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Research Article

Intermittent Ozone Insufflation as an Adjuvant Therapy for Ischemic Ulcers in Lower Extremity Arterial Occlusive Disease: A Prospective Randomized Controlled Study

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Abstract

Background: Lower extremity arterial occlusive disease (LEAOD), primarily arteriosclerosis obliterans (ASO), often leads to chronic, refractory ischemic ulcers, posing significant challenges in wound management and carrying high risks of amputation and mortality. This study aimed to evaluate the clinical efficacy and safety of intermittent local ozone insufflation as an adjuvant therapy for promoting wound healing and alleviating pain in patients with ASO-related ischemic ulcers. *Methods:* In this prospective randomized controlled trial, 62 eligible patients with LEAOD and lower limb ulcers admitted between July 2023 and December 2024 were randomly assigned to either an experimental group (n=32) or a control group (n=30). The control group received standard wound care (debridement, dressing changes), while the experimental group received standard care plus twice-daily local ozone insufflation (50 ml per session, 20 minutes each). Wound pain (Visual Analog Scale, VAS) and wound status (Bates-Jensen Wound Assessment Tool, BWAT) were assessed before intervention and on days 1, 3, and 7 post-intervention. Results: It showed that the experimental group had significantly lower VAS scores than the control group post-intervention ($P<0.001$). Similarly, BWAT scores in the experimental group were significantly lower ($P<0.001$), indicating better wound healing. Key healing indicators, including time to granulation tissue formation and time to exudate control, were also significantly shorter in the experimental group ($P<0.001$). No ozone-related adverse events were observed. *Conclusion:* Intermittent ozone insufflation is a safe, effective, non-invasive adjuvant therapy that significantly reduces pain and accelerates early wound healing in patients with ischemic ulcers due to LEAOD, offering a valuable clinical strategy for managing these challenging wounds.

Keywords

Ozone Therapy, Arteriosclerosis Obliterans, Ischemic Ulcer, Wound Healing, Pain Management, Adjuvant Treatment, Randomized Controlled Trial

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1. Introduction

Lower extremity arterial occlusive disease (LEAOD), with arteriosclerosis obliterans (ASO) as its most common etiology, is a prevalent vascular disorder characterized by limb ischemia, manifesting as intermittent claudication, rest pain, and tissue necrosis [1]. In advanced stages, critical limb ischemia often leads to refractory ulcers and gangrene, severely impairing patients' quality of life and carrying a substantial risk of amputation, reported to be as high as 45% [2, 3]. Epidemiological data indicate a high prevalence of ASO, particularly among the elderly, underscoring its significant clinical and socioeconomic burden [4]. Ischemic ulcers in ASO patients are particularly challenging to manage due to compromised microcirculation, persistent inflammation, and a hypoxic wound environment, which hinder conventional wound healing processes [5].

Ozone therapy has garnered attention in recent decades for its potential applications in managing chronic wounds, including diabetic foot ulcers [6, 7]. Its proposed mechanisms of action include antimicrobial effects, modulation of oxidative stress, enhancement of oxygen delivery, stimulation of angiogenesis, and anti-inflammatory properties [8, 9]. While some clinical studies and meta-analyses have suggested benefits of ozone in diabetic wound care [10], its specific efficacy and safety profile in the context of arterial ischemic ulcers remain less clearly established.

Therefore, this study was designed as a prospective randomized controlled trial to systematically evaluate whether adjunctive intermittent local ozone insufflation can improve wound healing outcomes and alleviate pain in patients with ischemic ulcers secondary to LEAOD, compared to standard wound care alone. We hypothesized that ozone therapy would significantly enhance the early healing trajectory and pain relief in this patient population.

2. Materials and Methods

2.1. Study Design and Participants

This was a prospective, randomized, controlled, single-center trial conducted at Zhuhai People's Hospital. The study protocol was approved by the Hospital's Ethics Committee (Approval No: 2023-伦审【研】-70). All participants provided written informed consent.

Patients admitted between July 2023 and December 2024 were screened for eligibility. Inclusion criteria were: (1) age >18 years; (2) clinical symptoms of LEAOD (intermittent claudication, rest pain, ulcer, gangrene); (3) diagnosis of lower extremity ASO confirmed by digital subtraction angiography (DSA) or other imaging modalities; (4) presence of a lower limb ischemic ulcer; (5) ankle-brachial index (ABI) between 0.5 and 0.9; and (6) post-interventional status (e.g., after percutaneous transluminal angioplasty) without major complications. Exclusion criteria included: (1) hyperthyroidism or glucose-6-phosphate dehydrogenase deficiency; (2) known allergy to ozone; (3) severe cardiac, hepatic, renal insufficiency, or coagulation disorders; (4) active infection or osteomyelitis at the ulcer site; (5) inability to cooperate with the study or follow-up.

2.2. Randomization and Intervention

Sixty-two eligible patients were randomly assigned using a random number table to either the experimental group (n=32) or the control group (n=30). Two patients in the ozone group withdrew from the study due to disease progression (n=1) and an unrelated severe adverse health event (n=1). Consequently, the per-protocol analysis included 30 patients in the ozone group and 30 patients in the control group. Both groups received standardized conventional wound care, which included wound debridement, cleansing with sterile normal saline, and dressing with alginate or foam dressings based on exudate levels, performed every 1-2 days. Patient education regarding limb protection, smoking cessation, and diet was also provided.

The experimental group received additional local ozone insufflation therapy. A specialized, transparent, ozone-resistant plastic bag was securely placed over and around the entire foot and ulcer area to create an airtight seal. Then, 50 mL of the prepared ozone-oxygen mixture was slowly introduced into the bag via a sterile syringe connected to the bag’s inlet port.The gas was allowed to remain in direct, topical contact with the wound bed for 20 minutes per session. This treatment was administered twice daily, with at least a 6-hour interval between sessions, for a total of 4 weeks. Patients’ vital signs (blood pressure, heart rate, oxygen saturation) and local skin reactions (erythema, blistering, pain) were closely monitored during and for 30 minutes after each treatment session. No systemic adverse effects related to ozone therapy were observed.

2.3. Outcome Measures

Primary outcome measures were wound pain intensity and wound healing status.

Pain Assessment: The Visual Analog Scale (VAS, 0-10 cm) was used to assess pain levels before intervention and on days 1, 3, and 7 post-intervention.

Wound Assessment: The Bates-Jensen Wound Assessment Tool (BWAT) was used to evaluate wound status at the same time points. The BWAT scores 13 items (size, depth, edges, undermining, necrotic tissue, exudate, surrounding skin, etc.) on a 1-5 scale, with a total possible score of 65 (higher scores indicate worse condition) [11].

Secondary Healing Indicators: Time to initial granulation

tissue appearance and time to complete control of wound exudate were recorded.

Safety: Adverse events related to ozone therapy or routine care were monitored throughout the study period.

2.4. Statistical Analysis

Data were analyzed using SPSS software (version 22.0). Continuous variables are presented as mean ± standard deviation (SD), and categorical variables as frequencies (percentages). Baseline characteristics were compared using independent samples t-tests or chi-square tests. For repeated-measures data (VAS and BWAT scores across time points), a repeated-measures analysis of variance (ANOVA) was performed, followed by simple effect analysis if a significant interaction was found. The significance level was set at $P < 0.05$.

3. Results

3.1. Baseline Characteristics

No significant differences were observed between the two groups at baseline regarding age, gender, ulcer area, baseline VAS score, or baseline BWAT score (all $P > 0.05$), indicating good comparability (Table 1).

Table 1. *Comparison of baseline characteristics between the two groups ($\bar{x} \pm s$).*

Characteristic	Experimental Group (n=30)	Control Group (n=30)	t/ χ^2	P-value
Age (years)	67.5 ± 8.2	66.8 ± 7.9	0.312	0.756
Gender (Male/Female)	18 / 12	17 / 13	0.105	0.746
Ulcer Area (cm^2)	4.8 ± 1.5	4.6 ± 1.3	0.521	0.604
Baseline VAS Score	5.6 ± 1.2	5.5 ± 1.3	0.521	0.775
Baseline BWAT Score	42.3 ± 5.6	41.8 ± 5.3	0.345	0.731

3.2. Pain Assessment (VAS Scores)

Repeated-measures ANOVA revealed significant main effects for time ($F=165.33$, $P<0.001$) and group ($F=28.94$, $P<0.001$), as well as a significant time × group interaction ($F=38.15$, $P<0.001$). Simple effect analysis showed no between-group difference at baseline ($P=0.775$). However, the experimental group demonstrated significantly lower VAS scores than the control group at day 1, day 3, and day 7 post-intervention (all $P<0.001$). Within the experimental group, VAS scores decreased significantly at each post-intervention time point compared to baseline (all $P<0.001$), whereas the control group showed a significant reduction only by day 7 (Table 2).

Table 2. *Comparison of VAS pain scores between groups at different time points ($\bar{x} \pm s$, points).*

Group	n	Pre-intervention	Day 1	Day 3	Day 7
Experim-ental	30	5.6 ± 1.2[4]	5.6 ± 1.2[4]	2.8 ± 0.8[1][2][4]	1.5 ± 0.6[1][2][3]
Control	30	5.5 ± 1.3[4]	5.0 ± 1.1[4]	5.0 ± 1.1[4]	3.2 ± 0.9[1][2][3]
F-values	$F_{time}=165.33$, $F_{interaction}=38.15$, $F_{group}=28.94$				
P-values	$P_{time}<0.001$, $P_{interaction}<0.001$, $P_{group}<0.001$				

[1] $P<0.05$ vs. pre-intervention; [2] $P<0.05$ vs. Day 1; [3] $P<0.05$ vs. Day 3; [4] $P<0.05$ vs. Day 7.

3.3. Wound Healing Assessment (BWAT Scores)

Similarly, repeated-measures ANOVA for BWAT scores showed significant main effects for time ($F=195.00$, $P<0.001$) and group ($F=17.55$, $P<0.001$), and a significant time × group interaction ($F=15.67$, $P<0.001$). The experimental group exhibited a significantly greater reduction in BWAT scores over

time compared to the control group (Table 3).

Table 3. Comparison of BWAT scores between groups at different time points ($\bar{x} \pm s$, points).

Group	n	Pre-intervention	Day 1	Day 3	Day 7
Experim-ental	30	42.3 ± 5.6[4]	36.5 ± 4.8[1][4]	28.7 ± 4.2[1][2][4]	15.2 ± 3.5[1][2][3]
Control	30	41.8 ± 5.3[4]	40.2 ± 5.1[4]	35.6 ± 4.5[1][4]	26.8 ± 4.1[1][2][3]
F-values	F_{time}=195.00, $F_{interaction}$=15.67, F_{group}=17.55				
P-values	P_{time}<0.001, $P_{interaction}$<0.001, P_{group}<0.001				

[1] P<0.05 vs. pre-intervention; [2] P<0.05 vs. Day 1; [3] P<0.05 vs. Day 3; [4] P<0.05 vs. Day 7.

3.4. Secondary Healing Indicators

The experimental group showed significantly better performance in key healing milestones:

Time to Granulation Tissue Formation: 3.2 ± 0.8 days vs. 5.6 ± 1.1 days in the control group (t=9.667, P<0.001).

Time to Exudate Control: 4.1 ± 1.0 days vs. 5.8 ± 1.2 days in the control group (t=5.961, P<0.001).

3.5. Safety Analysis

No adverse events related to ozone therapy (e.g., local irritation, allergic reactions, dizziness, nausea) were reported in the experimental group. No serious systemic complications occurred in either group. Vital signs remained stable throughout the study period.

4. Discussion

This randomized controlled trial provides evidence that intermittent local ozone insufflation is a safe and effective adjuvant therapy for ischemic ulcers in LEAOD patients. The therapy led to significantly faster pain relief and accelerated early wound healing compared to standard care alone.

The rapid and significant reduction in VAS scores observed in the ozone group may be attributed to ozone's multifaceted biological actions. Ozone can modulate oxidative stress by inducing endogenous antioxidant systems via the Nrf2 pathway, thereby reducing neuropathic pain associated with ischemia-reperfusion injury [12, 13]. Its potent anti-inflammatory effects, including the inhibition of pro-inflammatory cytokines like TNF-α and IL-1β, likely contributed to decreasing inflammatory pain [14, 15]. These findings align with previous research on ozone's analgesic properties in chronic wound management [10, 16].

The superior wound healing outcomes, evidenced by the sharper decline in BWAT scores and shorter times to granulation and exudate control, can be explained by several mechanisms. First, ozone's broad-spectrum antimicrobial activity helps reduce bacterial bioburden and biofilm, creating a cleaner wound bed conducive to healing [17]. Second, ozone decomposes to oxygen locally, directly improving tissue oxygenation. More importantly, it stimulates the release of signaling molecules like nitric oxide (NO) and vascular endothelial growth factor (VEGF), promoting vasodilation and angiogenesis, which are crucial for reversing the ischemic microenvironment in arterial ulcers [12, 18, 19]. Third, ozone appears to enhance fibroblast proliferation and collagen synthesis, facilitating the proliferative phase of healing [20].

The "intermittent insufflation" method used in this study offers practical advantages. It ensures direct, uniform, and three-dimensional contact of ozone gas with the entire wound surface, including undermining areas, which is superior to topical ozonated oils. Furthermore, as a strictly localized treatment, it avoids the systemic risks associated with major autohemotherapy, making it particularly suitable for elderly patients with multiple comorbidities [21].

This study has several limitations that should be acknowledged. First, the single-center design might introduce selection bias. Second, the absence of illustrative images of the procedure and wound outcomes, due to ethical constraints on patient photograph usage, limits the visual demonstration of the technique. Third, regarding the ozone technique itself, there is currently a lack of universally standardized parameters (e.g., optimal concentration, volume, and session frequency) for ischemic ulcers, which are areas for future research.

5. Conclusions

Intermittent local ozone insufflation, as an adjunct to conventional wound care, safely and effectively alleviates pain and promotes early wound healing in patients with ischemic ulcers due to lower extremity arterial occlusive disease. Its benefits are likely mediated through synergistic anti-inflammatory, analgesic, antimicrobial, pro-angiogenic, and tissue-regenerative mechanisms. This non-invasive, targeted therapy

presents a valuable clinical option for managing these challenging wounds. Future large-scale, multi-center studies with longer follow-up are warranted to confirm long-term outcomes, optimize treatment parameters, and further elucidate the underlying molecular pathways.

Abbreviations

ASO	Arteriosclerosis Obliterans
LEAOD	Lower Extremity Arterial Occlusive Disease
VAS	Visual Analog Scale
BWAT	Bates-Jensen Wound Assessment Tool
ABI	Ankle-Brachial Index
DSA	Digital Subtraction Angiography
PTA	Percutaneous Transluminal Angioplasty
ROS	Reactive Oxygen Species
Nrf2	Nuclear Factor Erythroid 2-related Factor 2
VEGF	Vascular Endothelial Growth Factor
NO	Nitric Oxide
TNF-α	Tumor Necrosis Factor-alpha
IL-1β	Interleukin-1 Beta

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Author Contributions

Qingxia He: Conceptualization, Data Curation, Investigation, Writing – original draft

Ming Xue: Formal Analysis, Methodology, Resources

Feiyan Deng: Data Curation, Investigation, Project Administration

Jing Xu: Validation, Visualization, Writing – review & editing

Xiaoling Han: Conceptualization, Funding Acquisition, Supervision, Writing – review & editing

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Conflicts of Interest

The authors declare no conflicts of interest.

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