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Invited Review

The temporal effect of intra-articular ozone injections on pain in knee osteoarthritis

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Abstract

Background: Osteoarthritis (OA) is a most common orthopaedic condition, often complicated by inflammatory features.

Sources of data: A systematic search in PubMed, Embase, Google Scholar and Scopus databases (to January 2019) was performed to define the effect obtained in patients with OA of the knee by injections of ozone, on pain and physical function. Six RCTs and 353 patients were included.

Areas of agreement: Recently, an increasing number of physicians have used ozone therapy to alleviate the symptoms of acute and chronic OA of the knee. Ozone can allow greater mobility of the knee joint, pain relief and decrease in effusion.

Areas of controversy: The volume and concentration of ozone injected are different in the various treatment protocols published.

Growing points: The action of ozone is unclear, but it is a promising therapeutic modality capable of impacting, favourably, function and quality of life.

Areas timely for developing research: The lack of a clear protocol of use is a major limitation, and to date there is no clear evidence of long-term efficacy.

Key words: osteoarthritis, knee pain, ozone therapy, injection therapy

Introduction

Osteoarthritis (OA) is a chronic, degenerative joint disorder affecting millions of people worldwide. OA is one of the most common causes of disability, affecting especially the joints subjected to impact loads such as the knee and hip.[1] The classic signs and symptoms of OA usually include joint stiffness, pain, muscle hypotrophy and swelling. The management of OA remains unsatisfactory, and there is no universally accepted successful treatment for OA.[2]

OA is a complex multifactorial condition, involving different factors such as genetic and epigenetic factors, obesity, dietary factors and sedentary lifestyle, and it is also associated with sport injuries, sex, ethnicity and age.[3,4] OA results in degenerative phenomenon complicated by inflammation, independently of which factor is the most involved in its development.[5]

The inflammatory process is supported by several proinflammatory cytokines released by chondrocytes. Among these most important are IL1, IL6, IL8, IL17, LIF, TNF-α and IFN-γ, which cooperate in destroying the articular cartilage.[6] Furthermore, IL1, which affects the production of ROS (reactive oxygen species), is implicated in the damage to chondrocytes DNA.[7] ROS accelerate the disintegration of the cartilage matrix, and narrowing of the joint space, inhibiting the synthesis of collagen and proteoglycans.[8] Another mechanism of joint destruction is proteolytic degradation.[6] Based on this, future therapy in OA should inhibit proteolytic enzymes such as metalloproteinases (MMPs), nitric oxide synthesis (NOs), proinflammatory cytokines (IL1, IL6, TNF-α) and apoptosis. On the other hand, OA treatment should stimulate the synthesis of anti-inflammatory cytokines (IL4, IL10, IL13) and growth factors (TGF-β, IGF-1).[9]

Ozone or O_3 is the allotropic form of oxygen. It is used in the management of various conditions such as infections and autoimmune and orthopaedic diseases.[10] Ozone has analgesic, anti-inflammatory, immunomodulatory and trophic properties.[11]

Ozone is not a homeopathic drug. On the contrary, ozone exhibits a dose/effect relationship.[12]

Most medical ozone-producing devices can generate an ozone concentration from 1 to 70–100 µg/mL.[12] The total ozone dose can be calculated by multiplying the gas volume (mL) for the ozone concentration (µg/mL). Different applications require different doses to achieve the optimal effects, with a therapeutic window between 10 and 80 µg/mL.[12]

Ozone therapy has long been used in the management of OA.[13] Furthermore, it has been shown to not cause a significant inflammation process or cartilage degradation.[14]

The positive effects of ozone therapy are given by the generation of ROS and lipid oxidative products (LOPs) in the synovial fluid once is injected. The anti-inflammatory action occurs through various mechanisms such as inhibition of the release of proteolytic enzymes or stimulating the liberation of the soluble receptor IL1 or other soluble receptors and antagonists able to block proinflammatory cytokines such as IL1, IL8, IL12, IL15 and TNF-α.[15] Ozone can inhibit the synthesis of inflammatory bradykinin/prostaglandins by favouring the decrease in pain and edema reabsorption.[15] One of the crucial points in the adaptive response to the chronic oxidative state of ozone is the induction of the synthesis of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase). This is the reason why ozone should initially be injected at low doses.[13] Ozone is able to stimulate the proliferation of chondrocytes and fibroblasts, with a synthesis of the matrix increased and possibly articular cartilage.[16]

An ever-increasing number of physicians use ozone therapy to alleviate the symptoms of chronic OA of the knee. Ozone therapy allows greater mobility of the joint, pain relief and decrease in effusion. The volume of the injectate and the concentration of the ozone are often different in the various protocols published.[17]

This review summarizes the current knowledge to give a critical perspective of the possible therapeutic effect of ozone injections in the management of the knee OA, particularly on pain and physical function.

Methods

The review was carried out following the Preferred Reporting Guidelines for Systematic Reviews and Meta-Analyses (PRISMA) (Fig. 1).[18]

All published studies evaluating the efficacy and/or safety in humans of ozone injection therapy for knee OA were subject to the inclusion criteria established a priori by the authors.

Randomized control studies (RCTs) that compared ozone injection therapy with other injection therapies and prospective studies in patients aged 18 years or older with symptomatic knee OA and a minimum 4-week follow-up were included. The studies where ozone was used in combination with or after surgical procedure were excluded, as were the studies where ozone was used in combination with other types of drug therapy. Technical note editorials, case reports, narrative and systematic review articles and meta-analyses were also excluded.

Search strategy

A systematic search, without language restriction, until January 2019, was carried out by two investigators in an independent manner using full-text archives of PubMed, Embase, Google Scholar and Scopus. Several keywords (Oxygen/Ozone; Intra-articular injections; degeneration of cartilage of osteoarthritis of the knee; Inflammatory regulation) were used in various combinations in the searches. Titles and abstracts were examined by the two investigators to remove duplicates and evaluate the eligible studies according to the inclusion criteria. Full-text examination was performed if ambiguity was detected. Where present, discrepancies were resolved through discussion with the senior investigator.

Evaluation tools

For data synthesis across studies, the primary outcome was evaluated by comparing the results of the visual analogue scale (VAS) and the arthritis index of the Western Ontario and McMaster Universities Arthritis Index (WOMAC). More specifically, scores at 1, 3, 6 and 12 months after treatment were recorded. The secondary outcome was to evaluate whether the therapy could be associated with any adverse effect.

VAS

The VAS allows to measure pain in a quantitative way; it is simple, sensitive and reproducible, and for these reasons it is widely used. The words ‘without pain’ and ‘unbearable pain’ are placed at the ends of a 100-mm-long line. The point indicated by the patient along this line to describe the pain is used to quantify the measure.[19]

WOMAC Index

The WOMAC scale consists of 24 questions. Each one has five possible answers (none, mild, moderate, severe and extreme). The parameters assessed through these questions are pain (5 questions), rigidity (2 questions) and physical function (17 questions) during the activities of daily life. The score can range between 0 and 96, with a high score indicating a worse condition of the patient.[20]

Data extraction

To produce a descriptive summary, two reviewers used a pre-developed table to organize the basic features extracted from the included studies, again in an independent manner. The extracted data were then evaluated for consistency, and the discrepancies were discussed until a unanimous consensus was reached.

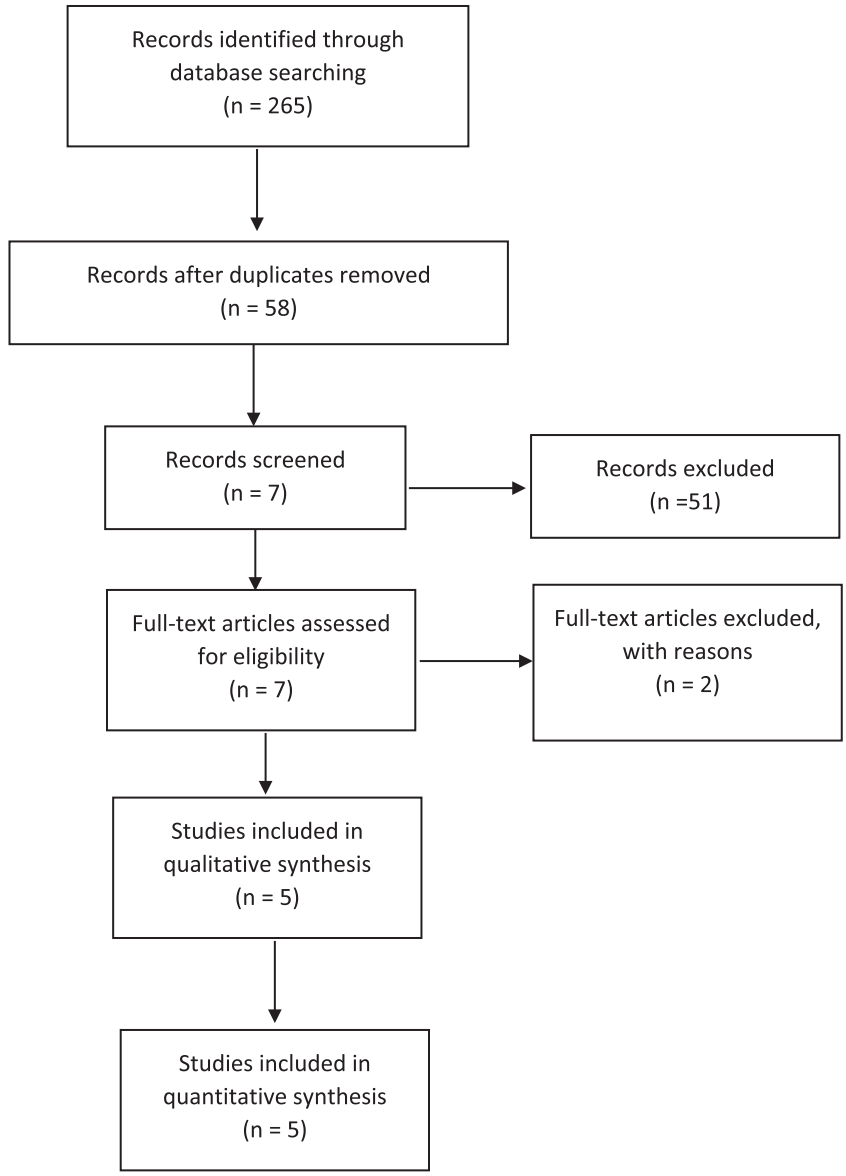

Fig. 1 PRISMA flow diagram.

Data analysis

For the continuous variables, the mean difference (MD) with 95% confidence interval (CI) was used, while the relative risk (RR) with 95% CI was adopted for dichotomous variables to express intervention effects. The data extracted from the different RCTs were organized and graphically represented using the forest plot.[21] A P value less than 0.05 was considered statistically significant.

Results

Study characteristics

Five studies met the inclusion criteria and were included in the analysis. The PRISMA flowchart shows the details of the search (Fig. 1)

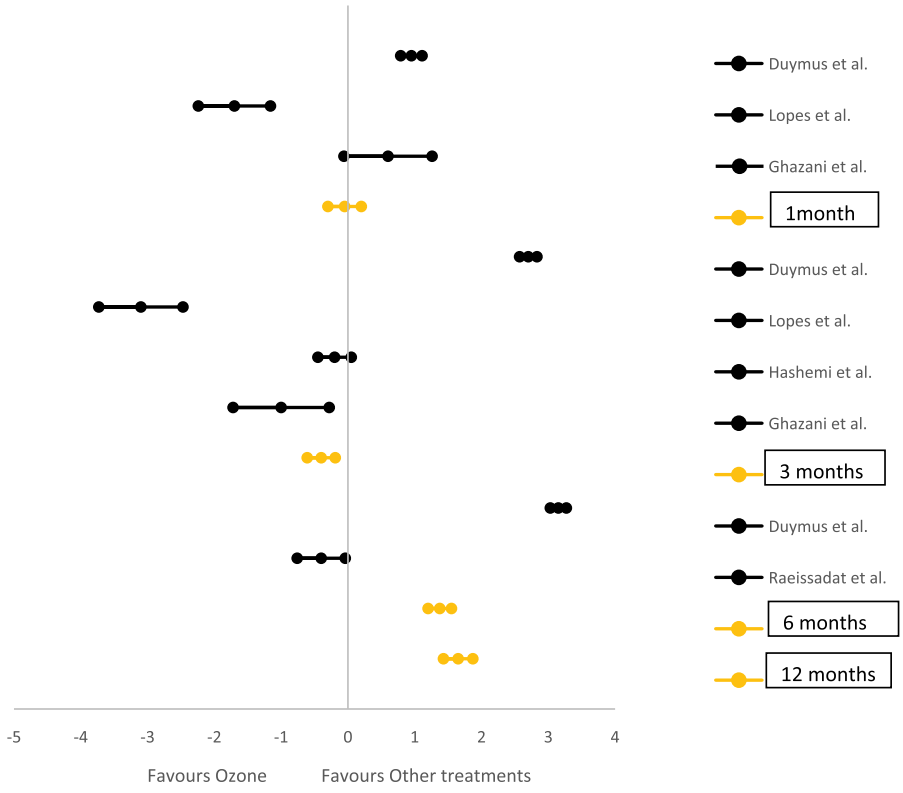

Fig. 2 Forest plot investigating the effect of ozone on VAS score at 1, 3, 6 and 12 months compared with other treatments.

A total of 353 patients were included for randomization (Table 1). The sample size of each study ranged from 31 to 119 patients.

VAS and WOMAC were the most commonly used outcome scales. All the five studies reported VAS,[22–26] and all five studies reported the WOMAC scores.[22–26]

Follow-up intervals and length of the follow-up were variable among studies. The shortest follow-up was 3 months,[24,25] and the longest was 12 months.[22]

Ozone treatment protocols varied among studies in terms of injection regimen of dose, times and intervals (Table 2).

The Kellgren–Lawrence grading (0–IV) OA grading system was used among the five studies. According to this grading system, most of the participants of all five studies who received ozone treatment were at the early or mid-stage of knee OA. Unfortunately, because the patients had the same degree of OA, it was not possible to stratify the results based on this.

The different studies compared ozone with other types of injection therapy (Table 3).

Knee pain

Figure 2 VAS (Table 4)

At 1 month, three studies reported the VAS score,[22–24] and a nonsignificant difference was found in favour of ozone treatment compared with control (MD, −0.05 [95% CI, −0.3 to 0.2] $P = 0.84$). Comparing the average VAS score before the ozone treatment of 7.6 with 1 month after the beginning of the treatment of 3.07, there was a 59.6% pain improvement.

At 3 months, the synthesis of four studies[22–25] demonstrated a nonsignificant difference in favour

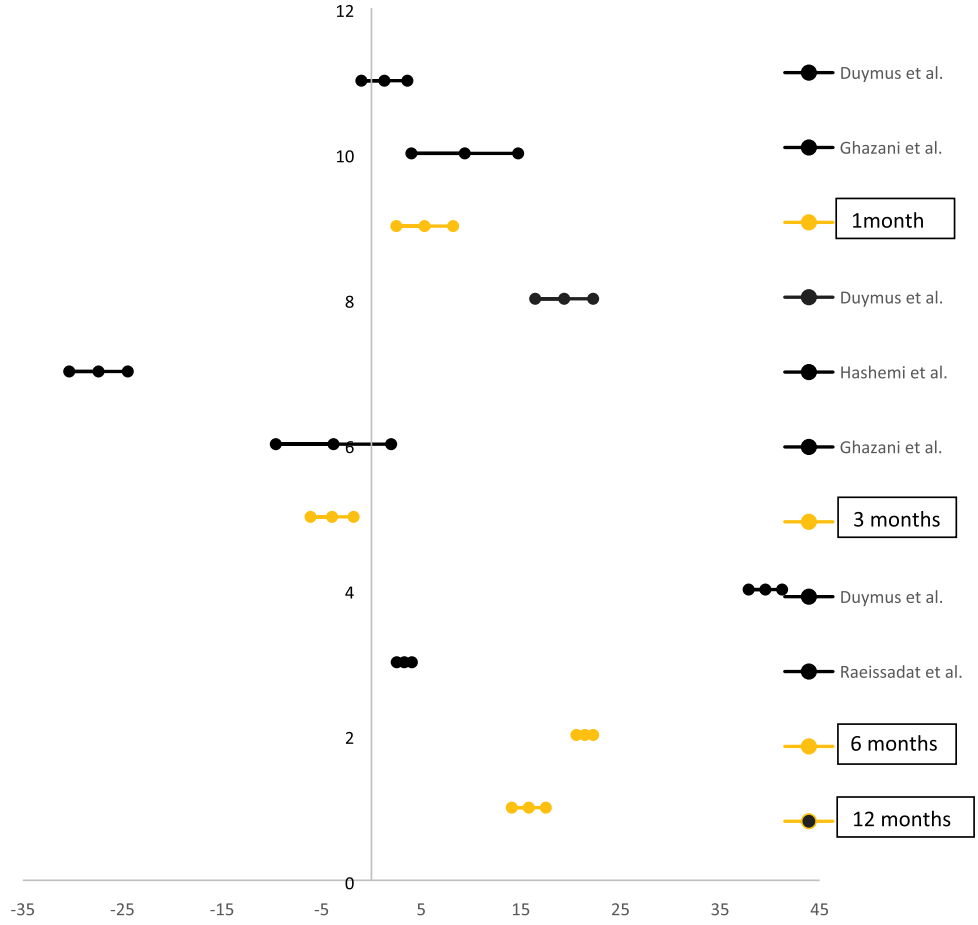

Fig. 3 Forest plot investigating the effect of ozone on total WOMAC score at 1, 3, 6 and 12 months compared with other treatments.

of ozone treatment compared with control (MD, −0.4 [95% CI, −0.61 to −0.19] $P = 0.0609$). Comparing the average VAS score before the ozone treatment of 7.39 with 3 months after the beginning of the treatment of 3.65, there was a 50.6% pain improvement.

At 6 months, the pooling results of two studies[22,26] demonstrated a statistically significant difference in favour of other treatments compared with ozone (MD, 1.375 [95% CI, 1.2 to 1.55] $P = 0.0001$) The average VAS score before the ozone treatment was 7.46, and 6 months after the beginning of the treatment it was 4.18, with a 43.9% pain improvement.

At 12 months, one study reported the VAS score.[22] A significant difference was found in favour of others treatment compared with ozone (MD, 1.65 [95% CI 1.43 to 1.87] $P = 0.0001$).

Considering the average VAS score before the ozone treatment of 7.2, and 1 year after the beginning of the treatment of 7.6, there was a 5.5% of pain worsening.

Figure 3 WOMAC (Table 5)

At 1 month, two studies reported the total WOMAC score.[22,24] A significant difference was found in favour of other treatments compared with ozone (MD, 5.33 [95% CI, 2.53 to 8.2] $P = 0.0464$). Considering the average WOMAC score before the ozone treatment of 69.18, 1 month after the

Table 1 Basic characteristics of included studies

Studies	Country	Sample size	Age (years) Mean ± SD	M/F	BMI (kg/m^2)	Outcome measurement	Follow-up
Duymus *et al.*	Turkey	35	59.4 ± 5.7	F = 31 (88.6 %)M = 4 (11.4 %)	27.6 ± 4.4	VAS, WOMAC	12 months
Raeissadat *et al.*	Iran	67	58.1 ± 6.4	F = 50 (75%)M = 17 (25%)	26.8 ± 1.95	VAS, WOMAC	6 months
Lopes *et al.*	Brazil	61	70.5 ± 7.2	F = 56 (91.8%)M = 5 (8.2%)	n.r.	VAS WOMAC GPM SF-36 Lequesne	4 months
Hashemi *et al.*	Iran	40	59.1 ± 12.3	F = 23 (57.5%)M = 17 (42.5%)	31.2 ± 1.1	VAS, WOMAC	3 months
Ghazani *et al.*	Iran	31	59.65 ± 10.25	F = 24 (77.4%)M = 7 (22.6%)	28.8 ± 2.5	VAS, WOMAC	3 months

beginning of the treatment, it had decreased to 29.36, a 57.5% improvement.

At 3 months, the synthesis of three studies[22,24,25] demonstrated a statistically significant difference in favour of ozone treatments compared with other treatments (MD, −3.95 [95% CI, −6.11 to −1.79] $P = 0.0812$). Considering the average WOMAC score before ozone treatment of 71.09 and 1 month after the beginning of the treatment of 53.26, there was a 25% improvement.

At 6 months, two studies reported the total WOMAC score[22,26]; a significant difference was found in favour of other treatments compared with ozone (MD, 21.42 [95% CI, 20.58 to 22.26] $P = 0.0001$). Considering the average WOMAC score before the ozone treatment of 52.87, and 6 months after its beginning of 39.68, there was a 24.9% improvement.

At 12 months, one study reported the total WOMAC score.[22] A significant difference was found in favour of other treatments compared with ozone (MD, 15.8 [95% CI, 14.09 to 17.51] $P = 0.0001$). Considering the average WOMAC score before ozone treatment of 76.0 and 12 months after the beginning of the treatment of 77.0, there was a 1.3% worsening.

Adverse effect

No severe complications were found in any studies. Raeissadat et al.,[26] after the first injection, reported a mild flare reaction in three patients, which self-resolved in few days.

Discussion

In knee OA, pain and joint deformity are caused by the progressive degeneration of the articular cartilage and inflammation. Decreasing pain and preserving joint mobility are the goals of therapy, and for this purpose different types of intra-articular injections have been used. Among these, the most commonly used in clinical practice are glucocorticoids, hyaluronic acid (HA), platelet-rich plasma (PRP) and non-steroidal anti-inflammatory drugs and ozone.[27]

Table 2 Details of ozone treatment protocols and control

Studies	Injection dose	Times	Intervals
Duymus *et al.*	20 μg/mL × 10 mL	4	1 week
Raeissadat *et al.*	30 μg/mL × 10 mL	3	1 week
Lopes *et al.*	20 μg/mL × 10 mL	8	1 week
Hashemi *et al.*	15 μg/mL × 6 mL	3	7–10 days
Ghazani *et al.*	15 μg/mL × 10 mL	3	10 days

Table 3 Other treatments compared with ozone

Studies	Control
Duymus *et al.*	PRP, HA
Raeissadat *et al.*	HA
Lopes *et al.*	Placebo (air)
Hashemi *et al.*	Hypertonic dextrose
Ghazani *et al.*	Corticosteroid

There are benefits of using intra-articular glucocorticoids in symptomatic relief,[28] but they should be used judiciously because their repeated use would further damage the articular cartilage.[29] HA is a complex polysaccharide containing glucosamine and glucuronic acid, which is present in joint fluid.[30] The benefits of HA are its visco-inductive and visco-supplementative effects. The pain reduction obtained in patients with knee OA after HA injections is comparable to that obtained with glucocorticoid injections.[31] Zhang *et al.*[32] reported that the intra-articular injection of PRP was not obviously superior to HA in knee OA. Therefore, the results obtained with the use of these three therapies are often comparable.

Ozone is a triatomic variety of oxygen, which is mainly used in rheumatoid arthritis and OA.[11] Ozone, through the activation of cell metabolism and the inhibition of prostaglandin synthesis, is able to generate antioxidant, anti-inflammatory and analgesic effects.[33] Furthermore, another beneficial effect of ozone therapy is the stimulation of angiogenesis and vasodilatation that allow an increase oxygen supply to tissues.[11] Intra-articular oxygen-ozone has been used for several decades. Many studies concluded that ozone was an effective and safe alternative to treat knee OA, although they used different treatment protocols and the times of follow-up were varied.

This meta-analysis included five RCTs and compared the temporal effect of O3 with other treatments on knee pain and physical function in patients with knee OA (Table 3). Data synthesis showed that intra-articular ozone injections reduce knee pain and total WOMAC scores.

Ozone therapy produces an improvement of the symptoms at 1, 3 and 6 months after treatment, although such improvement is not always statistically significant.

At 12 months, however, the evaluation scales have returned to the initial scores. No severe adverse events were recorded in any studies.

It remains unclear what is the best therapeutic protocol in terms of ozone concentration, volume to be injected, number of injections and time between one injection and another. In fact, it is possible that the worst results of ozone therapy compared to controls result from a non-uniformity of the protocol used for injections.

At 1 month, the greatest improvement in terms of pain was achieved by Lopes *et al.*[23] Using intra-articular injection of 20 μg/mL × 20 mL of O3, once a week for four consecutive weeks, they achieved a 72.11% improvement in the VAS score during a 3-month follow-up.

At 3 months, the greatest improvement in terms of pain was achieved by Lopes *et al.*[23] with a 76.3% improvement in the VAS score during a 3-month follow-up. Perhaps, to achieve rapid pain relief, it may be advisable to repeat the injection every 7 days

Table 4 The effect of ozone on VAS score at 1, 3, 6 and 12 months compared with other treatments

VAS	Ozone			Control					
study	Mean	SD	Total	Mean	SD	Total	Std diff in means	Lower limit	Upper limit
Duymus *et al.*	3.5	1.5	35	2.55	0.1	67	0.95	0.79	1.11
Lopes *et al.*	3.4	2.7	61	5.1	2.7	35	−1.7	−2.24	−1.16
Ghazani *et al.*	5.4	2.5	31	4.8	2.8	31	0.6	−0.06	1.26
At 1 month			127			133	−0.05	−0.3	0.2
P value = 0.8434 *t* = 0.1978 df = 258 SED = 0.253									
Duymus *et al.*	5.7	1.2	35	3	0.1	67	2.7	2.57	2.83
Lopes *et al.*	1.7	2.7	61	4.8	3.6	35	−3.1	−3.73	−2.47
Hashemi *et al.*	2.8	1.1	40	3	1.2	40	−0.2	−0.45	0.05
Ghazani *et al.*	5.3	2.67	31	6.3	3.1	31	−1	−1.72	−0.28
At 3 months			167			173	−0.4	−0.61	−0.19
P value = 0.0609 *t* = 1.8802 df = 338 SED = 0.213									
Duymus *et al.*	7.3	1.03	35	4.15	0.2	67	3.15	3.03	3.27
Raeissadat *et al.*	2.6	2	67	3	2.4	74	−0.4	−0.76	−0.04
At 6 months			102			141	1.375	1.2	1.55
P value = 0.0001 *t* = 7.6276 df = 241 SED = 0.181									
Duymus *et al.*	7.6	1.1	35	5.95	1.2	67	1.65	1.43	1.87
At 12 months			35			67	1.65	1.43	1.87
P value = 0.0001 *t* = 6.7795 df = 100 SED = 0.243									

SED = standard error of difference

using a concentration of 20 µg/mL and a volume of 10 mL.

Regarding the global improvement measured with the WOMAC scale, at 1 month the greatest improvement was achieved by Duymus *et al.*[22] with a 59.2% improvement in the WOMAC score during a 1-month follow-up. Perhaps, to achieve a better global improvement, it may be advisable to repeat the injection every 7 days using a concentration of 30 µg/mL and a volume of 15 mL.

Not all studies have shown long-term efficacy. From the results of our statistical analysis, ozone injections can temporarily reduce pain, but there is no evidence that they slow down the chronic degenerative evolution of OA in the long term.

Comparing the results obtained with ozone therapy with other injection therapy modalities, it is possible to notice how much the results are similar or sometimes inferior, even though this inferiority especially during the first months of treatment is not statistically significant. However, the cost of ozone is clearly lower compared to other therapies such as PRP or HA. Hence, optimization of the protocol could improve the cost effectiveness of such therapy.

This meta-analysis is not without its limitations; specifically, it is fundamentally limited by the weaknesses of each included study. Of note, there was marked heterogeneity in the outcome measures employed across studies. In addition, some articles presented only short-term data. Therefore, we do not have long-term patient outcome data. Lastly, the various articles had different treatment protocols, making it difficult to compare the results.

Table 5 The effect of ozone on total WOMAC score at 1, 3, 6 and 12 months compared with other treatments

Total WOMAC	Ozone			Control					
Study	Mean	SD	Total	Mean	SD	Total	Std diff in means	Lower limit	Upper limit
Duymus *et al.*	31.1	12.9	35	29.8	10.85	67	1.3	−1.01	3.61
Ghazani *et al.*	52.6	20.7	31	43.23	22.39	31	9.37	4.01	14.73
At 1 month			66			98	5.335	2.5	8.2
P value = 0.0464 *t* = 2.0071 df = 162 SED = 2.658									
Duymus *et al.*	53.1	15.9	35	33.75	14.15	67	19.35	16.44	22.26
Hashemi *et al.*	56.3	11.5	40	83.7	15.3	40	−27.4	−30.34	−24.46
Ghazani *et al.*	47.8	20.2	31	51.61	26.37	31	−3.81	−9.6	1.98
3 months			106			138	−3.95	−6.11	−1.79
P value = 0.0812 *t* = 1.7510 df = 242 SED = 2.256									
Duymus *et al.*	76.6	10.7	35	37.05	6.85	67	39.55	37.87	41.23
Raeissadat *et al.*	20.4	5	67	17.1	4.2	74	3.3	2.54	4.06
6 month ozone			102			141	21.42	20.58	22.26
P value = 0.0001 *t* = 25.4792 df = 241 SED = 0.841									
Duymus *et al.*	77	10.1	35	61.2	7.55	67	15.8	14.09	17.51
12 months			35			67	15.8	14.09	17.51
P value = 0.0001 *t* = 8.9093 df = 100 SED = 1.773									

Conclusions

Knee OA is a chronic degenerative condition. Although many non-surgical treatment options have been proposed, none is curative, as they all focus on symptom management. Genetic, biological and mechanical factors contribute to chronic inflammation of the joint. Ozone, acting on various inflammatory pathways, could be a promising therapeutic weapon capable of reducing pain and at the same time promoting the reacquisition of function and quality of life.

The data analysed shows that the most promising protocol in reducing pain is an intra- articular injection of 20 μg/mL × 20 mL of O_3, once a week for four consecutive weeks. However, appropriately powered studies with sufficiently long follow-up are necessary to establish which dose(s) and frequency of administration are optimal.

Ozone can be used as a safe, effective conservative therapeutic option with contained costs in the short-term management of knee OA.

Conflict of interest statement

The authors have no potential conflicts of interest.

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