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The Effectiveness of Ozone Therapy on Diabetic Ulcer Repair: A Systematic Review and Meta-analysis: Study of Changes in Wound Size

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ABSTRACT

Introduction: Diabetic foot ulcer (DFU) is a typical dermatological complication often found in patients with diabetes mellitus (DM). Chronic hyperglycemia state causes microvascular impairment that leads to loss of peripheral sensory, motor, and autonomous functions. The current diabetic ulcer therapy has not yet provided a satisfactory reduction of the ulcer area; thus, several adjuvant therapies were tried, such as using ozone therapy, either systematically or topically. Ozone increases the cell membrane permeability to glucose, increases oxygen metabolism, improves oxidative preconditioning, stimulates endogenous antioxidant systems, and has anti-inflammatory and antibacterial effects. **Methods:** This study was an analytic observational study, with systematic review and meta-analysis. Data searching were conducted online at Pubmed-MEDLINE, Scopus, EBSCOhost, ProQuest, Cochrane library, ClinicalTrials.gov, and Google Scholar, as well as hand-searching from libraries in Indonesia. Five most relevant articles included in the qualitative (systematic review) (n = 449) and three of those five articles included in the quantitative analysis (meta-analysis) (n = 211). Reduction of ulcer area was assessed. **Results:** The meta-analysis of effectiveness of ozone intervention group compared to the control group showed a homogeneity in data (Q=95.547, df=2; p=0.000, Tau2=5.276). Meta-analysis showed the value of Q statistic was z value = -9.478; (p=0.000). This shows that the overall administration of ozone can significantly improve the diabetic ulcer repair. The overall standardized mean difference showed an increase in the reduction of ulcer area following ozone therapy was -1.740 (95% CI [-2.100 to -1.380], p=0.00). These results indicate that the ulcer healing of the intervention group was significantly better than the control group. **Conclusion:** Systematic review and meta-analysis results show that the application of ozone therapy significantly improves DFU repair.

Keywords: ozone therapy, diabetic foot ulcers, diabetic ulcers repair

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Introduction

Diabetic foot ulcer (DFU) is a typical dermatological complication often found in patients with diabetes mellitus (DM). Diabetic ulcers are defined as full-thickness wounds extending to the dermis, below the ankle, on a weight-bearing or open surface in individuals with diabetes.[1] Chronic hyperglycemia conditions cause microvascular disturbances leading to peripheral neuropathy, including loss of sensory, motor, and autonomic function. Furthermore, the foot becomes unaware of the injuries, accompanied by balance disturbances and loss of skin protective function due to dry skin.[2]

The International Diabetes Federation Organization estimates at least 463 million cases of diabetes in people aged 20-79 years worldwide in 2019. While the prevalence of diabetes in Indonesia reached 2%, and this number continues to increase.[3] The increasing prevalence of diabetes lead to an increase in diabetic ulcer incidence. Diabetic ulcers are also the most frequent indication for hospitalization of patients with diabetes, of which around 25% of them were at risk of developing diabetic ulcers. Thus this creates a social-economic burden for sufferers.[4]

Treatments currently commonly performed for diabetic ulcer patients include glycemic index control, wound debridement, vascular surgery, antibiotic therapy (topical and systemic), and wound dressings using materials such as silver sulfadiazine, hydrogel, alginate, hydrocolloid, and foam. The current therapy is still unsatisfactory because, within one year, as many as 20% of ulcers do not heal, and 40% experience recurrences.[5] As many as 10-15% of diabetic ulcers are usually still active ulcers, and 5-24% lead to limb amputation in the 6-18 month period. Apart from the duration of ulcer healing and the incidence of amputation, an essential outcome as a parameter for clinical monitoring of diabetic ulcers is a change in the ulcer area.[6] Until now, diabetic ulcer therapy has not provided a satisfactory reduction of the ulcer area, so several adjuvant therapies have been tried, such as ozone therapy, hydro-surgery, acellular bioproducts, human growth factors, and oxygen therapy, as well as electrical or light-based therapies.[5]

Ozone therapy uses ozone gas to treat diseases or wounds, particularly diabetic ulcers.[4] This gas increases the permeability of cell membranes to glucose, increases oxygen metabolism, improves oxidative preconditioning, and stimulates endogenous antioxidant systems, all of which can ultimately prevent cellular neuropathies and increase tissue perfusion and oxygenation. In addition, ozone also has anti-inflammatory and antibacterial effects.[7,8] Ozone therapy for the diabetic foot can be administered systemically and topically. Forms of systemic therapy can be given through autohemotherapy or rectal insufflation, while topical therapy is generally used as a wound cleanser, pain injection, and a combination of local anesthetics. Several studies have demonstrated the effectiveness of ozone therapy in treating infections and wounds due to its antimicrobial properties.[4,8-10] Rosul et al. found that intravenous and topical ozone therapy showed positive results on the wound-healing process of diabetic ulcers.[11] Izadi et al. found that ozone therapy is effective in promoting diabetic ulcer healing and reducing the incidence of infection and amputation.[12]

The ultimate goal of diabetic ulcers is to reach complete wound healing. A completely healed ulcer is reached if the skin parameters are seen to be completely intact.[13] The ulcer parameter to be assessed was the surface area of the ulcer, which was carried out at the beginning and end of the study.

Material and Methods

Literature Search

The following databases were accessed until data analysis: Pubmed-MEDLINE, Scopus, EBSCO, Cambridge Core, ProQuest, Cochrane library, ClinicalTrials.gov, and Google Scholar.

The following MeSH terms were used for searching:" *Ozone therapy*" AND" *Diabetic foot ulcer*". The literature search was performed by three reviewers independently using the 2009 Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) flow diagram. Every dispute in determining papers and data extraction was settled by consensus.

Inclusion criteria were clinical studies or trials investigating the efficacy of ozone therapy for patients with DFU that carried out until 2022 with or without randomization; subjects were older adults (older than 18 years old) diagnosed as having DFU Wagner criteria 1, 2, or 3, or 4 post debridement; intervention was in the form of ozone therapy compared to placebo or standard therapy, such as antibiotics, topical agents or usual care; and outcome of the study were in the form of changes in the ulcer area.

Exclusion criteria were case reports, case serials, editorial letters, systematical reviews, literature reviews, articles written in languages other than Indonesian and English without any translation available, and research subjects who have gangrenous foot ulcers, osteomyelitis, or G6PD deficiency.

Study Selection

Three reviewers conducted the study selection independently. Duplicated articles were removed. The title and abstract, as well as full-texts, were reviewed for eligibility using the predefined inclusion and exclusion criteria. Differences in opinion were resolved between all reviewers to reach a consensus.

Data Extraction

Three reviewers performed data extraction independently using The Cochrane Collaboration data collection form for RCTs. Every dispute in determining papers and data extraction was settled with consensus.

Assessment of Risk of Bias

Risk of bias assessments was performed independently by three reviewers using The Cochrane Collaboration data collection form for RCTs only and The Cochrane Collaboration tool for assessing the risk of bias in randomized clinical trials.

Data Synthesis

Meta-analysis of the difference in weighted mean was conducted using *Comprehensive Meta-Analysis Version 3.0.* A descriptive synthesis was performed if data were unavailable to enable pooling.

Results

Research articles were searched based on the 2009 PRISMA flowchart (Figure 1).

Study Characteristics

Most of the included studies were carried out in China (n=3), followed by Israel (n=1) and Cuba (n=1). The total sample from those five studies was 449 subjects. All studies are randomized control trials. All study subjects were patients with DFU. Three studies use ozone as a topical immersion, one study uses ozone water flushing, and one uses a combination of local and systemic ozone. Three studies used a standard routine DFU therapy without additional therapy as a control. Two other studies use a placebo therapy in the control group. The characteristics of included studies are presented in Table 1.

Result of Qualitative Analysis (Systematic Review)

1. Martínez-Sánchez et al., 2005[14]

Martínez-Sánchez et al. conducted a randomized controlled clinical trial in 2005. The subjects were adult patients diagnosed with neuroinfectious diabetic ulcers. A total of 100 patients were randomized into two different treatment groups: 1) antibiotic therapy group, consisting of 49 patients who were treated with systemic antibiotic therapy (according to the microbes present) using conventional treatment methods, with topical application to the lesions (for 20 days), and 2) ozone therapy group; consist of 51 patients who were treated daily with ozone (generated by OZOMED equipment, Cuba), 20 sessions, with rectal insufflation (at 10 mg ozone dose, ozone concentration: 50 mg/l) and locally. For local ozone treatment, the lesion

is covered with a plastic bag, sealed to the leg, and placed under a vacuum to remove any air inside. Afterward, the bag was refilled with ozone at 60 mg/l. Patients receive therapy with a plastic bag for 1 hour. Afterward, the bag was removed, and the wound was covered with ozonized sunflower oil (Oleozon®). Repair of the lesion area with time was significantly greater in the ozone group (2.66 ± 0.03 cm^2/day) than in the antibiotic group (1.21 ± 0.01 cm^2/day). In addition, this study found that patients treated with ozone achieved complete recovery more quickly (21±10 days) than patients treated with standard antibiotics (45±11 days, p=0.002). This study concluded that medical ozone treatment could be an alternative therapy for diabetes and its complications.

2. Wainstein et al., 2011[15]

Wainstein et al. in 2011, conducted a multicenter placebo-controlled randomized clinical trial study. Included in this study were adult men and women (18 years and older) with type 1 and type 2 diabetes and Wagner's classification stage 2 or 3 or stage 4 diabetic ulcer post-debridement. Subjects were randomized into one of the two treatment groups, namely the ozone group and the control group. The ozone (active treatment) group received ozone treatment using the Ozoter 101 device in addition to the usual diabetic ulcer treatment. The control group received sham treatment (placebo) using an Ozoter 101 device set to the inactive mode in addition to the usual treatment of diabetic foot ulcers. Usual care includes debridement and daily wound dressings appropriate to the level of secretions and maintenance of wound moisture. Active ozone treatment is divided into two phases: first, patients receive treatment sessions four times per week for a maximum period of 4 weeks or until granulations appear in 50% of the wound area, whichever comes first. The interval between treatments did not exceed one day, five days a week, and the gas concentration was 96% oxygen and 4% (80 µg/mL) ozone. During the second treatment period, the session frequency was reduced to twice weekly to complete the 12 weeks of treatment, and the gas concentration was changed to 98% oxygen and 2% (40 µg/mL) ozone. Patients in the control group received sham treatments, and the ozone device circulated room air. Each treatment session lasts 26 minutes. This study found insignificant improvement in ulcer area reduction in the ozone group compared to the placebo group (4.2 ± 4.9 cm2 vs. 2.7 ± 1.5 cm2, p= 0.23).

3. Zhang et al., 2014[16]

Zhang et al. 2014 conducted a study on 50 patients aged 18 years or older with diabetic ulcers classified by Wagner stage 2, 3, or 4. Patients were randomized into the ozone group (n=25) and the control group (n=25). After debridement, the ozone group received non-invasive oxygen-ozone treatment with 52 µg/mL ozone (total volume: 20–50 mL) in a special bag for 30 minutes per day for 20 days using an ozone generator device (Humazon Promedic, Germany) in addition to standard treatment. The control group received only standard care, including debridement every other day and wound dressing appropriate for exudate levels and maintaining wound moisture. The wound size reduction in the ozone group was significantly greater than in the control group (6.84 ± 0.62 versus 3.19 ± 0.65 cm2, p<0.001).

4. Hu et al., 2019[17]

Hu et al. 2019 conducted a randomized controlled trial study of 136 patients with diabetic ulcers. Subjects were randomly divided into two groups, namely (1) the combined group in which patients received VAC (Vaccum-assisted closure) and ozone water flushing and (2) the VAC group in which patients received only VAC. In the VAC group, the wound surface was washed with 0.9% saline for 3-5 days after debridement, and the condition of the wound was monitored daily. Meanwhile, an ozone generator prepared 10 µg/mL O3 water for ozone rinsing. The O3 water that had been prepared was then put into the VAC system, and the treatment lasted for 60 minutes. Treatment is carried out twice daily until the ulcer is closed.

The results showed that the duration of therapy in the combination group was significantly shorter than in the VAC group (12.6 ± 4.2 vs. 25.8 ± 4.3, p<0.001). Meanwhile, the reduction in wound surface area was significantly greater after one week, two weeks, and three weeks of treatment in the combination group (p < 0.05, but no numerical value was reported); however, no significant difference was found after one month of treatment between the two groups. All these results indicate that O3 water rinsing treatment can facilitate DFU recovery.

5. Xinyuan et al., 2021[18]

Xinyuan et al. conducted a randomized controlled clinical trial study in 2019. A total of 100 type 2 diabetic foot ulcer patients with diabetic foot ulcers were included as research subjects and divided into two groups using the random number table method, namely the experimental group and the control group. In the experimental group, a sterile bag for medical ozone therapy was placed on the infected limb wound to fix and keep it airtight. First, vacuum the air inside the bag, then blow medical ozone gas into the bag (the concentration of ozone gas is 35 mg/L). Then after 30 minutes, the bag is removed. Patients in the control group were treated the same as the experimental group but with sham therapy (placebo). In the control group, a sterile bag was used to cover the infected limb/wound and closed tightly, blow air into the bag, remove the air in the bag after half an hour, take back the wound secretions for bacterial culture, clean the wound and wrap it with a sterile dressing. At the end of the third week of the observation period, the ulcer healing index in the experimental group (reduction in the wound area, wound pain visual analogy score, and degree of reduction in inflammation index) was better than the control group. The area of the ulcer before and after the procedure in the experimental group was found to be significantly different (25.85 ± 8.77 vs. 4.65 ± 1.93; p<0.05), whereas, in the control group, there was no significant difference (23.29 ± 7.91 vs. 21.47 ± 8.14; p>0, 05).

Table 1. Characteristics of Included Studies

No	Authors, Year	Country	Sample Size	Study Population	Interventions		Study Outcome	Frequency	Duration of intervention
					Intervention Group	Comparison Group			
1	Martínez-Sánchez et al., 2005[14]	Cuba	100	DFU	Standard therapy + ozone rectal insufflation and local immersion (ozone bath)	Systemic and topical antibiotic	Measurement area and perimeter of the lesion; Qualitative clinical evaluations of the lesion; LOS to obtain good lesion; Glucose level	Once daily for 20 days	20 days
2	Wainstein et al., 2011[15]	Israel	61	Diabetes Mellitus type 1 and type 2 with foot ulcer	Standard therapy + local ozone immersion (ozone bath)	Standard therapy + placebo	The proportion of subjects with complete closure of the wound;Wound size;The proportion of patients who had a reduction in wound size	Four times a week during week 1, followed by twice weekly	24 weeks
3	Zhang et al.., 2014[16]	China	50	DFU with Wagner classification stage 2, 3, or 4	Standard therapy + local ozone immersion (ozone bath)	Standard therapy	Wound condition;Length; Width;Depth;Healing progress;Infection; Need for debridement	Once daily for 20 days	20 days
4	Hu et al.., 2019[17]	China	136	DFU	Standard therapy + VAC + ozone water flushing	Standard therapy + VAC	Duration of treatment; Change of wound surface area; Dressing changing times; Pain VAS score; Bacterial clearance; Secretion of the wound; Bacterial culture	Twice a week until the ulcer healing	One month
5	Xinyuan et al., 2021[18]	China	100	DFU	Standard therapy + local ozone immersion (ozone bath)	Standard therapy + placebo	Bacterial control; Degree of the decline of inflammation index; Ulcer wound reduction; Wound healing rate; VAS; VEGF level	Once daily	Three weeks

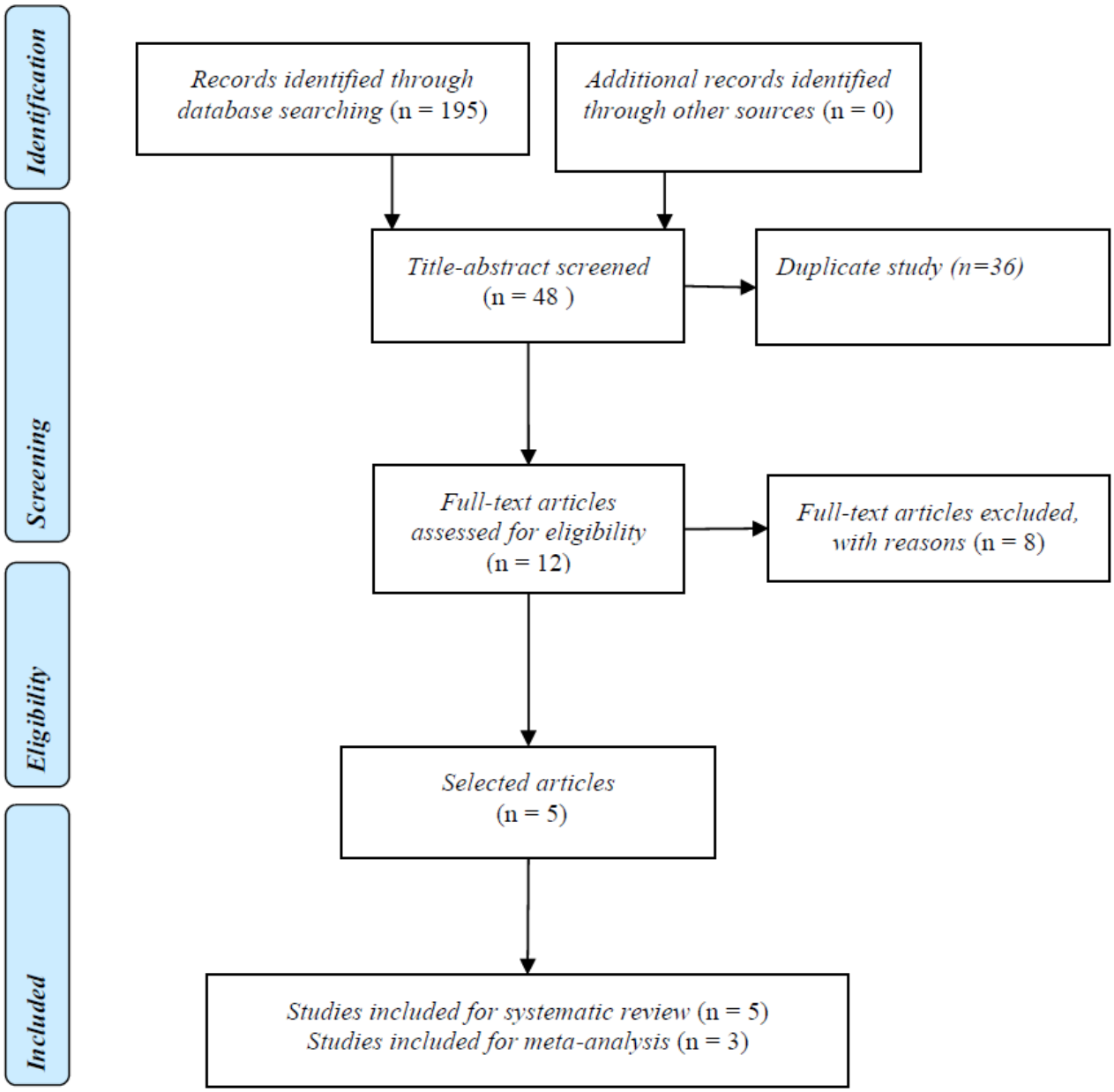

Figure 1. PRISMA Flow Diagram

Table 2. Changes in diabetic ulcer area in the ozone therapy group compared to the control group

No	Studies	Control Type	Ozone		Control	
			Mean±SD	n (subjects)	Mean±SD	n (subjects)
1	Wainstein et al, 2011	Standard therapy + Placebo	2.00 ± 3.90	32	1,60 ± 1,70	29
2	Zhang et al, 2014	Standard therapy	6.84 ± 0.62	25	3,19 ± 0,65	25
3	Xinyuan et al, 2021	Standard therapy + Placebo	21.2 ± 0.42	50	1,82 ± 0,31	50

Quantitative Data Result (Meta-Analysis)

Research articles included in the meta-analysis were the three studies of Zhang et al., 2014; Xinyuan et al., 2021; dan Wainstein et al., 2011; who assessed the surface area of diabetic ulcers pre and post-treatment with ozone. Meta-analysis of the parameter ulcer size in diabetic foot ulcer repair in groups with ozone intervention and control groups is presented in Table 2.

Meta-analysis of the effectiveness of ozone compared with the control group on diabetic ulcer repair showed the data were homogenous with I^2 <50% (Q=95.547, df=2; p=0.000, Tau^2=5.276). Meta-analysis showed the value of Q statistic was z value = -9.478; (p=0.000). This shows that the overall administration of ozone can significantly improve diabetic ulcer repair. The overall standardized mean difference in the reduction of ulcer area between the intervention group compared to the control was -1.740[-2.100 -1.380] (p<0.05). These indicated that the ulcer repair of the intervention group was significantly better than the control group (Figure 2).

Risk of Bias in Included Studies

The risk of bias from the study included in the analysis (both qualitative or quantitative) was assessed using The Cochrane Collecting data - form for RCTs only and The Cochrane Collaboration's tool for assessing the risk of bias in randomized trials, including the randomization technique, allocation concealment, participant blinding, blinding outcome, choice of outcomes reported, another source of bias, incomplete outcome data, and other biases. Table 3 shows the risk of bias in the included studies.

Discussion

This study is a meta-analytic observational study, a systematic review, and a meta-analysis of ozone therapy's effectiveness in improving ulcer areas in patients with diabetic ulcers. This study included five studies in a qualitative review (systematic review). In three of them, a quantitative review (meta-analysis) was carried out to determine the effect of ozone therapy on the improvement of ulcer areas in diabetic ulcer patients. Two studies, Martínez-Sánchez et al. (2005)[14] and Hu et al. (2019)[17], could not be included in the meta-analysis because of the lack of numerical data on the average ulcer area before and after treatment. This systematic review and meta-analysis provide evidence that ozone therapy has more beneficial outcomes as all clinical studies show increased wound healing compared to controls. Therefore, ozone is potentially an effective therapy in patients with diabetic ulcers.

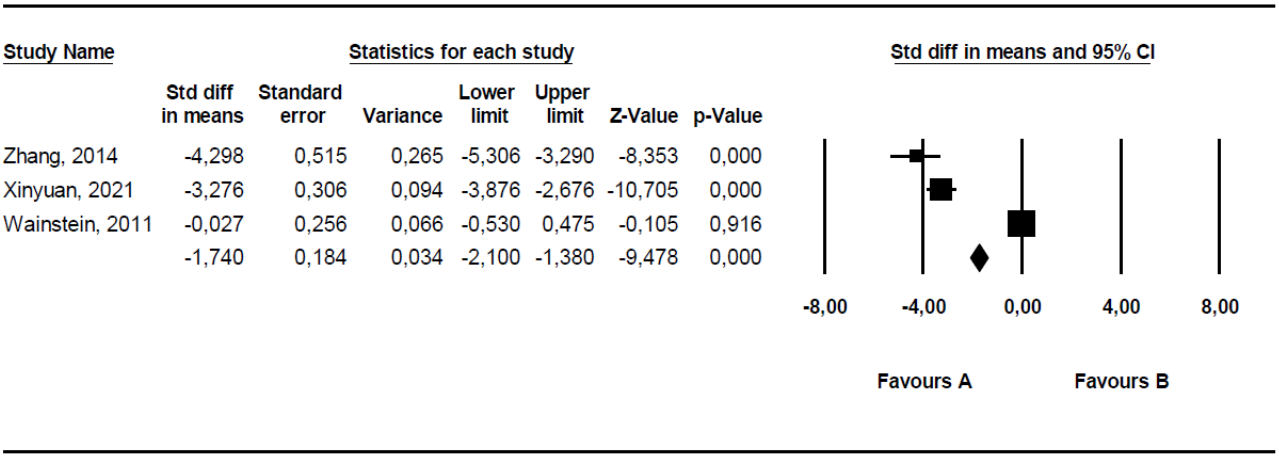

Figure 2. Results of a meta-analysis of the effectiveness of ozone therapy on diabetic foot ulcers. Favors A: Intervention group (ozone); Favors B: Control group

Table 3. Risk of Bias of Included Studies

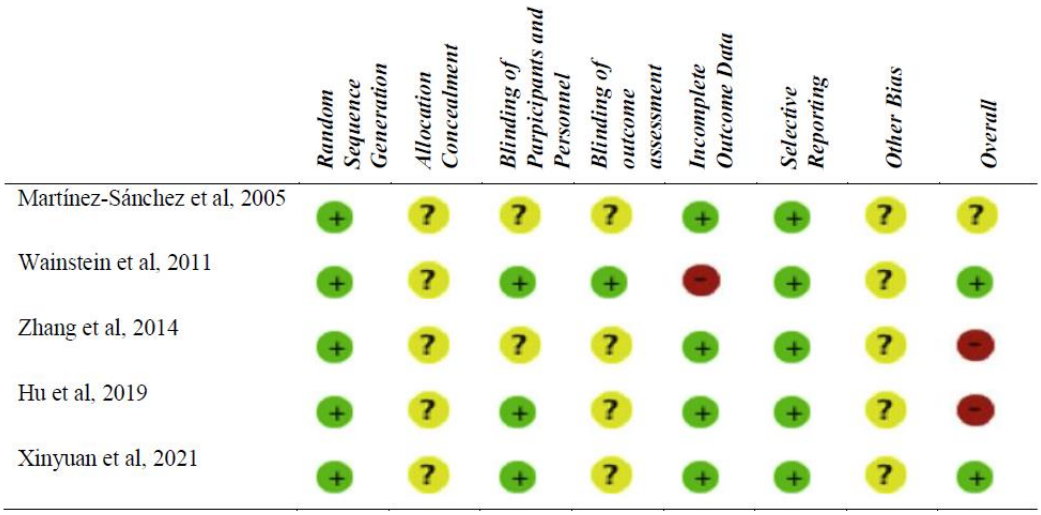

Figure 3. Risk of bias in studies used for systematic reviews and meta-analyses. Circle symbol of ● with a positive sign indicating a low risk of bias, circle symbol of ● with a question mark the risk of bias cannot be assessed, circle symbol of ● with a negative sign indicates a high risk of bias.

The five studies included in this review were mostly of unfavorable standard and may have a high degree of bias. However, when analyzed together, these studies cover a broad spectrum, such as a wide range of ulcer sizes and severity, an extensive age range, and different ozone application and dosage methods. Most studies provided detailed descriptions of participants' demographics and health status.

However, because several clinical trials did not report complete numerical research data regarding the average wound area after the intervention, not all clinical trials could be included in the meta-analysis. Therefore, this reduces the validity of the conclusions resulting from this study. Only three clinical trial studies were included in the meta-analysis. The duration and concentration of ozone used were at different doses in all these studies. In addition, one study failed to state or disguise the therapy given because it did not use a placebo. Current methods of ozone application can be complicated procedures and usually require trained professionals, leading to the observed bias in blinded allocation and treatment.

Subjects included in existing studies did not provide a specific age or gender limit. However, according to the prevalence of diabetic ulcer disease, the average age of the subjects was elderly (>60 years). Most studies did not limit the recruitment of subjects based on the severity of diabetic ulcer disease. As for the limitations made by several studies, they included diabetic ulcer patients with stage 2, 3, or post-debridement stage 4 Wagner classifications in the study. This is, of course, rational enough to limit ulcer conditions that do require intervention and exclude patients with advanced ulcer conditions who require definitive surgical therapy first. This study, in general, shows that the use of ozone in healing ulcers in patients with diabetic ulcers was beneficial in all age groups, including the elderly, and the severity of ulcers previously treated with conservative therapy.

Medical ozone therapy primarily contributes to anti-inflammatory effects, improves microcirculation, activates growth factors, and ultimately promotes wound healing when used to treat infected wounds, especially diabetic ulcers. Ozone has been considered a potent germicide since the 19th century. Ozone has broad, effective, and fast bactericidal capabilities. According to research, 20 minutes of exposure to high ozone concentrations can efficiently destroy 63 clinical pathogenic microorganisms, such as fungi and Gram-

positive and Gram-negative bacteria. When eliminating microorganisms that cause diabetic foot ulcers, ozone shows special benefits, including preventing antibiotic resistance and side effects of medication.[19,20]

Based on the type of application of ozone therapy, most of the studies applied local ozone using the ozone bath or local immersion method, such as the studies by Xinyuan et al., Zhang et al., and Wainstein et al. In the same context, these three studies are included in the meta-analysis. This strengthens the evidence that ozone therapy, specifically local immersion therapy with ozone baths, has good efficacy benefits in healing diabetic ulcers. Transcutaneous ozone immersion is the method of choice for extensive and deep topical infections. Its microbicidal and virostatic effects can occur at lower concentrations (<40 µg/ml). Once wound healing has begun, the concentration can be reduced again (<20 µg/ml), thus taking full advantage of ozone's metabolic stimulant and immunomodulatory effects while the healing process continues. Low-pressure ozone treatment is not monotherapy because other forms of wound care must be continued simultaneously, according to the condition of the wound and following standard conventional therapy. The considerable local hyperemic effect due to the mild subatmospheric conditions plus the properties of ozone contributes to the healing process.[3,4]

The results of this study proved that medical ozone therapy significantly increased the wound healing rate compared to the control group. In wound tissue, ozone can increase platelet-derived growth factor, transforming growth factor, and vascular endothelial growth factor.[21] These growth factors are crucial in controlling how quickly tissue repair cells proliferate. The ability of cells to regenerate is enhanced by the activity of these growth factors, which help accelerate wound healing.[22] In addition, ozone can scavenge free radicals, activate antioxidant enzymes and free radical scavenging mechanisms and increase the activity of catalase, superoxide dismutase, oxidized catalase, and glutathione reductase, as well as increase local tissue metabolism. The growth of granulation tissue and epithelial cells is stimulated by forming collagen fibers, stimulating capillary regeneration, tissue repair, and faster wound healing.[23]

Ozone treatment improves glycemic control and prevents oxidative stress in STZ-induced diabetic rats.[24] Recently, ozone administration was reported to prevent the development of atherosclerosis and improve antioxidant systems in New Zealand white rabbits.[25] In patients with coronary artery disease, ozone treatment with rectal insufflation significantly increased prothrombin time, reduced protein biomarkers and lipid oxidation, and increased total antioxidants.[26] This is in line with our study's evidence that ozone treatment significantly promotes wound healing in diabetic ulcer patients. Zhang et al. found that the expression of VEGF, TGF-β, and PDGF was significantly higher in the ozone group than in the control group.[16] This suggests that the efficacy of ozone treatment on diabetic ulcer healing is due to an increase in endogenous growth factor in the wound.

Oxygen can increase the amount of 2,3-DPG and ATP in red blood cells, thereby increasing their capacity to transport oxygen, increasing red blood cell metabolism, moving the oxyhemoglobin dissociation curve to the right, and increasing oxygen release. Ozone eventually decomposes into oxygen while exerting its therapeutic effect, and these reactive oxygen species remain in local tissues and further aggravate the local hypoxic environment. This effect helps to offset the ischemia-hypoxia caused by diabetic vascular disease partially.[27] This increases the oxygen concentration of nearby cells, accelerating the healing of the lesion.

Most studies on this subject apply only to local or systemic ozone therapy. However, as previously found, each method of local and systemic use has a particularly beneficial effect

on diabetes. Among the five studies included in the qualitative analysis, only one by Martinez-Sanchez used a combination of local and systemic therapy. The study showed that combination therapy significantly improves lesions resulting in a reduction in ulcer area compared to the control group. Additionally, they demonstrated that ozone treatment improves glycemic control, prevents oxidative stress, normalizes organic peroxide levels, and activates superoxide dismutase. The pharmacodynamic effects of ozone in treating diabetic foot neuroinfection can be ascribed to its possible superoxide scavenger. Superoxide is considered a link between the four metabolic routes associated with diabetic pathology and its complications.

One of the studies that took part in the qualitative review, namely the study by Hu et al., demonstrated a relatively unique method of ozone treatment, namely the combination of using vacuum-assisted closure (VAC) therapy and ozone water rinsing. The results showed that the combined use of VAC-negative pressure wound therapy and ozone water rinsing could improve the recovery of diabetic ulcers, shorten the duration of treatment, and reduce pain during treatment. VAC-negative pressure wound therapy has been reported in the treatment of diabetic ulcers in many studies. Muhammad et al.. compared the efficacy and safety of VAC-negative pressure wound therapy and advanced moist wound therapy in treating diabetic ulcers. They found NPWT using VAC more effective than traditional wet wound therapy.[28] Hu et al. demonstrated for the first time that ozone water rinses effectively treat diabetic ulcers. Combined ozone water rinses and VAC treatment may improve diabetic ulcer healing. The script also has some limitations. First, the study sample size is small. Second, all cases originate from a single center. Third, the precise numerical data of the mean and standard deviation before and after treatment were not reported.

Studies have not been conducted to compare the effects of different types of ozone treatments directly. Such research will allow the most effective method of application of ozone treatments to be determined. Accordingly, specific methods and guidelines for ozone application have not been defined, and guidelines and recommendations for ozone therapy have not been consolidated, potentially leading to highly variable concentrations and the number of doses in the studies submitted for this review.

Ozone has many beneficial functions, but it also risks human health, primarily because it stimulates the respiratory system. When the amount of ozone in the air exceeds a certain threshold, it can result in symptoms including dry mouth, dry tongue, coughing, chest tightness, loss of appetite, fatigue, dizziness, and body aches, as well as temporary changes in lung function, which can damage lung tissue.[29] As a result, when using this ozone/trioxygen gas, it is essential to keep the room ventilated. The depleted ozone/trioxygen gas must be absorbed as much as possible for safe disposal.

It has been observed that the action of ozone is proportional to the concentration, but that does not mean that the higher the concentration, the better. The concentrations used in medical ozone therapy applications are non-uniform.[30] Studies have shown that ozone concentrations greater than 80 mg/L can damage tissue structures, whereas ozone concentrations less than 50 mg/L are safe for clinical practice.[31] In the included studies, ozone concentrations were 35-60 mg/L.

The dose of ozone used by Wainstein et al. was started at 80 µg/mL and then continued with 40 µg/mL. Study by Zhang et al. used a dose of 52 µg/mL ozone, while the study by Xinyuan et al. used a dose of 35 mg/L. The doses of ozone from the 3 following studies was not uniform, but in the study by Wainstein who used the highest dose, the result was not significant in the ozone group when compared to the control group. Meanwhile, study by Zhang et al. and Xinyuan et al., using lower doses and the results showed significant changes in ulcer area in the ozone

group. This data supports the theory that using the maximum dose of ozone does not mean that the results will be better.

Furthermore, the existing studies also did not undertake or clearly state any attempt to identify unwanted side effects during follow-up. Due to the unknown toxicity of ozone, further studies should be carried out to determine the specific application method of this therapy, including possible long-term side effects that have not been reported previously. Such investigations will allow the most efficacious doses and application methods to be developed to balance the destructive oxidizing effects with their potential benefits. Future research is expected to improve the quality of the study by recording and reporting these unwanted outcomes.

Conclusion

Based on the data of a systematic review and meta-analysis, it can be concluded that there was a more significant reduction in ulcer area after being given ozone therapy compared to the placebo group. Based on this, it can be concluded that ozone therapy is effective in improving ulcer healing in patients with diabetic ulcers.

Abbreviations:

DFU: Diabetic Foot Ulcer

DM: Diabetes Mellitus

MeSH: Medical Subject Headings

NPWT: Negative Pressure Wound Therapy

PRISMA: Preferred Reporting Items for Systematic Review and Meta-Analysis

PDGF: Platelet-Derivied Growth Factor

RCT: Randomized Controlled Trial

TGF-β: Transforming Growth Factor beta

VAC: Vaccum Assisted Closure

VEGF: Vascular Endothelial Growth Factor

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