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Original Article

Efficacy of comprehensive ozone therapy in diabetic foot ulcer healing

Morteza Izadi [a], Ramin Kheirjou [a,*], Roya Mohammadpour [b], Mohammad Hassan Aliyoldashi [b], Saeedreza Jamali Moghadam [c], Farzin Khorvash [d], Nematollah Jonaidi Jafari [e], Shahram Shirvani [f], Nahid khalili [e]

[a] Ozone Therapy Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
[b] Department of Physiology, Faculty of Veterinary Medicine, University of Tabriz, Iran
[c] Ziaeian Hospital, Teheran University of Medical Sciences, Teheran, Iran
[d] Nosocomial Infection Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
[e] Health Research Center, Baitullah University of Medical Sciences, Tehran, Iran
[f] Microbiology Research Center, Baqiyatallah University Medical of Sciences, Tehran, Iran

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ABSTRACT

Background: Diabetic foot ulcer is one of the common complications of diabetes disease that is costly and difficult to treat. This problem can lead to morbidity and even mortality. Ozone is a gas that can optimize cellular metabolism and, because of its antioxidant and antibacterial effects, can help the better healing of diabetic foot ulcer.

Method: Two hundred patients, aged 18–85 with diabetic foot ulcers ranging from grade 1 to 4 according to Wagner classification in two groups were studied. Group 1 was treated by full ozone therapy besides the standard regular DFU treatment while group two just was received routine diabetic foot care. Wound size, wound grade, healing time, Fasting blood sugar and inflammatory biomarker before and after treatment were checked.

Results: All patients have had complete wound closure in the ozone group. The mean age of the patients included in the results was 59.03 ± 12.593 and 53.5 ± 10.212 for ozone group and control group. The baseline average surface area of ulcers was $13.41 \pm 14.092\ cm^2$ (range $1–70\ cm^2$) in ozone group and 12.72 ± 0.911 (range $1\_64\ cm^2$) in the control group. Average healing time was 69.44 ± 36.055 days (range 15–180 days), which is significantly lower than the median healing time measured in the control group and some previous studies.

Conclusion: Our study results support the efficacy of ozone therapy especially in its comprehensive use in DFU healing and reduction in the chances of infection and amputation.

1. Introduction

Diabetes is a disease identified by high blood glucose caused by the impairment of the insulin function and its production or both. High blood glucose affects many parts of the body and causes various complications [1]. With a growing prevalence of diabetes, secondary complications arising due to this disease are increasing, too. Diabetic foot ulcers (DFU) are one of the complications resulting from multiple contributing causes such as neuropathy, ischemia, and infection that contribute to morbidity and amputation [2,3]. It has been found that lower limb amputations increase the risk of mortality [4]. While the incidence of foot ulcer has been estimated to be 19–34% [5], the prevalence of DFU was found to be 6.3% in the overall population [6] and is expected to grow more in future [7]. The average 3-year cumulative cost of DFU in 2011 was estimated to be $52,360 [8], which is pretty high.

The above data show that DFU is a very common, complicated, and costly problem that draws researchers' interest to find effective means to prevent or treat it and help its better healing.

Ozone is a gas made of three atoms of oxygen with a cyclic structure [9] and can be applied to treat many diseases due to its known multiple effects such as antioxidant functions and antibacterial action [10,11]. For instance, it can be used in the treatment of chronic infections caused particularly by antibiotic-resistant pathogens. Recently, the beneficial effects have been found of

* Corresponding author.
E-mail address: dk_ramin@yahoo.com (R. Kheirjou).

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treating a vascular ulcer with ozone [12]. Moreover, ozone administration can induce tolerance of oxidative stress and prevent damage mediated by free radical [13].

There are only a few studies to verify the efficacy of the local and systemic ozone therapy on the human DFU. So we decided to assess ozone therapy effects as a comprehensive treatment and inflammatory biomarker changes in DFUs.

2. Methods

This single-blind randomized clinical trial study was aimed at identifying the safety and effectiveness of ozone on the healing of foot ulcers among diabetes patients. The study was conducted from April 2016 to November 2017 at the Baqiyatallah ozone therapy center in Tehran, Iran, Golpayegani hospital in Qom, Iran, and the Najafabad-Esfahan, Iran, ozone therapy center. Two hundred Type 1 and Type 2 diabetic patients with foot ulcer were divided into two groups. Each group consists of 100 patients; 50 male and 50 female. Group 1: Patients treated with ozone, while routine conservative treatment was done alongside. Group 2: Patients just received routine cares. Inclusion criteria were patients aged 18–85 years and diagnosed with diabetes mellitus with DFU Wagner 1 to 4 and who underwent the treatment course regularly. Exclusion criteria were patients with abnormal thyroid functional test and abnormal coagulation test, pregnant or nursing patients, G6PD patients, and patient with hypersensitivity to ozone.

The baseline demographic information consisted of wound grade and wound size was recorded. The necessary laboratory tests such as complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), fasting blood sugar (FBS), prothrombin time (PT), partial thromboplastin time (PTT), and creatinine were required. Samples were obtained after 8 h of overnight fasting. The wound grade was marked according to Wagner criteria and wound surface measured by a ruler in the longest-length and widest-width of the wound. Appropriate medical and surgical treatments (administration of appropriate antibiotics, regular sterile dressing, and the use of surgical debridement) were carried out in all patients. Group 1 patients also were treated by local (bagging and ozonized olive oil and solution) and systemic ozone (minor and major ozone therapy) twice a week (at an interval of at least 24 h) until wound closure and epithelialization was confirmed by the doctor as an end of the study. In local forms, wounds remain in special bag consist of ozone gas for 30 min. Ozonated gel (Ozolive) was applied on the wounds every 12 h and then dressed in sterile gauze. We used the subcutaneous injection of ozone-oxygen around the wound, too. In systemic use, patients took a mixture of ozone and oxygen through rectal or intravenous administration after necessary preparations. Patients took vitamin c tablets immediately after every systemic procedure.FBS, ESR, CRP were checked again after the healing of the ulcer. Moreover, the healing time and the sessions of ozone therapy and surgery for amputation were recorded. All patients gave informed and signed consent before being involved in the study. Ethical approval for this study was obtained from the Ethical Committee of Baqiyatallah University of Medical Sciences.

3. Statistical

All statistical analyses were performed with SPSS software (SPSS for Windows, Version 16.0). The results were expressed as the mean ± standard deviation. Differences in parameters before and after treatment were analyzed by the pair *t*-test. We used independent T-test to calculate a difference value between ozone and control group. The value was assumed to be significant at p-value < 0.05.

4. Result

Two hundred patients (100 male and 100 female) with DFU divided into two groups were studied. All patients completed the study. The mean age of the patients included in the results was 59.03 ± 12.593 and 53.5 ± 10.212 for group 1 and group 2. The baseline average surface area of ulcers in the intervention group was 13.41 ± 14.092 cm^2 (range 1–70 cm^2) and 12.72 ± 0.911 in control group and there was no significant difference between them (p: 0.609). As for the Wagner criteria, there were 20.9% grade 1, 42.7% grade 2, 18.2% grade 3 and 18.2% grade 4 in ozone group and 25.3% grade 1, 44.7% grade 2, 23.1% grade 3 and 6.9% grade 4 in control group. The average healing time was 69.44 ± 36.055 days (range 15–180 days) in the ozone group while In control group after 180 days 25% of patient did not completely heal, so mean healing time in ozone group is significantly lower than the mean healing time measured in control group (p: 0.012) and also some previous studies [14] ($P < 0.001$) [15] (P: 015) [16] (P: 0.019).

There was no significant difference in the length of healing between male and female. As expected, the healing time increased as the wound size increased and, subsequently, the need for ozonetherapy increased, too. It was found that the amputation rate in high-grade and the large wounds were more than in low-grade and the small wounds.

FBS, ESR, and CRP for the before and after treatment groups can be seen in Table 1.

According to Table 1, in control group, FBS after therapy was increased while the other variables were decreased, but in ozone group not only all variables were decreased but also it's after treatment values were less than those in control group.

The 20 sessions cut off point was founded critical because it showed a significant inverse correlation with the amputation rate (p-value: 014).

Fig. 1 shows the amputation frequency according to the wound grade. As can be seen, the amputation rate in the ozone group is less than the control group and increases with an ascending wound grade in both groups.

More patients were amputated in the control group (57%) than in the group treated with ozone (19.1%) (P value < 0.05).

No obvious side effects occurred during the treatment.

5. Discussion

However, the ozone therapy in the treatment of ulcer, especially diabetic foot ulcer, is not a new approach but needs more research regarding its efficacy to find the best ozone therapy methods. The main finding of our study is that amputation decline after treatment with ozone in patients with a diabetic foot infection, In addition, we found routine treatment have adequate performance, but lower in comparison with ozone therapy for the treatment of diabetic foot ulcer.

In a diabetic patient with poorly controlled blood glucose, mitochondrial damage happens in repair cells and wounds apoptosis increases, impairing wound healing [17,18]. Moreover, the high glucose level in diabetic patients generates high levels of free radicals; besides, the low level of antioxidants in these patients makes vascular damage [19]. It has been demonstrated that in diabetic patient high oxidative stress and antioxidant reduction characterize the mechanisms that may lead to foot ulcer and its progress [20,21]. Our result shows that ozone therapy reduces the FBS level significantly; so, this effect, as well as the antioxidant effect can be one of our improvement factors to achieve wound healing. As understood before, this effect was due to a systemic use of ozone therapy and shows the importance of this type of administration. This finding is in conformity with previous studies

Table 1
Comparison of two groups according to FBS, CRP and ESR at baseline and after treatment.

Parameters		baseline	After treatment	p value
FBS	CONTROL	166.01 ± 46.01	188.01 ± 46.02	0.001
	OZONE	178.36 ± 80.314	135.58 ± 44.151	<0.001
CRP	CONTROL	11.9 ± 0.8	4.2 ± 2.7	<0.001
	OZONE	12.3 ± 3.5	3.6 ± 1.4	<0.001
ESR	CONTROL	61.8 ± 5.1	39.1 ± 4.4	<0.001
	OZONE	52.72 ± 29.238	33.53 ± 21.624	<0.001

Data are a mean ± standard deviation.

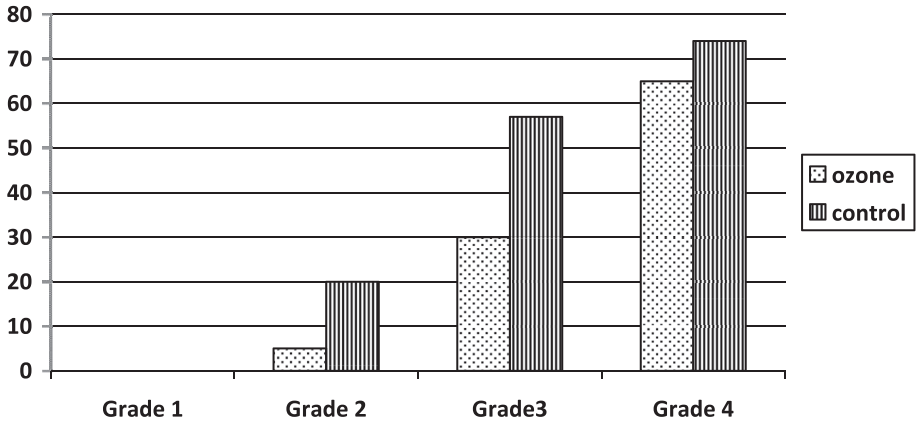

Fig. 1. Amputation percent in different wound grades.

[22,23]. Drug-resistant infection is common in DFU and a history of previous antibiotic treatment can increase its risk in diabetic patients and needs special attention in treatment. It was found earlier that ESR was a strong diagnostic factor of osteomyelitis and severe infection [24–26], and high CRP levels could be a prognostic risk factor for foot amputation in DFU [27]. Our finding shows that ozone therapy significantly reduces CRP and ESR and as expected by decreasing this diagnostic and prognostic factors; amputation rate decrease significantly too.

Most of the available articles on this subject just use local or systemic ozone therapy [28–31], but, as found before, every method of local and systemic use have special beneficial effect on diabetes. So, we decided to assess the efficacy of full ozone therapy (local and systemic) on the healing of DFU and achieved positive results.

Unlike most articles that suggest a special treatment for DFU, in our study all participants had complete wound closure. In addition, there were significant differences in the healing time compared to other custom treatment.

Several challenges were faced in this study. Treatment by different operators at different centers can affect results. Therefore, we were training special operators to reduce operator bias. Moreover, biochemical parameters were tested in different laboratories but the same standard methods were used. On the other hand, since the patients were from various economic situations and, because of the absence of a fine supportive medical system, providing the same appropriate condition such as standard diabetic foot shoes for wound healing to all patients seems to be impossible, and that can affect the results.

6. Conclusion

In spite of various probable biases, our study results support the efficacy of ozone therapy especially in a comprehensive use in DFU healing and reduction of infection and amputation. According to the results, we suggest ozone therapy use as complementary medicine in treating DFUs.

Conflicts of interest

The authors declare no conflict of interest and have not received any funds for this study.

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