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The effectiveness of ozone therapy for diabetic foot treatment: A systematic review

Eficácia da ozonioterapia para o tratamento do pé diabético: Uma revisão sistemática

La eficacia de la ozonoterapia para el tratamiento del pie diabético: Una revisión sistemática

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Abstract
*Introduction***:** Diabetic foot ulcer is a local wound in patients with diabetes. Ozone therapy is becoming a treatment option as it accelerates healing. *Objective***:** To verify the effectiveness of ozone therapy for the treatment of diabetic foot ulcers. Methodology: We performed searches in PubMed, SciELO, LILACS, MEDLINE, Web of Science and CINAHL databases until October 2021. The search included article references and clinical trial registry websites without limitation of language or publication date. Randomized and non-randomized clinical trials addressing ozone therapy in patients with diabetic foot ulcers were included. The Risk of Bias assessment tool and the Review Manager software, version 5.4 were used. *Results***:** Six randomized and non-randomized clinical trials met the inclusion criteria (608 patients). Only two studies were considered to be at low risk of bias, with adequate randomization, allocation concealment, and blinded evaluation of results. One study compared two IPC regimens where there was evidence of a statistically significant treatment effect in favor of the rapid IPC regimen (hazard ratio 0.74, 95% CI 0.56 to 0.90). Five trials evaluated CPI as adjunctive therapy to standard therapies and agreed that CPI increases the rate of ulcer healing and a higher cure rate. Two trials evaluated CPI alone compared to other therapies. *Conclusion***:** Ozone therapy for diabetic foot ulcers has been shown to be effective as an adjunct to standard treatment, although the included studies were classified as having a high risk of bias.
Keywords: Complementary therapies; Diabetic foot; Ozone.

Resumo
*Introdução***:** A úlcera do pé diabético é uma ferida local em pacientes com diabetes. A ozonioterapia está tornando-se uma opção de tratamento, pois acelera a cicatrização. *Objetivo***:** Verificar a eficácia da ozonioterapia para o tratamento da úlcera do pé diabético. *Metodologia***:** Realizamos buscas nas bases de dados PubMed, SciELO, LILACS, MEDLINE, Web of Science e CINAHL até outubro de 2021. A busca incluiu referências de artigos e sites de registro de ensaios clínicos sem limitação de idioma ou data de publicação. Incluiu-se ensaios clínicos randomizados e não randomizados que abordassem a ozonioterapia em pacientes com úlcera do pé diabético. Utilizou-se a ferramenta de avaliação Risk of Bias e o software Review Manager, versão 5.4. *Resultados***:** Seis ensaios clínicos randomizados e não randomizados atenderam aos critérios de inclusão (608 pacientes). Apenas dois estudos foram considerados com

baixo risco de viés, com randomização adequada, ocultação de alocação e avaliação cega dos resultados. Um estudo comparou dois regimes de CPI, nos quais houve evidência de um efeito de tratamento estatisticamente significativo em favor do regime de CPI rápido (taxa de risco 0,74, IC 95% 0, 56 a 0,90). Cinco ensaios avaliaram a CPI como terapia adjuvante às terapias padrão e concordaram que a CPI aumenta a taxa de cicatrização da úlcera e uma taxa de cura mais alta. Dois ensaios avaliaram CPI sozinho em comparação com outras terapias. *Conclusão***:** A ozonioterapia para úlcera do pé diabético mostrou-se eficaz como adjuvante ao tratamento padrão, embora os estudos incluídos classifiquem-se com alto risco de viés.
Palavras-chave: Terapias complementares; Pé diabético; Ozônio.

Resumen
*Introducción***:** La úlcera del pie diabético es una herida local en pacientes con diabetes. La ozonoterapia se está convirtiendo en una opción de tratamiento ya que acelera la cicatrización. *Objetivo***:** Verificar la efectividad de la ozonoterapia para el tratamiento de las úlceras del pie diabético. *Metodología***:** Realizamos búsquedas en las bases de datos PubMed, SciELO, LILACS, MEDLINE, Web of Science y CINAHL hasta octubre de 2021. La búsqueda incluyó referencias de artículos y sitios web de registros de ensayos clínicos sin limitación de idioma o fecha de publicación. Se incluyeron ensayos clínicos aleatorios y no aleatorios que abordaran la terapia con ozono en pacientes con úlceras del pie diabético. Se utilizó la herramienta de evaluación de Riesgo de Sesgo y el software Review Manager, versión 5.4. *Resultados***:** Seis ensayos clínicos aleatorizados y no aleatorizados cumplieron los criterios de inclusión (608 pacientes). Solo dos estudios se consideraron con bajo riesgo de sesgo, con asignación al azar adecuada, ocultación de la asignación y evaluación cegada de los resultados. Un estudio comparó dos regímenes de PCI en los que hubo pruebas de un efecto del tratamiento estadísticamente significativo a favor del régimen de PCI rápido (cociente de riesgos instantáneos 0,74; IC del 95%: 0,56 a 0,90). Cinco ensayos evaluaron la CPI como terapia adyuvante a las terapias estándar y acordaron que la CPI aumenta la tasa de cicatrización de la úlcera y una tasa de curación más alta. Dos ensayos evaluaron la CPI sola en comparación con otras terapias. *Conclusión***:** La terapia con ozono para las úlceras del pie diabético fue eficaz como complemento de la atención estándar, aunque los estudios incluidos se clasifican como de alto riesgo de sesgo.
Palabras clave: Terapias complementarias; Pie diabético; Ozono.

1. Introduction

Diabetes Mellitus (DM), increasingly and on a global scale, has become an important health problem that affects the population, regardless of the economic and social development of a country. (Cubas et al., 2013) Patients with this disease have a 25% risk of developing foot ulcers over a lifetime. These ulcerations, when not treated properly, can cause a complication known as the diabetic foot, which results from a previous infection that may or may not lead to the destruction of deep tissues, associated with changes in neurological physiology and peripheral vascular diseases. Diabetic foot ulcers are classified, according to their etiopathogenesis, as neuropathic, vascular or mixed. (M. da S. S. de A. à S. D. de A. B. Brasil, 2016)

Approximately 15% of diabetics will develop ulcers, of which 15 to 20% will require some form of amputation. Chronic ulcers also represent almost 50% of the causes of hospitalization in diabetic patients. et al., 2010) In this scenario, there is a significant financial impact on the public and private health system due to outpatient costs, greater occupation of hospital beds, and prolonged hospital stays. (Rezende et al., 2008) However, new studies with ozone therapy seek to prove a better efficacy in diabetic foot treatment compared to conventional treatments, an improvement in quality of life with reduced rates of hospitalization, amputation, and treatment time. (Izadi et al., 2019) Consequently, a reduction in public hospitals' expenditure on treatment of patients with a diabetic foot would be expected. (Rezende et al., 2008)

Clinical trials show that ozone has the function of optimizing cell metabolism due to its antioxidant and antimicrobial effects. The administration of ozone seems to induce tolerance to oxidative stress and prevent damage mediated by free radicals. In addition, it enables the arrangement of new vessels in the affected region, which increases local irrigation, being responsible for accelerating the development of granulation tissue. All these factors reduce healing time and improve the healing of diabetic foot ulcers. (Kushmakov et al., 2018) This treatment, in turn, can be delivered to patients via local

application of a mixture of oxygen-ozone directly to the wound, in the form of ozonized oils, or administered systemically via the rectal, intravenous, or subcutaneous route.

This study seeks to investigate the benefits of treatment with ozone therapy, commonly used by developed countries pursuing to enhance the treatment of diabetic foot ulcers. Since 1906, the United States has used this technique. However, in Brazil, it has not yet been enforced with a therapeutic purpose, since there is a lack of evidence about its effectiveness, although its harmfulness is also not evident for the treatment of the most diverse diseases or aggravations that it can cure. (C. dos deputados do Brasil, 2017) Ozone therapy is a therapy used since World War I to treat soldiers with infected wounds. (Larissa, et al., 2019) It is a technique that is much compared to a hyperbaric oxygen chamber, a therapeutic modality that takes place in a pressurized environment with 100% oxygen. (V. Bocci & Aldinucci, 2006; Costa Val, et al., 2003) On the other hand, ozone therapy, compared to the hyperbaric chamber, proves even more effective, with a longer-lasting effect and at a lower cost. (V. Bocci & Aldinucci, 2006; Costa Val et al., 2003)

The action that occurs in ozone therapy is, as the name says, resulting from the action of ozone, which, in turn, will offer several processes that positively imply diseases associated with ischemia, infection, delayed healing, and oxidative stress. (Anagha V.S, et al., 2006) For scholars, there are three methods by which ozone can exert its action: interruption of the integrity of the cell envelope in bacteria, inhibition of cell growth in fungi, and damage to the viral capsid in viruses. (Anagha V.S, et al., 2016; V. A. Bocci, 2006)

Thus, ozone therapy is a therapy that has benefits such as the prevention of oxidative stress, among others. (V. Bocci, et al., 2015; Elvis & Ekta, 2011) However, this also has the disadvantage of being related to excessive oxidation and the consequent generation of free radicals. (Elvis & Ekta, 2011) It is worth noting that ozone still has a potent bactericidal effect, resulting from this direct attack by microorganisms and consequent oxidation of amino acids and nucleic acids. (Gurley, Gurley, B. (1985). Ozone: pharmaceutical sterilant of the future? Journal of Parenteral Science and Technology, 1985)

Following this line of thought, several diseases can be treated with ozone therapy, alone or associated with other methods such as acute and chronic infectious diseases caused by parasites, diseases with chronic ischemia, skin diseases, and degenerative diseases, among many others. (Paulo, 2008) This is because, as previously described, ozone improves the body's oxygenation and metabolism, in addition to having bactericidal and fungicidal effects and improves blood circulation. (Guerra X.V., et al., 1999; Pino, E.; et al., 1999)

Based on this scenario, this systematic review aimed to verify the effectiveness of ozone therapy in the treatment of diabetic foot ulcers.

2. Methodology

2.1 Study design

This systematic review aimed to verify the effectiveness of ozone therapy in the treatment of diabetic foot ulcers. This study was carried out in accordance with the recommendations established by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Moher et al., 2009) and the Cochrane Handbook for Systematic Reviews of Interventions. (Library, n.d.) The protocol for this systematic review was submitted to the International Prospective Register of Systematic Reviews, (Research, n.d.) with the following registration number: CRD42020158472.

2.2 Elegibility criteria

Types of studies

Randomized, quasi-randomized, non-randomized clinical trials (RCTs) and pilot studies that evaluate the effectiveness of ozone therapy in the treatment of patients with diabetic foot ulcers were included.

Types of participants

People with diabetic foot ulcers in any age group, ranging between 18 and 85 years of age, and with all grades of ulcers, according to Wagner ulcer Classification System, were included.

Types of interventions

We considered any type of isolated ozone therapy, for systemic or local use, applied intravenously or topically, or associated with standard treatment compared to routine treatments, such as gauze dressings**,** debridement and topical agents.

Types of outcome measures

We included any type of clinically relevant measure that could be considered patient-centered. We did not consider biochemical analysis (e.g. glucose levels, serum levels of fructosamine, and advanced oxidation protein products, among others). The primary outcomes considered were the degree of the wound, healing time, wound size, and complete wound closure. The secondary outcomes considered were pain and quality

2.2 Search methods for identification of studies

Electronic searches

We searched for randomized controlled trials from the following electronic databases, without restrictions on language or date of publication. Searches were performed by two independent reviewers (F.H and L.B.) in the databases PubMed, SciELO, LILACS, MEDLINE, Web of Science, and CINAHL via PERIÓDICOS CAPES, CENTRAL (Cochrane Central Register of Controlled Trials, The Cochrane Library, which contains the Back Group Trials Register), and PEDro (Physiotherapy Evidence Database).

Searching other resources

We also searched the reference lists of eligible papers, as well as trial registry websites: ClinicalTrials.gov and the Brazilian Registry of Clinical Trials (ReBEC). An active search and consultation with experts in the field were also carried out.

2.3 Eligibility Criteria

The studies included covered from 2005-2021 and they were collected from the electronic databases mentioned above The inclusion criteria were randomized controlled trials, quasi-randomized, non-randomized clinical trials (RCTs), and pilot studies that had any type of isolated ozone therapy, for systemic or local use, applied intravenously or topically, or associated with standard treatment compared to routine treatments, such as gauze dressings**,** debridement, and topical agents. We did not consider biochemical analysis (e.g. glucose levels, serum levels of fructosamine, and advanced oxidation protein products, among others). The articles or bibliographic materials were the ones that did not attend the inclusion criteria.

2.4 Data collection and analysis

Selection of studies

Two reviewers (F.H and L.B.) independently screened titles and abstracts for potentially eligible studies. We used full-text papers to determine the final inclusion in this review. Disagreements were resolved between review authors through discussion or by the arbitration of a third review author (M.E.M or A.Q.L) when consensus could not be reached. We included only full-text papers, written in any language, regardless of the date of publication. We sent all remaining papers that were written in languages other than Portuguese and English to translators.

Search strategy

To establish the descriptors used in the searches for the articles, as well as their synonyms, a query was made to DeCS (Descriptors in Health Sciences) and MeSH (Medical Subject Headings). We used custom Boolean operators (AND, OR and NOT). The search strategy was:

("ozonated"[All Fields] OR "ozonating"[All Fields] OR "ozonation"[All Fields] OR "ozonations"[All Fields] OR "ozone"[MeSH Terms] OR "ozone"[All Fields] OR "ozone s"[All Fields] OR "ozonization"[All Fields] OR "ozonized"[All Fields] OR "ozonizer"[All Fields]) AND ("ozone"[MeSH Terms] OR "ozone"[All Fields] OR ("tropospheric"[All Fields] AND "ozone"[All Fields]) OR "tropospheric ozone"[All Fields]) AND ("ozone"[MeSH Terms] OR "ozone"[All Fields] OR ("ozone"[All Fields] AND "tropospheric"[All Fields]) OR "ozone tropospheric"[All Fields]) AND ("ozone"[MeSH Terms] OR "ozone"[All Fields] OR ("low"[All Fields] AND "level"[All Fields] AND "ozone"[All Fields]) OR "low level ozone"[All Fields]) AND ("ozone"[MeSH Terms] OR "ozone"[All Fields] OR ("level"[All Fields] AND "ozone"[All Fields] AND "low"[All Fields])) AND ("ozone"[MeSH Terms] OR "ozone"[All Fields] OR ("ozone"[All Fields] AND "low"[All Fields] AND "level"[All Fields])) AND ("ozone"[MeSH Terms] OR "ozone"[All Fields] OR ("ground"[All Fields] AND "level"[All Fields] AND "ozone"[All Fields]) OR "ground level ozone"[All Fields]) AND ("ozone"[MeSH Terms] OR "ozone"[All Fields] OR ("level"[All Fields] AND "ozone"[All Fields] AND "ground"[All Fields])) AND ("ozone"[MeSH Terms] OR "ozone"[All Fields] OR ("ozone"[All Fields] AND "ground"[All Fields] AND "level"[All Fields])) AND (("therapeutical"[All Fields] OR "therapeutically"[All Fields] OR "therapeuticals"[All Fields] OR "therapeutics"[MeSH Terms] OR "therapeutics"[All Fields] OR "therapeutic"[All Fields]) AND ("therapeutical"[All Fields] OR "therapeutically"[All Fields] OR "therapeuticals"[All Fields] OR "therapeutics"[MeSH Terms] OR "therapeutics"[All Fields] OR "therapeutic"[All Fields]) AND ("therapeutics"[MeSH Terms] OR "therapeutics"[All Fields] OR "therapies"[All Fields] OR "therapy"[MeSH Subheading] OR "therapy"[All Fields] OR "therapy s"[All Fields] OR "therapys"[All Fields]) AND ("therapeutics"[MeSH Terms] OR "therapeutics"[All Fields] OR "therapies"[All Fields] OR "therapy"[MeSH Subheading] OR "therapy"[All Fields] OR "therapy s"[All Fields] OR "therapys"[All Fields]) AND ("therapeutics"[MeSH Terms] OR "therapeutics"[All Fields] OR "treatments"[All Fields] OR "therapy"[MeSH Subheading] OR "therapy"[All Fields] OR "treatment"[All Fields] OR "treatment s"[All Fields]) AND ("therapeutics"[MeSH Terms] OR "therapeutics"[All Fields] OR "treatments"[All Fields] OR "therapy"[MeSH Subheading] OR "therapy"[All Fields] OR "treatment"[All Fields] OR "treatment s"[All Fields])) AND (("ozonated"[All Fields] OR "ozonating"[All Fields] OR "ozonation"[All Fields] OR "ozonations"[All Fields] OR "ozone"[MeSH Terms] OR "ozone"[All Fields] OR "ozone s"[All Fields] OR "ozonization"[All Fields] OR "ozonized"[All Fields] OR "ozonizer"[All Fields]) AND ("therapeutics"[MeSH Terms] OR "therapeutics"[All Fields] OR "therapies"[All Fields] OR "therapy"[MeSH Subheading] OR "therapy"[All Fields] OR "therapy s"[All Fields] OR "therapys"[All Fields])) AND (("diabetic foot"[MeSH Terms] OR ("diabetic"[All Fields] AND "foot"[All Fields]) OR "diabetic foot"[All Fields]) AND ("diabetic foot"[MeSH Terms] OR ("diabetic"[All Fields] AND "foot"[All Fields]) OR "diabetic foot"[All Fields] OR ("foot"[All Fields] AND "diabetic"[All Fields]) OR "foot diabetic"[All Fields]) AND ("diabetic foot"[MeSH Terms] OR ("diabetic"[All Fields] AND "foot"[All Fields]) OR "diabetic foot"[All Fields] OR ("diabetic"[All Fields] AND "feet"[All Fields]) OR "diabetic feet"[All Fields]) AND ("diabetic foot"[MeSH Terms] OR ("diabetic"[All Fields] AND "foot"[All Fields]) OR "diabetic foot"[All Fields] OR ("feet"[All Fields] AND "diabetic"[All Fields]) OR "feet diabetic"[All Fields]) AND ("diabetic foot"[MeSH Terms] OR ("diabetic"[All Fields] AND "foot"[All Fields]) OR "diabetic foot"[All Fields] OR ("foot"[All Fields] AND "ulcer"[All Fields] AND "diabetic"[All Fields]) OR "foot ulcer diabetic"[All Fields]))

Selection process

Articles found through the search strategy or additional sources were initially selected by reading their titles and abstracts. They were independently identified by two reviewers who defined which studies met the above inclusion criteria. Potentially qualified studies were read in full and independently selected for eligibility evaluation.

A standardized form was used to extract data from the included studies to assess study quality and evidence. The extracted information included: Bibliometric data (authors, year of publication, language); Study characteristics (study design, sample size, description of the sample, country, recruitment modality, funding); Characteristics of the population and participants (gender, age); Details of the intervention and control conditions; Duration of follow-up assessments; Outcomes assessed; and Study results.

Data collection process

All numerical data provided by the studies were considered as mean, standard deviation, odds ratio, relative risk, confidence interval, and follow-up time. In regards to trials with multiple publications or sub-studies, the study was included only once. To avoid possible double-counting of patients, articles from the same group of authors were evaluated considering the time/place of recruitment of patients and the time of intervention. In case of doubt, the authors were contacted for clarification.

Assessment of risk of bias in included studies

We assessed the risk of bias in the included studies using the 'Risk of bias' assessment tool, as recommended by The Cochrane Collaboration, (Training, n.d.) which includes randomization, concealment of allocation, masking, follow-up, selective description of the outcome and other sources of bias (the sample size calculation was considered). We rated each of the six items of the 'Risk of bias' assessment as 'high', 'low' or 'unclear' risk. We defined a study with an overall low risk of bias as having a low risk of bias in five or more of these items.

Two review authors (F.H. and M.E.M.) independently performed the 'Risk of bias' assessment. Possible disagreements between review authors were resolved by discussion, or arbitration by a third review author (L.B.), when consensus could not be reached. Data were represented by graphs made by Review Manager 5.4.

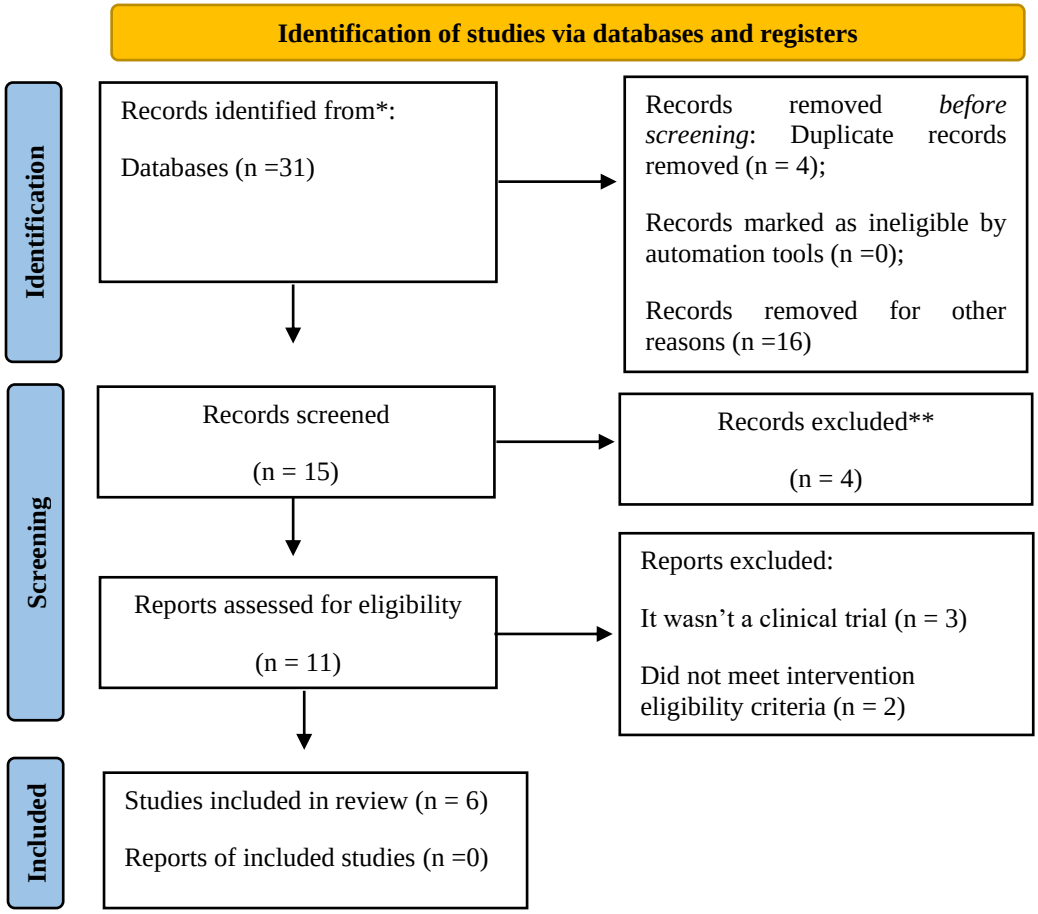
Data synthesis

It was not possible to combine studies' results through the meta-analysis due to discrepancy.

3. Results

The search retrieved nine trials, of which six fulfilled the inclusion criteria and were included in this review (a total pooled sample of 608 participants).

Figure 1- Flow diagram.



Source: Authors.

3.1 Study population

The population of the included studies was composed of adults and elders. All studies included men and women with diabetic foot ulcers.

3.2 Technique: number and duration of treatments

Three studies compared the use of ozone therapy in the rectal with other therapies. One study conducted a clinical trial with three comparison groups, two of which were considered intervention groups. The first intervention group used rectal insufflation of ozone while, in group three, participants used topical ozone therapy plus antibiotics. The second group, the control group, was subjected to usual care, based on oral and systemic antibiotics.

Two trials compared local ozone application through the use of a vacuum-sealed device. All studies used usual care measures for their control groups.

The duration of therapy in the included trials ranged from 20 days to 24 weeks. We considered pain and quality of life as secondary outcomes in this review; however, none of the included studies reported these outcomes.

3.3 Risk of bias in included studies

The results from the 'Risk of bias' assessment for the individual studies are summarized in Figure 2. In total, we considered 16,67% of the studies to have a low risk of bias, which represents one study out of six.

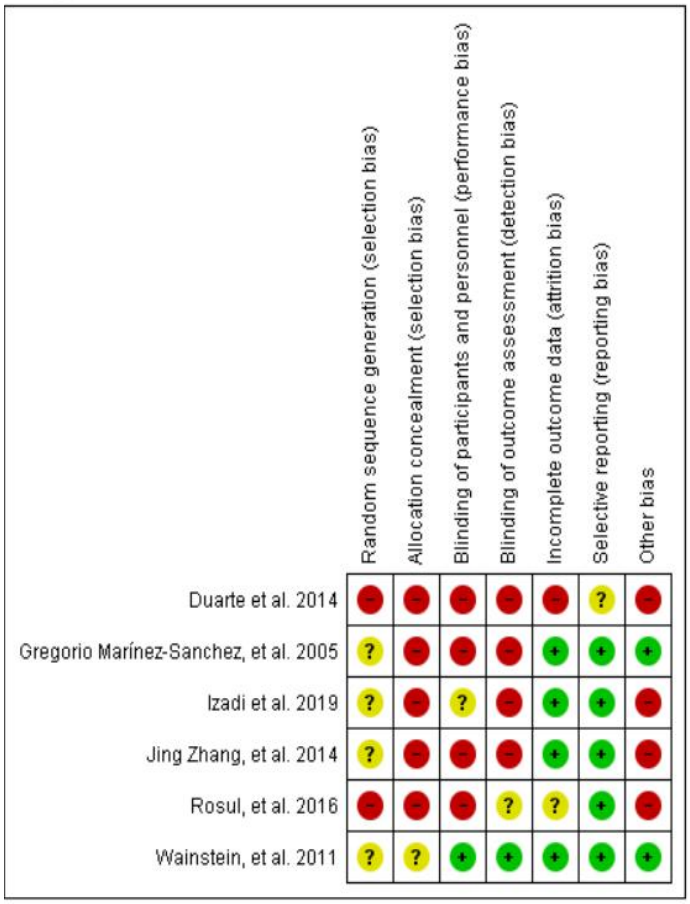
Allocation - In all studies, there was no detailed information about the randomization and allocation procedures.

Blinding - In one trial the information about blinding was nuclear. (Wainstein, et al., 2011) The additional studies did not blind both the evaluator and patients. Presumably, blinding of therapists was not possible considering the intervention proposed.

Incomplete outcome data - A total of four studies provided information on missing data, although none of the studies reported long-term follow-up.

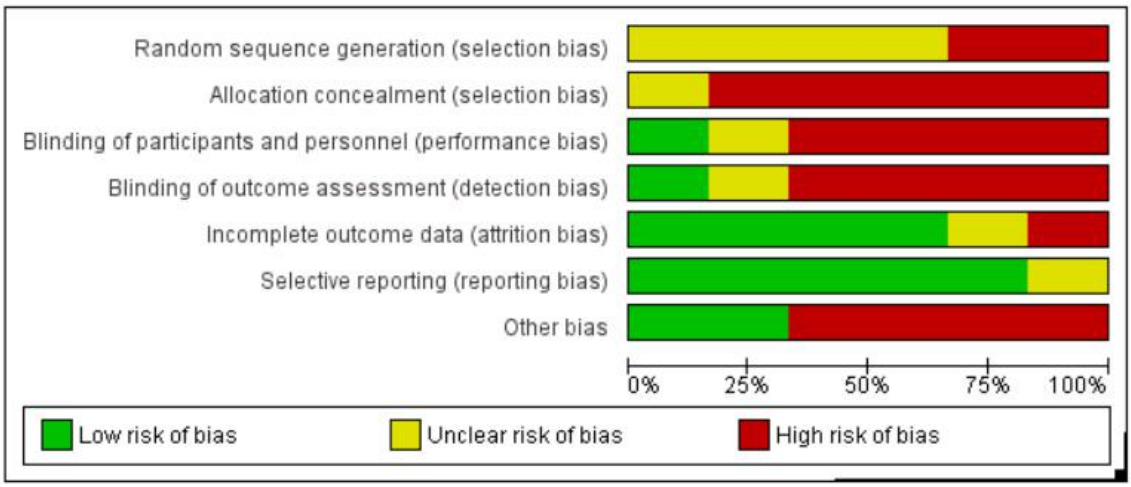
Selective reporting - Protocols for all trials were not available, but it was clear that all expected outcomes that were included or reported in advance met this criterion. We considered five studies included with a low risk of bias for this criterion. Only one study had an uncertain bias, as it did not indicate all pre-established outcomes in the results.

Figure 2 - Risk of bias summary: review authors' judgments about each risk of bias item for each included study.



Source: Authors.

Figure 3 - Risk of bias graph: review authors' judgments about each risk of bias item, presented as percentages across all included studies.



Source: Authors.

3.4 Description of included studies

(Izadi et al., 2019) compared ozone treatment for foot ulcers in 100 patients with DM, applied through two different routes. Local administration was performed using ozonized gel (Ozolive), applied to the wounds every 12 hours, and closed with sterile gauze, in addition to the subcutaneous application of an oxygen-ozone mixture around the wound. On the other hand, ozone therapy associated with oxygen was administered through rectal insufflation or intravenously. This treatment regimen was compared to usual care in 100 control patients. Patients were followed until wound closure and tissue re-epithelialization were confirmed by the physician. Treatments, such as antibiotics, sterile dressing, and debridement, were performed on all patients. Time to wound recovery and mean reduction in ulcer area from baseline. The period of the healing and mean baseline surface area showed no difference between groups. Time to wound recovery and amputation rate in the ozone group were lower. The trial did not report the methods used to generate the randomization sequence, or the methods used to conceal allocation.

(Martínez-Sánchez et al., 2005), applied ozone (OZOMED) at a concentration of 50mg/L through rectal insufflation in 51 patients, along with ozone at a concentration of 60mg/L administered through a vacuum-sealed plastic bag on the patient's leg, and ozonized sunflower oil (Oleozon®) application after bag removal. This group was compared to 49 patients who received systemic antibiotics and conventional treatment. The mean reduction in ulcer area from baseline and perimeter was greater in the intervention group than in the control group. Length of hospital stay was reduced in patients treated with ozone. No significant difference was observed in the subjective clinical evolution of the two groups, but, in the ozone group, there was an increase in the number of cured patients and a consequent decrease in the number of uncured patients.

(Álvarez Duarte, 2014) performed a trial with two intervention groups. The control group was composed of 50 patients treated with oral antibiotics (ciprofloxacin 250 mg, cephalexin 500 mg, and cotrimoxazole 480 mg) and systemic antibiotics (ceftriaxone 1g, metronidazole 0.5%, cefotaxime 1g, meropenem 1g, bulb ciprofloxacin 200 mg). The two intervention groups were composed of 50 patients each, and intervention was based, respectively, on the use of ozone therapy for 21 days, at a concentration of 40-50mg/L, according to the extent of the wound, or ozone therapy for 21 days plus antibiotics. Patients were followed until complete ulcer healing. Weekly visits and wound size measurements were performed. In the end, a reduced length of hospital stay was noticed in the intervention group that used the combined therapy (ozone

therapy associated with antibiotics), as well as a higher frequency of improvement in ulcers, being 75% higher in those who received ozone plus antibiotics.

(Zhang et al., 2014) compared two types of treatment for diabetic foot ulcers. In the intervention group, patients received debridement, followed by non-invasive oxygen-ozone treatments at a total volume of 20 to 50mL, applied through a special bag for 30 minutes per day, followed by 20 days using the ozone generating device (Humazon Promedix, German), in addition to standard treatment. The control group received only standard treatment, which included debridement once every two days and wound dressings appropriate for the degree of exudate and moisture maintenance of the wound. For the therapeutic effect criterion, wound conditions were evaluated into one of the four grades by the criteria as follows, where Grade 0: indicates no change or worse than before; Grade 1: wound size reduction to less than 1/2; Grade 2: wound size reduced by more than 1/2, secretion obviously concentration less than before, with little necrosis, and newly generated granulation; Grade 3: wound healing completely epithelialized with dimensions of 0 x 0 x 0 cm. Finally, the reduction in wound size was significantly greater in the intervention group. An increase in collagen fibers was also observed in the intervention group.

(Wainstein et al., 2011) conducted a double-blind clinical trial, using as an intervention an active ozone treatment from the vice Ozoter 101 ® device (OZ Recovery Technologies, Ramat Gan, Israel), designed to treat ulcers using an oxygen-ozone mixture. The study selected 61 patients with type 1 or 2 diabetes, in stage 2 or 3 of the Wagner classification, recommended to assess the progression of diabetic foot impairment. Included participants (a total of 61) were randomized into two groups and the researchers recruited a technician trained to operate the Ozoter 101 device. The technician determined the mode of action for each patient according to randomization so that 32 patients were treated in the active model (ozone-oxygen treatment), and 29 in the idle mode (ambient air only). Both groups received the usual care for diabetic foot ulcers, which included debridement and daily dressings for wound closure. Some of the participants did not complete the full follow-up and had the collected data imputed using the last observation performed. The wound surface area of each participant was evaluated, with the method described by Bohannon and Pfaller, before and after the start of treatment. Sixteen patients treated with ozone had a significantly higher rate of achieving complete wound closure than patients included in the control group (81% vs. 44%, $P = 0.03$).

(Rosul M.V, 2016) , in a clinical trial, compared two groups of patients with stage I and II diabetic foot, corresponding to superficial and deep ulcers without involving the subcutaneous tissue, ligaments, tendons, and muscles. The first group received standard therapy, specified by blood sugar correction, antibacterial therapy, antiaggregant therapy, anticoagulants, infusion of rheological drugs, and detoxifying preparations, associated with systemic and local ozone therapy. Systemic intravenous infusion of 200ml of ozonized saline solution was performed, at an ozone concentration of 1000−1300 mcg / l. Topical ozone was used in a 0.9% NaCl solution with ozonized sea buckthorn oil at a 4000 mcg concentration. The authors concluded that the use of ozone treatment was responsible for a decrease in a burning pain in the foot (from 69.57% to 39.13%), constant coldness of the feet (30.43% to 8.70 %), and paresthesia (from 69.57% to 34.78%). In addition, the wound healing process was accelerated in the intervention group, compared to patients who received only standard care. A reduction in swelling and hyperemia of the skin around the wound was observed in 10.17 ± 0.74 days.

Table 1 - Characteristics of the included studies.

Author, year	Study type	Number of participants (study and control group)	Inclusion and exclusion criteria	Outcomes
Izadi et al. 2019	Single blind randomized clinical trial	Control group: 100 patients, 50 men and 50 women. Intervention group: 100 patients, 50 men and 50 women	Inclusion: patients aged 18-85 years and diagnosed with DM with Wagner 1 to 4 diabetic foot and who took the course of treatment regularly. Exclusion: patients with abnormal thyroid function test and abnormal coagulation test, pregnant or lactating patients, patients with G6PD, and patients with hypersensitivity to ozone.	Mean surface area from baseline ulcer there was no difference between control and intervention groups. Healing time Duration of healing Amputation rate
Duarte et al. 2014	Clinical trial	150 patients Control group (1): 50 Control group (2): 50 Intervention group: 50	Inclusion: Over 40 years old, both sexes, Type II Diabetic with diagnostic criteria for neuroinfectious diabetic foot, followed up at the angiology service Exclusion: patients who presented with severe septic status; Patients with liver disease, Patients with nephropathy; Hypersensitivity to O3 and antibiotics; Women with ongoing pregnancy.	Extent of injury
Gregorio Marínez-Sanchez, et al. 2005	Randomized controlled clinical trial	Control group: 49 patients Intervention group: 51 patients	Inclusion: Adult patients of both sexes and ethnic origins with a diagnosis of neuroinfectious diabetic foot according to the classification of McCook et al (1971) who were suffering from ulcer of the feet and lower extremities and who were hospitalized at the Institute of Angiology and Vascular surgery Exclusion: severe septic conditions, hypersensitivity to the drug being used, liver dysfunction, renal failure (N1 creatine level, 32umol/L), pregnancy, cancer or other serious illness, inability to comply with study requirements, recent history of abuse of alcohol or drugs, current therapy with any immunosuppressive or anticonvulsant agent, concurrent participation in another clinical trial, or current treatment with an investigational drug.	Primary: Clinical evaluation of the lesion by measuring the area and perimeter of the lesions using a tracing made on an acetate plate at the beginning and end of the study and the change over time; qualitative clinical assessment of lesions; length of stay. And glucose levels. Secondary: Serum levels of fructolysin, advanced protein products, nitric oxide, reduced glutathione, glutathione peroxide, catalase, superoxide dismutase, total hydroperoxides, potent peroxidation and malondialdehyde. And the side effects.
Jing Zhang, et al. 2014	Prospective randomized clinical trial	Control group: 25 patients Intervention group: 25 patients	Inclusion: 18 years or older with Wagner classification 2,3 or 4 diagnosed with diabetic foot. Exclusion: gangrenous skin ulcers all over the foot; active osteomyelitis; history of collagen diseases; hyperthyroidism; pregnancy or breastfeeding; hemoglobin A1c (HbA1c) levels > 10.5%; ankle-brachial index (ABI) <0.70; hemoglobin less than 90 g / L; liver function tests (alanine transaminase, aspartate transaminase, or c-glutamyl transpeptidase)	Wound size

			elevated to more than three times the upper limit of normal; serum creatinine > 133 μmol/L or dialysis and known ozone allergy.	
Rosul, et al. 2016	Clinical trial	Total: 47 Intervention group: 23 Control group: 24	Patients with stages I and II of the diabetic foot which corresponds to superficial and deep ulcers without involving subcutaneous tissue, ligaments, tendons, and muscles in the process, without bone lesion, phlegm and abscess	Primary - cytological of wound secretion, lipid peroxidation status and antioxidant protection status Secondary: Pain, swelling and hyperemia
Wainstein, et al. 2011	Double-blind, randomized clinical trial	Total: 61 Intervention group: 32 Control group: 26	Included in the study were adults (18 years of age or older), men and women with type 1 and type 2 diabetes and a stage 2 Wagner classification or stage 3 or post-debridement 4-foot ulcer. The size of the wound was ≤ 40 cm 2, and the wound was at least 8 weeks old in the study initiation. Excluded patients with (1) gangrenous foot ulcers, (2) active osteomyelitis, (3) a history of collagen diseases, (4) hyperthyroidism, (5) pregnancy or breastfeeding, (6) hemoglobin, A1c (HbA1c) levels > 10.5%, (7) ankle-brachial index (ABI) <0.65, (8) hemoglobin less than 8 g/dL, (9) liver function tests (alanine transaminase, aspartate transaminase or c-glutamyl transpeptidase) elevated to more than three times the normal limit, (10) serum creatinine > 2.5 mg/dL or dialysis, and (11) a known ozone allergy	Primary – complete wound closure Secondary: wound size and (2) the proportion of patients who had a reduction in wound size.

Source: Authors.

Table 2 - Characteristics of the included studies

Author, year	Intervention	Control	Follow-up	Outcomes
Izadi et al. 2019	Routine care + ozone therapy. Ozone therapy was performed in 2 ways, locally and systemically. Local: ozonized gel (ozolive) applied to the wounds every 12 hours and closed with sterile gauze + application of oxygen-ozone around the wound subcutaneously. Systemic: a mixture of ozone and oxygen rectally or intravenous administration after the necessary preparations. Patients took vitamin C immediately after the systemic procedure. They were performed twice a week with an interval of at least 24 hours until wound closure and re-epithelization were confirmed by the physician.	Routine care	20 sessions (2x per week), with an interval of at least 24 hours, until the wound is completely closed and epithelialization is confirmed by a doctor	200 patients were analyzed (100 men and 100 women). All patients completed the study. The mean age of patients was 59.03 ± 12,593 and 53.5 ± 10,212 for groups 1 and 2. Mean surface area from baseline ulcer there was no difference between control and intervention groups. Healing time in the intervention group was shorter than the average healing time in the control group. Healing duration was not different in the groups. Amputation rates for high-grade and large wounds were higher than for low-grade and small wounds. FBS increased, while the other variables decreased. A cut-off point of 20 sessions was set. The amputation rate in the ozone group was lower and this rate increased as the wound worsened. There was no side effect.
Duarte et al. 2014	Intervention group - patients who received ozone therapy for 21 days associated with antibiotic therapy.	Control Group (1) - patients who received oral antibiotics (ciprofloxacin 250 mg, cephalexin 500 mg and cotrimoxasol 480 mg) and systemic (ceftriazone 1g, metronidazole 0.5%, cefotaxime 1g, meropenem 1g, ciprofloxacin bulb 200 mg). Control group	21 days	Reduction in the length of hospital stay of patients in the intervention group. The frequency of improvement of lesions exceeded 75%, being higher in those who received the combined treatment;

		(1) - ozone therapy for 21 days. O3 was applied locally with a concentration of 40-50mgL according to the wound extension.		
Gregorio Marínez-Sanchez, et al. 2005	Ozone by rectal insufflation with an ozone dose of 10mg, ozone concentration of 50mg/L, and locally. The lesion was covered with plastic, and the leg was vacuum sealed. Then the bag was recharged with ozone at a concentration of 60mg/L. The patient remains with the bag for 1 h and after that, ozonized sunflower oil is applied to the lesion. NOTE: debridement was indicated for all wounds and gauze dressings were used.	Systemic antibiotic therapy according to the pathogen present, with the application of conventional topical treatment for 20 days.	20 days	The groups were similar (p>0.05). 44% of patients in both groups were over 60 years of age and the majority were white. The medical history was mainly characterized by hypertension and the concomitant treatments used were those to control hypertension (captopril, nifedipine), in addition to blood glucose (glibenclamide) and cardiovascular disease (ASA). More patients were treated with hypoglycemic drugs in the intervention group (80%) than in the control. Area and perimeter of the lesion: at the beginning of the study there was no difference between the groups, at the end, the intervention group achieved a significant decrease in area and perimeter. 4 patients in the control group had increased area and perimeter. The reduction in the area with time and the reduction in perimeter with time were greater in the intervention group than in the control. Clinical evolution: there was no significant difference between the 2 treatments, but there was an increase in the number of cured patients and a decrease in the number of uncured patients in the intervention group compared to the control. The duration of hospitalization was shortened in ozone-treated patients.
Jing Zhang, et al. 2014	After debridement, the ozone group received non-invasive oxygen-ozone treatments with 52 μg/mL ozone (total volume: 20–50mL) in a special bag for 30min daily for 20 days using the ozone generating device (Humazon Promedic, German)	Standard care only, which included debridement once every two days and dressings appropriate for the degree of exudate and		The reduction in wound size was significantly greater than in the control group; In the ozone group, collagen fibers were larger than in the control group; On days 7 and 11, VEGF and PDGF levels were significantly higher in the ozone group; On day 11, the ozone group had a significantly higher TGF-β level than the control group; on day 11, VEGF, TGF-β and PDGF contents

	in addition to standard treatment.	maintenance of wound moisture.		in wounds were significantly higher in the ozone group than in the control group.
Rosul, et al. 2016	Traditional therapy + systemic and regional ozone therapy for 12-14 days, one session per day. Systemic-Intravenous 200ml ozonized saline solution (ozone concentration 1000−1300 mcg/l) Regional- ozone in 0.9% NaCl solution and 4000 ozonized sea buckthorn oil mcg/l concentration	Traditional therapy includes blood sugar correction, antibacterial therapy, antiplatelet therapy, anticoagulants, infusion of rheological drugs and detoxifying preparations.		Decreased burning pain in the foot (from 69.57% to 39.13%), constant coldness of the feet (from 30.43% to 8.70%) and paresthesia (from 69.57% to 34.78%). Reduction of swelling and hyperemia of the skin around the wound in 10.17 ± 0.74 days phase acceleration of the wound process course swelling and hyperemia around the wounds were reduced
Wainstein, et al. 2011	Ozone treatments using Ozoter 101 ® from vice (OZ Recovery Technologies, Ramat Gan, Israel), in addition to the usual treatment for diabetic foot	Treatments using the Ozoter 101 device configured to inactive mode, in addition to the usual treatment (daily dressing appropriate ingredients for the degree of secretion and moisture wound maintenance)	24 weeks	A significant difference between the groups in the proportion of patients with complete wound closure were not detected (41% vs. 33%, P=0.34). The difference between the groups in the proportion of patients with complete wound closure was not significant

Source: Authors.

4. Discussion

Although studies regarding the treatment of ozone therapy in patients with diabetic foot ulcers are still very scarce, the association between ozone therapy and a reduction in ulcer size is evident in the available studies. Among the six articles included in this review, five achieved this conclusion as an outcome.

Of the six treatment effect estimates provided in this review, only three had a decreased time to wound healing compared to usual care as an outcome. However, it is noteworthy that this outcome is not addressed in the other three selected articles.

In achieving complete wound closure, ozone treatment is more effective than routine treatment, according to only one study. No results were found for the observation of the degree of the wound as an outcome. Although one study mentioned the selection of patients in stage 2 or 3 of the Wagner classification, it does not include in its results any improvement considering this scale.

No studies associating alternative therapy with ozone were found when it comes to considering outcomes within the biopsychosocial sphere. This finding completely disregards the high prevalence of the disease and its impact on a patient's quality of life.

Finally, the evidence on ozone therapy in patients with diabetic foot ulcers has a low to moderate quality, mainly because there is a gap in the possibilities of treatment with this particular therapy.

4.1 Overall completeness and applicability of evidence:

The studies included in this review were conducted in Iran, Cuba, China, Ukraine, and Israel with adult participants diagnosed with Diabetes Mellitus and diabetic foot ulcers. The studies did not address the applicators of ozone therapy and outpatient care. Regarding the clinical relevance of the selected studies, a clear description of the patients, results, and interventions used was observed. However, none of the articles included managed to cover all the expected outcomes for the construction of this review.

4.2 Quality of the evidence

In summary, most of the selected studies demonstrated a high risk of bias regarding allocation, randomization, and blinding of investigators and participants, which may have compromised the quality of the evidence provided, despite the relevant sample size (from 47 participants to 200).

4.3 Potential biases

The main limitation found in this review is the low number of available trials that addressed the subject, as it is a topic of current relevance in medicine. The included studies revealed a high risk of selection bias and performance bias, when considering the generation of random sequence and allocation concealment, and blinding of participants and professionals at risk.

5. Conclusion

In view of the objective to assess the effectiveness of ozone in the therapy of patients with diabetic foot ulcers, after analyzing the data provided by carefully selected clinical trials, there is no sufficient evidence to conclude that monotherapy with ozone, used both systemic or locally, is responsible for completely healing ulcers. However, when associated with

standard therapy, a significant reduction in wound degree, wound size, and pain, as well as a decrease in healing time, which interferes with the patients' quality of life, were observed. Health care costs are, thus, also reduced.

It is important to emphasize the scarcity of studies in the medical literature. These findings highlight the need for additional clinical trials with well-designed methodologies and low risk of bias, which include longer exposure to ozone therapy, standardization of ozone therapy management, as well as long-term follow-up of patients undergoing therapeutic practice. Ozone therapy can be an important adjuvant element in the treatment of diabetic patients who have chronic ulcers located in the lower limbs.

Therefore, it is worth mentioning that future research and articles must do additional clinical trials with more appropriate methodologies. Subsequently, when a new systematic review gets conducted, those articles will support ozone therapy as an essential adjuvant treatment for patients with diabetic foot.

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