAC, respectively). Eventually, from a safety point of view, two methods had similar minor adverse events (occurrence rate of 4.0% vs 5.4% for ozone and control groups, respectively). In fact, ozone was as safe as other intra-articular injections (HA or dextrose) and none of them had major complications (Table 3).

A

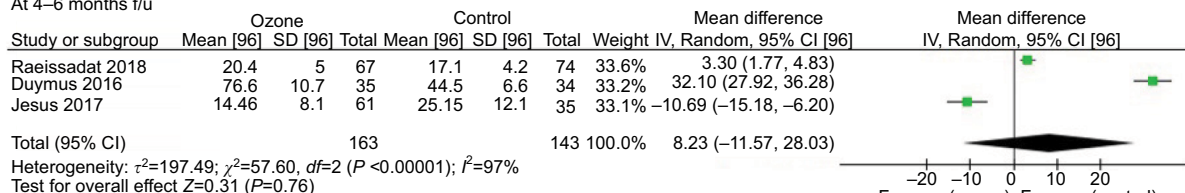
At 1 month f/u

Results after sensitivity analysis performed for removing data of Jesus et al.: MD=-2.1 (-8.02, 3.82), $I^2=NA$, P -value=0.49**B**

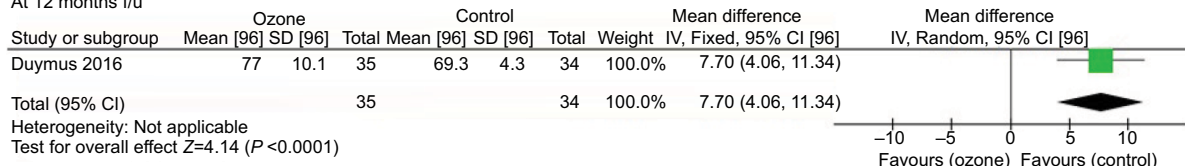
At 2–3 months f/u

Results after sensitivity analysis performed for removing data of Jesus et al.: MD=8.85 (-8.69, 26.39), $I^2=93\%$, P -value=0.32**C**

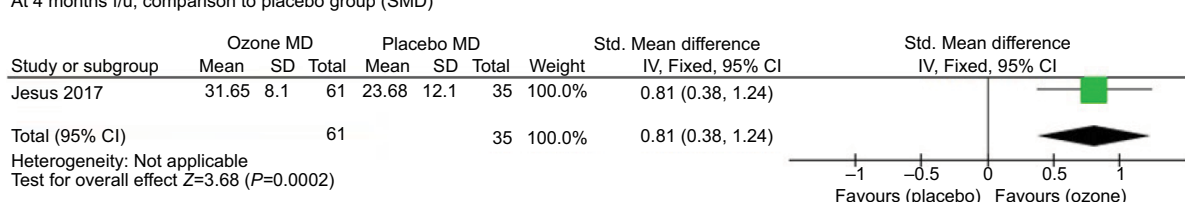
At 4–6 months f/u

Results after sensitivity analysis performed for removing data of Jesus et al.: MD=17.63 (-10.59, 45.85), $I^2=99\%$, P -value=0.27**D**

At 12 months f/u

**E**

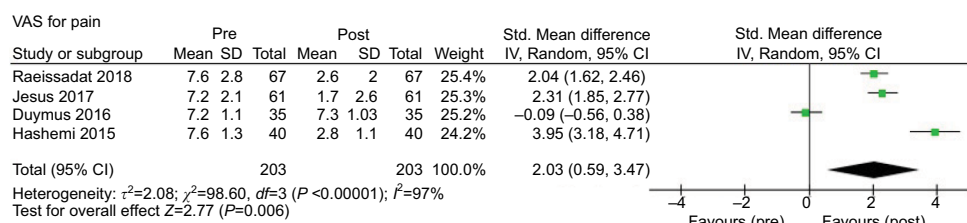
At 4 months f/u, comparison to placebo group (SMD)

**Figure 6** Comparison of total WOMAC improvement between ozone and control groups.**Abbreviations:** F/U, follow-up; MD, mean difference; SMD, standardized MD; WOMAC, Western Ontario and McMaster Universities Arthritis Index.**Limitations**

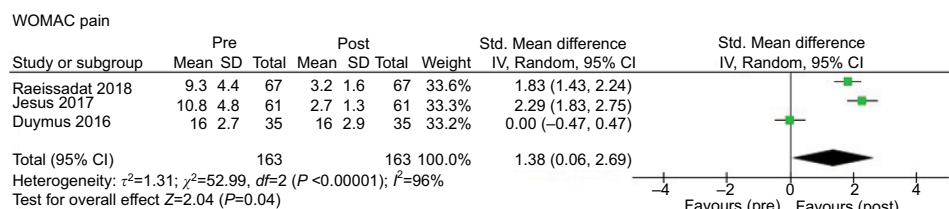
An important limitation of this meta-analysis was the large variation among different studies' protocols and settings that will certainly result in various patients' responsiveness to treatments. Due to this heterogeneity, we applied the random-effect model for meta-analysis, but there have still been many

considerations to be emphasized. In this systematic review, females were the majority in both treatment and control groups (80% and 77%, respectively), with an overall mean age of about 64.5 years (from 57.3 to 70.5). The grade of OA among studies was another issue that should be addressed; except for Hashemi et al's study,¹⁹ which just included patients with

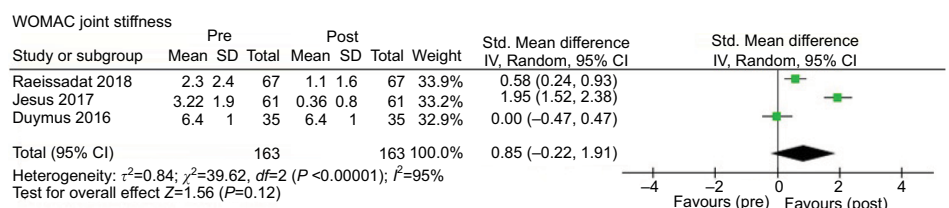
A



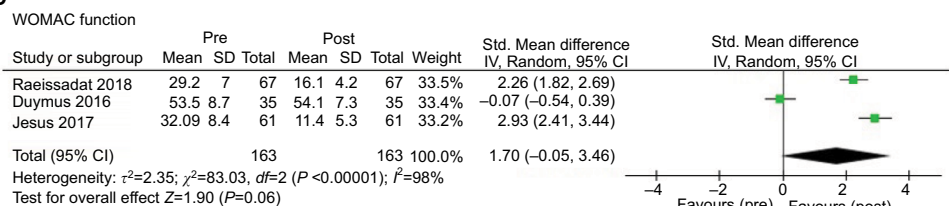
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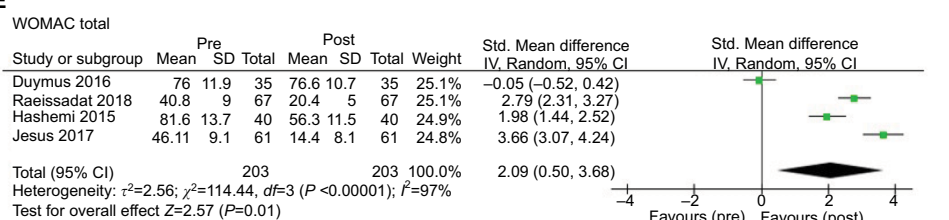


Figure 7 Comparison of VAS and WOMAC subscales (pre-and post-treatment) in the ozone group at 3–6 months.

Abbreviations: F/U, follow-up; MD, mean difference; SMD, standardized MD; WOMAC, Western Ontario and McMaster Universities Arthritis Index.

Table 3 AEs after injection

| Study (sample size) | AEs in ozone group | AEs in control group |
|-------------------------------------------|--------------------------|--------------------------|
| Lopes de Jesus et al ³⁷ (n=96) | 1:61 | 2:35 |
| Invernizzi et al ³⁸ (n=42) | 2:22 | 3:20 |
| Raeissadat et al ²⁰ (n=141) | 3:67 | 2:74 |
| Total (n=279) | 6:150 (rate=4.0%) | 7:129 (rate=5.4%) |

Note: P-value=0.31.

Abbreviation: AE, adverse event.

mild grades (I and II), the other four RCTs were grade II and III KLS and altogether ~40% of the participants had grade III knee OA. In addition to these variations in demographics and selection parameters, the included studies used certain models of ozone generator machines and HA brands with different molecular weights (MWs) and other biochemical characteristics that could lead to additional heterogeneity. For example, in one¹³ of three HA studies, a single injection of

high MW-HA was compared to four weekly rounds of ozone injection; another two HA RCTs compared equal sessions of low MW-HA (three injections in Raeissadat et al's study¹³ and four in Invernizzi et al's study⁴⁷). Furthermore, people in different countries are not the same in their anthropometric and psycho-socioeconomic factors and even the coherence to advised exercise program, which has certainly a crucial role in any treatment effectiveness and durability. Included RCTs were from India,⁵ Brazil,³⁷ Italy,^{34,38} and the Middle East^{13,19,26} (Table 2); therefore, present results could probably be extrapolated to knee OA patients worldwide. Future reviews will be more comprehensive if larger high-quality RCTs would evaluate similar outcome-measuring tools among their well-selected patients, at longer periods, to better describe the long-term efficacy of this emerging treatment.

Conclusion

Based on the current meta-analysis, intra-articular ozone injection efficacy was superior to placebo and equal to other control injections; therefore, ozone could be recommended as an efficient non-surgical treatment, durable for at least 3–6 months, in mild or moderate knee OA management, particularly among middle-aged women.

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Disclosure

The authors report no conflicts of interest in this work.

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