Impact of ozone concentration on the treatment effectiveness of diabetic foot syndrome: a pilot single-centre study

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Abstract

Introduction: Diabetic foot syndrome (DFS) a severe complication of diabetes which can result in ulcers, infections, or tissue damage in the feet.

Aim: To compare the treatment effectiveness in patients with DFS using local O_3 therapy depending on the O_3 concentration.

Material and methods: The study included 50 patients, 24 male and 26 female ones, in the age range between 39 and 84 years, with DFS. In group 1 (25 patients), 30 µg/ml doses of ozone were applied, and in group 2 (25 patients) doses of 60 µg/ml. A total of 30 local ozone therapy procedures, lasting 30 min each, were performed in both groups, in two sessions (15 procedures), with a 4-week break between sessions. The effectiveness of wound healing was evaluated by computerized planimetry, and pain intensity was assessed with the use of the VAS scale.

Results: After treatment, a statistically significant reduction in the area of wounds and the intensity of pain was achieved in both groups. The median (IQR) wound size after treatment in group 1 was: 4.5 (4–5) cm^2, and in group 2: 4 (3–4.5) cm^2; ($p = 0.027$). The median (IQR) pain intensity (VAS) after treatment in group 1 was: 5 (4–5) points, and in group 2: 4 (3–4.5) points ($p = 0.002$).

Conclusions: The use of a higher concentration ozone increased the effectiveness of the therapy in terms of reducing the wound surface area and alleviating the pain. Therefore, the possibility of using higher ozone concentrations in the treatment of diabetic foot syndrome is worth considering.

Key words: dose, ozone concentration, diabetic foot ulcers, wound healing, computerized planimetry, pain intensity.

Introduction

Diabetic foot syndrome (DFS) is an infection and/or ulceration and/or destruction of deep tissues of the foot (e.g. bones) occurring in the course of diabetes. The syndrome is caused by damage to the peripheral nerves and/or vessels of the foot, of varying degrees. The resulting ulcers, if not treated properly, can lead to a complication called diabetic foot ulcer syndrome [1, 2].

The International Diabetes Federation (IDF) estimates that DFS, as a serious complication of diabetes, affects 40 to 60 million diabetic patients worldwide (6.3%) [2, 3]. In Poland, according to statistical data, the incidence of DFS is estimated at some 3.5 million patients [1, 2, 4, 5].

The risk of developing DFS increases with age, which phenomenon is closely related to the longer duration of diabetes, cumulative effects of hyperglycaemia and more frequent occurrence of micro- and macro-vascular complications [6–9]. Due to the aetiology, DFS is divided into three types: neuropathic (35%), ischemic (15%), and mixed neuropathic-ischemic (50%) ones [10]. If treatment is delayed, vascular and neuropathic complications lead to DFUs, which may result in gangrene or even foot amputation [11, 12].

The treatment of DFU is difficult and expensive, causing a psychological and financial burden for patients and their families. Due to the complex pathomechanism, the

treatment of diabetic foot syndrome should be managed by a multidisciplinary team [13–16].

In addition to pharmacological treatment, patients suffering from DFS can also use various alternative treatment methods, such as hyperbaric oxygen therapy or ozone therapy, which are recommended in the treatment of DFS [16–18].

The use of ozone (O_3) within the medical community began in the 19th century. The research conducted so far has confirmed that ozone can increase the efficiency of wound healing by improving skin microcirculation in the case of pressure sores, burn wounds and other ulcers associated with peripheral vascular disease. Currently, increasing evidence has confirmed that O_3 can also be used to treat DFS [19–26].

Although O_3 therapy with the application of appropriate doses is safe and does not cause side effects, when used outside the "therapeutic window" it may be toxic. It has been proven that at ozone concentrations of 5–10 mg/l and lower there is a therapeutic effect with a large margin of safety for the patient [27–31].

Aim

The aim of the study was to compare the effectiveness of treatment in patients with DFS using local O_3 therapy depending on the O_3 concentration, based on planimetric assessment of changes in the ulcer and the intensity of accompanying pain.

Material and methods

This was a single-centre, parallel-group study comprising 50 patients in the age range between 39 and 84 years with type 2 diabetes complicated with DFS (neuropathic-ischemic type), treated with the use of local O_3 therapy from 2023 to 2024. The patients were divided into two study groups:
1. The group of patients subjected to a cycle of local O_3 therapy using ozone at 30 μg/ml concentration – 25 patients (10 women and 15 men).
2. The group of patients subjected to a cycle of local O_3 therapy using ozone at 60 μg/ml concentration – 25 patients (16 women and 9 men).

Inclusion criteria for the study were as follows: age between 18 and 85 years, diabetic foot ulcer diagnosed in the right or left foot area, ankle-brachial index between 0.7 and 1.2, ulcer surface area > 2 cm², Wagner grade 2, 3 or 4, patient's disqualification for endovascular ablation for medical reasons, informed and voluntary consent of the subject to participate in the study.

Exclusion criteria comprised: limb ulcer not associated with diabetic foot syndrome, pregnancy; hypersensitivity to ozone, contraindications to O_3 therapy, patients with malignant transformation of diabetic ulcers – skin cancer, patients who did not comply with the treatment or had incomplete clinical data, comorbidities (hyperten-sion, smoking habit), Charcot joint, infectious diseases, critical limb ischemia and/or or previous vascular or orthopaedic procedures, as well as lack of informed and voluntary consent of the subject to participate in the study.

The diagnosis of DFS was based on the diagnostic criteria set out in the International Diabetic Foot Working Group guidelines [4].

The randomization procedure consisted of tossing a coin of PLN 1. This method is widely considered to be the simplest randomization procedure [29]. The obverse of the coin meant assignment to group 1, and the reverse of the coin meant assignment to group 2. Study participants were not informed which group they were assigned to.

Before starting a cycle of local O_3 therapy, surgical wound debridement was conducted to remove necrotic tissues or purulent infiltration accompanying the ulcer. Before the study, patients' baseline clinical and demographic characteristics were also recorded, including height, weight, and duration of diabetes.

Before and after the end of the therapy (10 weeks), a planimetric assessment of the wound surface area was performed. A computer software for planimetric assessment of wound surface area was used for digital image processing [32].

Additionally, before the beginning and after the end of the therapeutic cycle the assessment of pain intensity was performed with the use of VAS scale.

Oxygen ozone therapy procedures

Local O_3 therapy with the use of a gaseous oxygen-ozone mixture (5% O_3 and 95% O_2) with close monitoring of patients was performed once a day for 5 days a week, with a weekend (Saturday–Sunday) break, in two series consisting of 15 treatment procedures each (for a total of 10 weeks). Each treatment lasted 30 min. The intermission between the two series of treatments was 4 weeks, introduced in order to counteract the potential risk of exposing patients to the negative effects of long-term exposure of the skin around the ulcer and deeper tissues to O_3.

The following treatment procedure was used: patients were a semi-sitting position and wound dressings were removed. The treated limb was placed in a polyethylene bag and sealed (Figure 1). At the end of the treatment, the wound was covered with sterile dressings, in order to maintain proper humidity and improve wound cleanliness. During the procedures, good ventilation of the rooms was ensured so as not to expose patients to additional O_3 inhalation.

During the examination, all patients continued their anti-hyperglycaemic therapies. Moreover, during a cycle of physical procedures, in both groups of patients similar conventional pharmacological treatment was applied, including the administration of: sulodexide, micronized pu-

Figure 1. Local O_3 therapy in a 75-year-old female patient with DFUs of the left foot

rified flavonoid fraction, pentoxifylline, and acetylsalicylic acid in standard doses. Patients from both groups were also treated with insulin therapy to maintain the proper fasting serum glucose level below 6 mmol/l.

Statistical analysis

Statistical analysis was performed with the use of Statistica13 package (StatSoft, Poland). The Shapiro-Wilk test was used to test the normality of data. The distribution of all data was not normal. The Mann Whitney U test and Wilcoxon test were used to compare the two, unmatched and matched, groups of non-parametric data, respectively. Qualitative variables were assessed using the χ^2 test. The level of statistical significance was set at $p < 0.05$. The effect size was calculated, with $r = 0.1$ indicating a small effect, $r = 0.3$ indicating a medium effect, and $r = 0.5$ indicating a large effect. G*power software version 3.1.9.7 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) (http://www.gpower.hhu.de) was used to determine the power using 2-sided testing, with $\alpha = 0.05$, and sample size of 25 per group [33]. The outcome used for power analysis was ulcer surface area (cm^2). The effect size was 0.72. The power (1-β err prob) was calculated as 0.70. The test family was "t-test" and statistical test was the difference between two independent means (two groups). The type of power analysis was "Post hoc: Compute achieved power – given α, sample size, and effect size".

Results

The patient characteristics, including the division into study groups, are presented in Table 1.

The mean age of all treated patients was 67.1 ±10.6 years, 66.6 ±12.6 years in group 1 and 67.5 ±8.5 years in group 2. The difference in the age of patients between group 1 and group 2 was not statistically significant

Table 1. Demographic profile patients of studied groups

Parameter	Total n (%)	Group 1 n (%)	Group 2 n (%)	*P-value
Gender				
Male	24 (48)	15 (60)	9 (36)	0.089
Female	26 (52)	10 (40)	16 (64)	
Age [years]				
< 70	26 (52)	13 (52)	13 (52)	1
≥ 70	24 (48)	12 (48)	12 (48)	
Body mass index (BMI) [kg/m²]				
18.5–24.99	24 (48)	11 (44)	13 (52)	0.571
25.0–29.99	26 (52)	14 (56)	12 (48)	
Wound ulcer duration [years]				
< 3	22 (44)	10 (40)	12 (48)	0.568
≥ 3	28 (56)	15 (60)	13 (52)	
Wound ulcer location				
Left leg	24 (48)	11 (44)	13 (52)	0.571
Right leg	26 (52)	14 (56)	12 (48)	

*χ^2 test.

(p = 0.891). The BMI index was 25.74 ±1.81 kg/m² in group 1 and 24.73 ±2.30 kg/m² in group 2, on the average. Also in this case, the difference between the groups was not statistically significant (p = 0.196). The average duration of diabetes was: 3.12 ±6.4 years in group 1 and 2.89 ±5.48 years in group 2. The difference was not statistically significant (p = 0.231).

Before the beginning of therapy, the mean values of the ulcer surface area in both groups did not differ statistically significantly (p = 0.174) and were: median (Q1–Q3): 7 (7.0–8.0) cm² in group 1 and median (Q1–Q3): 7 (6.1–7.5) cm² in group 2. After the treatment, the average values of the ulcer surface area in group 2 were statistically significantly smaller than in group 1. The median (Q1–Q3) of the ulcer surface area in group 1 was 4.5 (4.0–5.0) cm², and in group 2 it was 4 (3.0–4.5) cm² (p = 0.027), and in both study groups the mean values of the ulcer surface area after the end of treatment were statistically significantly lower as compared to the baseline values before the start of therapy (p < 0.001) in both groups (Table 2).

Before commencing the therapy, the mean values of pain intensity assessed on the VAS scale in both groups did not differ statistically significantly (p = 0.361) and were as follows: median (Q1–Q3) 8 (7–8) points in group 1 and median (Q1–Q3): 7 (7–8) points in group 2. After treatment, the mean values of pain intensity in group 2 were statistically significantly lower than in group 1. The median (Q1–Q3) of pain intensity in group 1 was 5 (4–5) points, and in group 2 it amounted to 4 (3–4.5) points (p = 0.002), and in both study groups the mean pain intensity values after the end of treatment were statisti-

cally significantly lower compared to the baseline values before the start of therapy (p < 0.001) in both groups (Table 3).

In both study groups, the percentage changes in the area of ulcers and the intensity of pain after the end of treatment compared to the baseline values were also calculated. In group 1, the mean values of the ulcer surface area after treatment decreased by median (Q1–Q3): 37.50 (28.57–42.86)%, and in group 2 by 44.44 (37.50–51.61)% (p = 0.013). In group 1, the mean pain intensity values assessed using the VAS scale decreased by median (Q1–Q3): 37.50 (28.57–50.00)%, in group 2 by 50.00 (42.86–62.5)% (p = 0.003) (Table 4).

None of the assessed patients from groups 1 and 2 achieved 100% improvement in the form of complete wound healing. In 3 (12%) patients in group 1, and 9 (36%) patients in group 2, the wound area was reduced by more than 50%. The slightest improvement in the reduction of the ulcer surface area, i.e. 14.28%, was recorded in group 1, and the biggest, i.e. 81.81%, in group 2. In no patient, either in group 1 or 2, was there any increase in the wound surface area after the application of treatment.

No patient in either group 1 or group 2 achieved 100% improvement in the form of complete relief from pain. In 7 (28%) patients in group 1 and 14 (56%) patients in group 2, the intensity of pain experienced was reduced by more than 50%. The least significant improvement in reducing the intensity of pain, i.e. 12.5%, was achieved in group 1, and the most significant, i.e. 71.42% in group 2. In none of the patients, either in group 1 or group 2, the

Table 2. Comparison of mean values of the ulcer surface area between groups 1 and 2 and before and after treatment, along with statistical analysis

Variable	Ulcer surface area [cm²]		Effect size	**P-value (before vs. after)
	Pre-treatment median (Q1–Q3)	Post-treatment median (Q1–Q3)		
Group 1	7 (7–8)	4.5 (4–5)	0.79	< 0.001
Group 2	7 (6.1–7.5)	4 (3–4.5)	0.84	< 0.001
Effect size	0.19	0.32		
*P-value (Group 1 vs. Group 2)	0.174	0.027		

*Mann Whitney U test, **Wilcoxon test.

Table 3. Comparison of the intensity of pain experienced between groups 1 and 2 and before and after treatment, along with statistical analysis

Variable	VAS score [points]		Effect size	**P-value (before vs. after)
	Pre-treatment median (Q1–Q3)	Post-treatment median (Q1–Q3)		
Group 1	8 (7–8)	5 (4–5)	0.83	< 0.001
Group 2	7 (7–8)	4 (3–4.5)	0.87	< 0.001
Effect size	0.14	0.4		
*P-value (Group 1 vs. Group 2)	0.361	0.002		

*Mann Whitney U test, **Wilcoxon test.

Table 4. Comparison of the percentage of improvement after treatment between groups 1 and 2 in terms of reducing the size of wounds and pain, along with statistical analysis

Variable	Group 1	Group 2	Effect size	*P-value (Gr. 1 vs. Gr. 2)
	Median (Q1–Q3)	Median (Q1–Q3)		
Percentage change of the wound surface area post-treatment	37.5 (28.57–42.86)	44.44 (37.5–51.61)	0.22	0.013
Percentage change of VAS score post-treatment	37.5 (28.57–50)	50 (42.86–62.5)	0.42	0.003

*Mann Whitney U test.

intensity of pain symptoms was noted to increase after the treatment, and in 1 (4%) patient in group 1 it did not change.

Discussion

Diabetes has increasingly been becoming a serious health problem on a global scale. One of the best ways to reduce mortality and morbidity among patients suffering from complications of diabetes, including diabetic foot syndrome, is early diagnosis [4, 13, 34]. Prevention and early detection of diabetes through multidisciplinary, guideline- and experience-based care is key to reducing the incidence of DFS [35–37].

In the present study, patients from the group treated topically with O_3 at a higher ozone concentration of 60 μg/ml demonstrated a significantly higher rate of ulcer healing compared to the group treated with O_3 applied at a lower concentration of 30 μg/ml. After the end of treatment, a significant reduction in pain was also noted in the group of patients treated with O_3 at a higher concentration. No possible toxic effects of O_3 were observed in any of the examined groups.

Based on the review by Fitzpatrick *et al.*, it has been shown that O_3 concentrations ranging from 5 to 60 mg/l have been determined to be safe and effective in the treatment of skin wounds, in accordance with medical standards [38]. Moreover, other authors point to the beneficial therapeutic effects of O_3 at concentrations exceeding 60 mg/l, which may require more intensive studies to analyse the effects of its toxicity [27, 29]. Currently, no studies provide a clear answer as to why different O_3 concentrations are more or less effective on human tissues. One plausible explanation may be that ozone does not damage the eukaryotic cell wall of the host. This is probably due to the ability of human cells to compensate better for oxidative load than for pathogenic microbial cells in the therapeutic O_3 concentration range of 5–60 mg/l.

There is little information provided in the available literature regarding the optimal dosage of O_3 used in gaseous form in the local treatment of DFS. This limits the translation of the results into evidence-based clinical practice. Routine is required, along with universal reporting of O_3 dosing parameters in future studies, in order to optimize the therapeutic effects of this method and to enable uniform use of precise concentrations in clinical practice.

Among the increasingly more often proposed forms of treatment for diabetes and its complications, including DFS, there are selected physical treatments, including O_3 therapy [21, 37, 38]. O_3 applied topically in the form of a gas mixture has a direct disinfecting and trophic effect. By stimulating the formation of peroxides, it exerts a systemic antibacterial and antiviral effect; increases the deformation of red blood cells, thus improving blood circulation; promotes the supply of oxygen to tissues; increases the efficiency of glucose metabolism, in erythrocytes among others. Finally, thanks to the activation of antioxidant enzymes responsible for the elimination of peroxides and free radicals, it improves the metabolism of fatty acids, supporting the repair mechanisms of treated tissues [20, 24, 39].

Some studies suggest that O_3 therapy is more effective than standard treatment for DFS. An example may be provided by the results of the work of Kushmakov *et al.* who emphasize that thanks to the rapidly developing clinical research on O_3 therapy and its rapid inclusion in comprehensive treatment procedures, O_3 therapy has been gaining popularity and is at the forefront of DFS treatment methods [18]. In the study of Sun *et al.* conducted in patients with DFS, the O_3 therapy group had a higher wound healing rate after a 12-week follow-up as compared to controls ($p < 0.05$). Kaplan-Meier curves indicated a higher cumulative wound healing rate in the O_3 therapy group ($p < 0.05$). Additionally, the O_3 therapy group had less in-patient days, lower duration of antibiotics application, re-infection rate, and readmission rate compared to the control group ($p < 0.05$) [37]. In another study, Lavery *et al.* has indicated that O_3, as a topical treatment for infected skin wounds, deserves serious consideration and further research. It can be used as an alternative to traditional forms of therapy due to its broad antimicrobial activity profile, strong wound healing stimulating effect, and relatively low treatment costs [40]. The observations presented above may be confirmed by the study conducted by Astasio-Picado *et al.*, i.e. a review of the following databases: PubMed (Medline), Dialnet, Google Scholar, Web of Science (WOS), SciELO and Scopus, which considered articles published between November 2014 and June 2023. The results of the analysed

studies proved O_3 therapy to be a promising method in the treatment of wounds in patients with DFS, due to its high therapeutic effectiveness and low number of side effects [41].

There are also studies indicating that O_3 therapy or hyperbaric oxygen therapy have no significant effect on wound healing in DFUs [19, 42].

The Italian Scientific Society of Oxygen-Ozone Therapy (SIOOT), in Bergamo (Italy), suggests using a range of O_3 concentration from 10 to 50 μg/ml (very rarely 60–80 μg/ml) in different approaches (or administration routes), with which O_3 is introduced into the patient body: minor autohemotherapy, infiltration of ozonated blood, major autohemotherapy, rectal ozone, topical ozone in lipophilic media, ozonated water or oil. In the oxygen-ozone major autohemotherapy (O_2-O_3-MAHT), where usually the concentration of 45 μg/ml O_3 (in the O_2-O_3 gaseous mixture) is introduced in the autologous blood (200 ml), previously withdrawn in a sterile bag and then reintroduced, the "actual" concentration of O_3 entering the blood depends on the medium (plasma water) and on blood temperature (about 37°C). So, following Henry's law, if in air there is 1% O_3 = 7.824 μg/ml O_3 at 25°C, then 45 μg/ml of O_3 in the preparation mixture is the equivalent of 5.75% O_3 (weight), yet in water and at 37°C the O_3 concentration should drop to less than 8 μg/ml, actually amounting to 7.165 μg/ml [27].

In line with the current recommendations of scientific societies, and thanks to appropriately developed guidelines regarding the methodology of treatments and the quality of devices for generating O_3, O_3 therapy may in the future play a significant role in the treatment of chronic wounds, constituting a safe and effective alternative method of treating DFS, especially in the light of the growing resistance of pathogenic bacteria to the antibiotics used [4, 5, 14].

Therefore, in order to introduce O_3 therapy into the set of routine treatment methods for DFS, it is necessary to conduct multi-centre, randomized, double-blind clinical trials on large patient populations, in order to clearly determine the optimal concentrations and duration of exposure to O_3 and, consequently, develop the standardization of recommendations and clinical practice guidelines in this regard.

The strength of the study is the collection of complete data and the controlled course of the study, which allows obtaining reliable results. Although this is a pilot study, it initially confirmed the clinical usefulness of local ozone therapy treatments in the management of DFS, which is generally very resistant to previously used treatment methods. The data presented in this study indicate that ozone therapy as a form of local treatment of infected skin wounds which deserves further research as an alternative to traditional therapies due to its potentially high therapeutic effectiveness, lack of significant side effects and relatively low cost of treatment. The authors believe that

supportive treatment using local ozone application, after confirming the presented preliminary results in randomized trials on larger patient populations, will in the future constitute an element of comprehensive therapeutic treatment for patients with diabetic foot syndrome.

The study has some limitations. Firstly, it is a pilot study (with a limited number of patients studied), while prospective randomized controlled studies provide stronger evidence. Secondly, the study was conducted at a single centre. Lastly, our follow-up period was limited to 10 weeks, and longer-term follow-up would be of greater significance. The study is characterized by absence of a control group of patients, which would have allowed for comparison with patients of similar characteristics treated with the use of routine methods. It seems that the value of the conducted research would also be increased by a comparison of neuropathic and ischemic foot ulcers, which was not possible in this study due to the limited sample size. The sample size was not calculated in the analyzed group of patients. The study also did not include the analysis of previous standards of care.

Conclusions

The study reported here investigated the short-term effects of local O_3 therapy in the treatment of DFS, depending on the ozone dose concentration. Higher doses of O_3 resulted in faster healing of ulcers and reduced pain. The possibility of using higher O_3 dose concentrations is therefore worth considering because the results appear to be encouraging. Therefore, promoting the clinical use of O_3 therapy in the treatment of DFS is justified.

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Ethical approval

The study was conducted in accordance with the Declaration of Helsinki (1964) and its protocol has been accepted by the local bioethical committee at the Medical University of Silesia in Katowice, Poland (approval reference number: KNW/0022/KB1/102/II/16/19, date of approval: 02.07.2019). Every patient enrolled to the study has signed written informed consent approval for all procedures conducted in the study.

Conflict interest

The authors declare no conflict of interest.

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