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REVIEW ARTICLE

Ozone therapy as a complementary treatment for diabetic foot

La ozonoterapia como tratamiento complementario del pie diabético

A ozonioterapia como tratamento complementar do pé diabético

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Abstract

The aim of this paper was to present the use of ozone therapy as an adjuvant treatment in cases of diabetic foot ulcers and to compare traditional treatment and traditional treatment associated with ozone therapy. This is an integrative review in which searches were carried out in the PubMed, VHL, ScienceDirect and SciELO databases, without date or language restrictions. The results demonstrate that ozone, as a complementary measure to conventional treatment, has shown promise in the treatment of diabetic foot and ulcers, as it helps with healing, circulation, the immune system and has no adverse effects. Diabetes Mellitus, if left untreated, can lead to complications, such as diabetic foot - a syndrome that causes neuropathy, ischemia and infections that lead to the development of diabetic foot ulcers. Conventional treatment for diabetic foot ulcers consists of the use of antibiotics, debridement of the lesions and, in some cases, amputation of the limbs; therefore, ozone therapy has been used to complement the treatment. The results obtained showed that the use of ozone is promising in patients with diabetic foot ulcers.

Descriptors: Ozone; Diabetic Foot; Combined Therapy; Diabetes Mellitus; Focal Infection.

Resumén

El objetivo de este trabajo fue presentar el uso de la ozonoterapia como tratamiento adyuvante en casos de úlceras del pie diabético y comparar el tratamiento tradicional y el tratamiento tradicional asociado a la ozonoterapia. Se trata de una revisión integradora en la que se realizaron búsquedas en las bases de datos PubMed, VHL, ScienceDirect y SciELO, sin restricciones de fecha ni de idioma. Los resultados demuestran que el ozono, como medida complementaria al tratamiento convencional, se ha mostrado prometedor en el tratamiento del pie diabético y las úlceras, ya que ayuda a la cicatrización, la circulación, el sistema inmunológico y no tiene efectos adversos. La diabetes mellitus, si no se trata, puede provocar complicaciones, como el pie diabético, un síndrome que causa neuropatía, isquemia e infecciones que conducen al desarrollo de úlceras del pie diabético. El tratamiento convencional de las úlceras del pie diabético consiste en el uso de antibióticos, desbridamiento de las lesiones y, en algunos casos, amputación de las extremidades, por lo que se ha utilizado la ozonoterapia como complemento del tratamiento. Los resultados obtenidos demostraron que el uso del ozono es prometedor en pacientes con úlceras del pie diabético.

Descriptores: Ozono; Pie Diabético; Terapia Combinada; Diabetes Mellitus; Infección Focalizada.

Resumo

O objetivo deste trabalho foi apresentar o uso da ozonioterapia como tratamento coadjuvante em casos de úlcera de pé diabético e comparar o tratamento tradicional e o tratamento tradicional associado à ozonioterapia. Trata-se de uma revisão integrativa em que foram realizadas pesquisas nas bases de dados PubMed, BVS, ScienceDirect e SciELO, sem restrições de datas ou idiomas. Os resultados demonstram que o ozônio, como medida complementar ao tratamento convencional, se mostrou promissor no tratamento do pé diabético e das úlceras, por auxiliar na cicatrização, circulação, no sistema imunológico e não apresenta efeitos adversos. A Diabetes Mellitus, se não tratada, pode acarretar complicações, como o pé diabético - uma síndrome que ocasiona neuropatia, isquemia e infecções que cursam com o desenvolvimento das úlceras do pé diabético. O tratamento convencional para as úlceras do pé diabético consiste na utilização de antibióticos, desbridamento das lesões e, em alguns casos, a amputação dos membros, assim, a ozonioterapia vem sendo utilizada para complementar o tratamento. Os resultados obtidos mostraram que o uso do ozônio é promissor nos pacientes portadores de úlcera do pé diabético.

Descritores: Ozônio; Pé Diabético; Terapia Combinada; Diabetes Mellitus; Infecção Focal.

Introduction

Diabetes mellitus (DM) is a comorbidity considered as one of the great epidemics of the twenty-first century. According to the World Health Organization (WHO), forecasts for the year 2030 consider that this disease will be classified as the seventh cause of death in the world[1].

It is a pathology that manifests itself especially in three groups: type 1, gestational and type 2 diabetes. Type 1 diabetes mainly affects children, gestational diabetes requires medical care and follow-up during pregnancy. Type 2 diabetes, on the other hand, affects about 90% of patients and tends to evolve if a treatment is not effective. The lack of treatment of this chronic and silent disease can lead to serious complications, such as diabetic foot, defined by the WHO as a syndrome that generates neuropathy, ischemia and infections, which can cause ulcers and tissue damage[1,2].

Ulcers are the result of both case angiopathy and neuropathy, which cause a loss of sensation in the foot, predisposing to a localized increase in pressure, callus formation, tissue rupture and lesion formation. In addition, metabolic lack of control threatens the integrity of the foot and can lead to amputation, especially in limbs that have poor perfusion. Thus, diabetics who have foot ulcers are associated with a high healing time, wound infection and repeated hospitalizations[3].

To assist in the treatment of diabetic foot ulcers (DFU) a classification system was created, which has ten nomenclatures, such as the Wagner-Meggitt system, ischemic depth classification, the University of Texas system, SAD classification, PEDIS classification, King classification, Kobe classification, Amit Jain classification, Van Acker/Peter-Riesch classification (VA/P) and other classifications[4].

The therapeutic resource used for DFU consists basically of cleaning the affected site, debridement of the wound, use of dressing, antibiotic therapy for cases with infection and revascularization of the limb, when indicated. Metabolic control is also a treatment that has been shown to be effective[3].

However, due to the high cost for the treatment of diabetic foot, new therapeutic strategies began to be adopted. In the mid-nineteenth century, ozone began to be used as a treatment, because it has the property of releasing nascent oxygen, which has bactericidal action and stimulates antioxidant enzymes, thus contributing to wound healing[5].

In this way, ozone therapy has currently been used in several hospitals around the world. However, orthodox medicine does not value this form of treatment due to misunderstanding and prejudice based on the known toxicity of ozone. However, this view is wrong, because the toxicity of chemical compounds depends on the dosage, so both ozone and other gases are used only in therapeutic doses. Thus, the idea that ozone is always toxic is unrealistic[2].

The benefits of using this gas outweigh the harms, as it allows the inactivation of bacteria, fungi and viruses, improves the circulatory state, activates the immune system and the formation of peroxides. Thus, the use of ozone has been promising, for being able to treat ulcerative lesions of the diabetic foot and for not having toxic effects[6]. The aim of this study was to analyze the literature and compare traditional treatment and traditional treatment associated with ozone therapy for DFU.

Methodology

In this integrative literature review, search strategies were carried out for Case Reports and for published Randomized Clinical Trials (RCTs), which investigated the efficacy of the use of ozone therapy as a complementary therapy to the treatment of diabetic foot ulcers, that is, used as an additional therapy to the conventional treatment recommended for such individuals. The searches were made through searches in the PubMed, VHL, ScienceDirect and SciELO databases, in April 2021, without restrictions of dates or language. The final search strategy was: *("Diabetic Foot" OR* "Diabetic Foot Ulcer") *AND (Ozone) AND (Therapeutics OR* Treatment).

It was considered as "intervention with Ozone Therapy" those interventions that used ozonated oils (sunflower oil or ozonated olive oil), or by mixing oxygen and ozone applied directly to the wound or through rectal insufflation (blown into the final portion of the intestine / intestines through the anus), in patients diagnosed with DFU and undergoing conventional treatment.

Published RCTs were included, including patients in different stages of diabetic foot ulcer and older than 18 years, who received intervention with the use of ozone therapy, combined with active intervention treatments or other interventions.

The outcomes of interest used were the analysis of the extent of the lesion, disease progression, glucose levels and oxidation by-products.

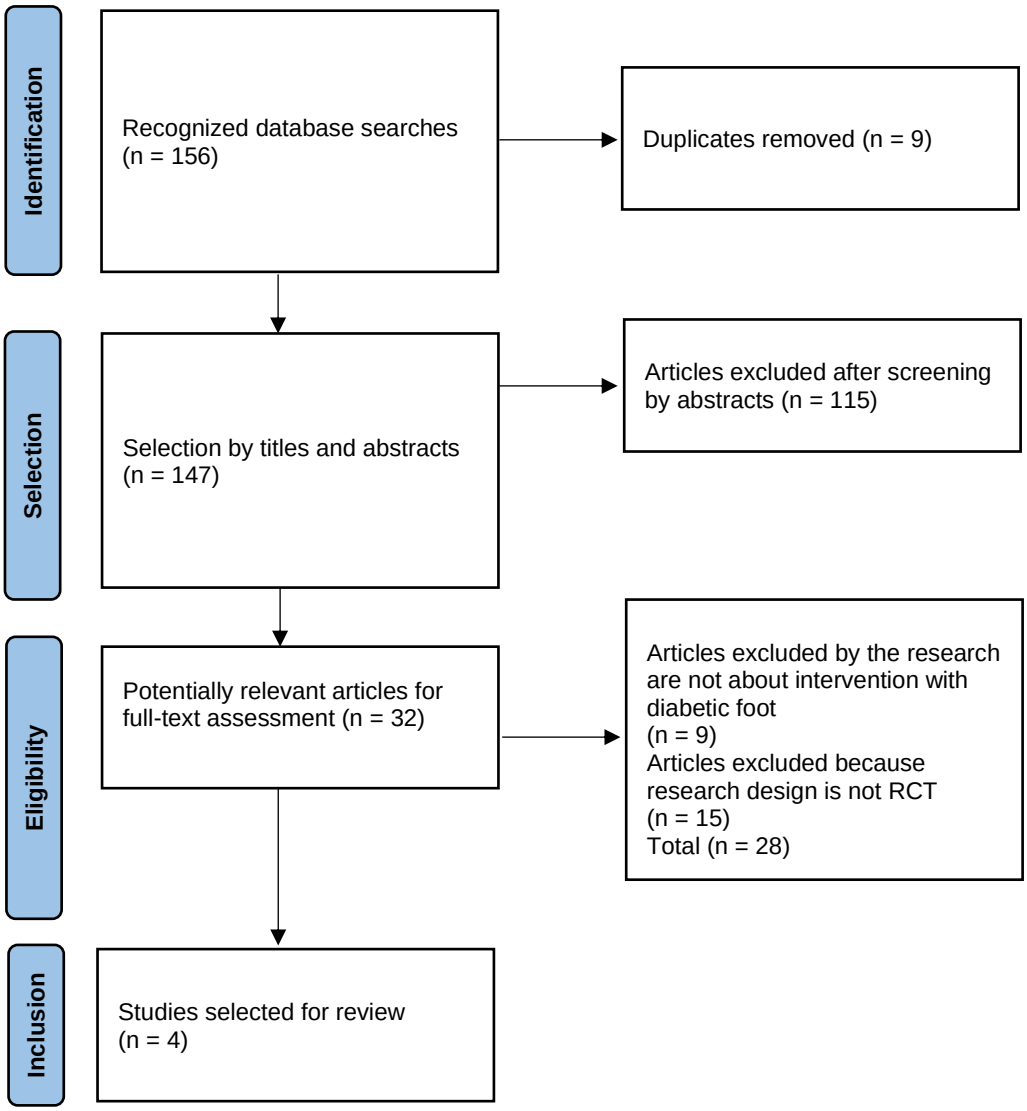
First, from the searches in the selected databases, 156 studies were found. Through the reading of the titles and abstracts by independent reviewers (TCA and RAP), from these the duplicates were removed, 32 potential articles were selected, leaving 4 papers, all of which were RCTs. The inclusion and exclusion criteria were established and followed, including patients with diabetic foot ulcers, RCTs, without restriction of date or language, in addition to ozone therapy. Those excluded were diabetics without foot ulcers, *in vitro tests*, animal studies and studies that did not have ozone therapy as an intervention. The articles that established according to the inclusion criteria were included in the review, the importance of 4 randomized clinical trials. There was no discrepancy in the choice of studies.

The methodological quality of the included trials was assessed using the 0-10 PEDro scale. Two independent investigators (RAP and TCA) evaluated each study, and a third investigator (DLMA) resolved the discrepancies.

Descriptive data from the included studies (i.e., characteristics of participants, type of intervention, and outcome) and outcomes for all groups of interest (extent of injury, disease progression, glucose levels, and oxidation byproducts) were extracted by two independent investigators (TCA and RAP), with discrepancies resolved by a third and fourth investigator (JSRN and LFA). The data collected were from the evaluation of the studies and their results.

For the evaluation of the extent of the lesion, the progression of the disease from the treatment cycles was analyzed, for blood glucose analysis, serum tests were done, as well as they were made to analyze the by-products of oxidation and in relation to the progression of the disease, a comparison was made between the groups and intergroup comparison, about the need or not for amputation and, again, size of the lesions, i.e. progression of the lesion.

Figure 1. Flowchart of review studies. Paracatu, MG, Brazil, 2021



Results

In the search strategy, 156 references were identified. After removing the duplicates, titles and abstracts, 147 articles were read and analyzed, leaving 32 potential articles. The 32 potential articles were read in full and the importance of 27 articles was excluded from this review. Remaining in this work, four RCTs.

The four included studies were published in English and Spanish in 2005, 2014 and 2018. They included patients of both sexes, without age restriction, all with established diagnosis of diabetic foot ulcers, who were aware and consented to the research that would be done. Both groups, placebo and intervention, were on conventional treatment (antibiotic therapy and debridement).

Chart 1. Synoptic chart of the selected studies. Paracatu, MG, Brazil, 2021

Authors/ Year	Source	Research	Participants	Intervention	Measurement and Outcome
Duarte et al. (2014)	Patients with DM2 with neuroinfectious diabetic foot; age over 40 years; and informed consent to participate in the study.	Evaluate the advantages of ozone therapy in type 2 diabetic patients with neuroinfectious diabetic foot.	N = 150 Age = > 40 years	Exp 1= Ozone therapy for 21 days with bagging at a concentration of 40-50 mgL -1 O3 volume + rectal insufflation (150 mL at a concentration of 30-40 mgL-1) (n=50)	Disease progression. Glucose levels.

				Exp 2= Ozone therapy for 21 days and oral and systemic antibiotic therapy (n=50) Con= Oral and systemic antibiotic therapy (n=50)	
Izadi et al. (2018)	Patients with type 1 and 2 diabetes mellitus with diabetic foot ulcers with Wagner stage 1-4; age between 18 and 85 years; and informed consent to participate in the study.	Evaluate the effects of ozone therapy as a comprehensive treatment and inflammatory biomarker changes in diabetic foot ulcers.	N = 200 Exp: Age = 59.03 (SD = 12,593) With: Age = 53.5 (SD= 10,212)	Exp= Ozone therapy with bagging for 30 minutes followed by application of ozonated olive oil every 12 hours + rectal insufflation 2 times a week + standard treatment of diabetic foot (n=100) Con= Conventional treatment of diabetic foot (n=100)	Size of wounds. Glucose levels.
Martínez-Sánchez et al. (2005)	Patients with DM2 with neuroinfectious diabetic foot; age over 20 years; and informed consent to participate in the study.	Investigate the therapeutic efficacy of ozone therapy in the treatment of diabetic foot in patients with T2DM and to compare O3 with antibiotic therapy.	N = 100 Exp: Age = 50 (SD = 17) Con: Age = 30 (SD = 18)	Exp= 20 sessions of ozone therapy by rectal insufflation with 10 mg of O3 at a concentration of 50 mg/L + bagging at a concentration of 60 mg/L of O3 for 1 hour + ozonated sunflower oil in the wound (n=52) Con= Oral and systemic antibiotic therapy (n=49)	Extent of injury. Glucose levels. Levels of oxidation by-products in each section.
Zhang et al. (2014)	Patients with T2DM with Wagner stage 2-4 diabetic foot ulcer; over 18 years; and informed consent to participate in the study.	Evaluate the effects of ozone therapy on healing and expressions of VEGF, TGF and PDGF in wounds in the initial phase and after treatment in complicated diabetic patients with diabetic foot ulcers.	N= 50 Exp: Age = 59,72 (SD=12,2) Con: Age = 61,12 (SD=10,9)	Exp= Ozone therapy for 21 days with bagging of 52 g/mL of O3 in a total volume of 20-50 mL for 30 minutes + standard treatment of diabetic foot (n= 25) Con= Standard treatment of diabetic foot	The dimensions of the wounds were evaluated. Glucose levels.

Note: DM2 = Type 2 diabetes mellitus; n = sample size; SD = standard deviation; Exp = experimental group; Con = control group, VEGF = vascular endothelial growth factor; TGF = transforming growth factor beta; PDGF = platelet-derived growth factor.

The four RCTs evaluated the benefits of the use of ozone therapy in the treatment of PU in stages 1 to 4 of the Wagner classification, in patients with type 1 and 2 DM, older than 18 years without distinction of sex. All participants signed an informed consent to participate in the study. The main reasons for exclusion from the RCT were patients who had a severe septic condition, liver disease, nephropathy, hyperthyroidism, hypersensitivity to O3, pregnant women, nursing mothers, severe diseases such as cancer, and alcoholism[5,7-9].

A total of 501 participants were selected who were separated into a randomized group in which ozone therapy was performed and a control group where the standard treatment of diabetic foot was done, 36 could not complete the study, due to the extension and progression of the ulcer or because they needed other types of treatment. Ozone was applied locally through plastic bags and ozonated oils, and systematically through rectal insufflation. Concentrations and duration of treatment varied between studies[5,7-9].

In an RCT[7], one group received ozone therapy for 21 days, being applied locally through bagging at a concentration of 40-50 mgL -1 of O3 volume associated with systemic application by rectal insufflation (150 mL at a concentration of 30-40 mgL-1). The other group underwent oral and systemic antibiotic therapy, and group 3 received a combination of the two treatments, ozone therapy for 21 days and oral and systemic antibiotic therapy. The results of the group that performed the combination of ozone therapy with oral and systemic antibiotic therapy did not differ significantly from those that used only ozone.

In the study[8], the randomized group performed ozone therapy by bagging for 30 minutes followed by application of ozonated olive oil every 12 hours, associated with the systemic use of ozone rectally twice a week with an interval of at least 24 hours until wound healing and standard treatment of diabetic foot. While the control group was performed only regular treatment of diabetic foot.

In the RCT[9], one group was treated with 20 sessions of systemic ozone therapy by rectal insufflation with 10 mg of O3 at a concentration of 50 mg/L and locally through plastic bags at a concentration of 60 mg/L of ozone for 1 hour, after which ozonated sunflower oil was used in the wound. The control group performed the conventional method of diabetic foot therapy.

In an article[5], the randomized group after wound debridement performed bagging ozone therapy with 52 g/mL of ozone in a total volume of 20-50 mL for 30 minutes for 20 days, associated with standard treatment of diabetic

foot. In the control group, only conventional treatment of diabetic foot was performed.

In none of the RCTs were there any adverse effects. Patients who used ozone in the treatment of PU had a better wound evolution, with a significantly shorter hospitalization and healing time compared to those who underwent the standard treatment of diabetic foot. In addition, the groups that underwent ozone therapy had a lower number of amputations, as in the study[8], in which only 19.1% of patients in the randomized group had to undergo amputations, compared to the control group with 57% of patients.

There was a significant decrease in fasting blood glucose levels, compared to the group that underwent conventional treatment in which there was an increase after therapy[5,7-9].

In a paper[8], after treatment with ozone therapy, the levels of fasting glucose, C-reactive protein and erythrocyte sedimentation rate decreased in relation to the control group.

A RCT[5] evaluated the expressions of vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF-β) and platelet-derived growth factor (PDGF) in both groups. The randomized group had an increase in proteins on the 11th day of treatment compared to the control group, concluding that healing of diabetic foot ulcers was through the potential induction of these proteins in the primary stage of treatment.

Chart 2. Methodological Assessment - PEDro Scale. Paracatu, MG, Brazil, 2021

PEDro Criteria	Duarte, et al. (2014)	Izadi, et al. (2019)	Zhang, et al. (2014)	Sánchez, et al. (2005)
Random Allocation	No	Yes	Yes	Yes
Hidden Allocation	No	No	No	No
Blind Participants	No	No	Yes	Yes
Blind Therapists	No	No	No	No
Similar groups at baseline	Yes	Yes	Yes	Yes
Blind Researcher	No	No	No	No
< 15% dropout	No	Yes	No	Yes
Intention-to-treat analysis	Yes	Yes	Yes	Yes
Difference between groups reported	Yes	Yes	Yes	Yes
Point estimation and reported variability	Yes	Yes	Yes	Yes
Eligibility	Yes	Yes	Yes	Yes
Total (0 to 10)	5	7	7	8

Discussion

The DFU has been one of the most common complications of DM and is associated with high morbidity and mortality.[5] The management of DFU is a major problem for the world, a study was done with 312,744 wounds of all types, the wounds caused by DM were among the two most common causes of ulcers[10].

The conventional treatment of DFU is done using antibiotics, to eradicate bacterial infections in the wounds and consequently worsens the picture, it is also performed the debridement of the lesions or even the amputation of parts of the limbs or the limb, when conservative treatment with antibiotics is inefficient, it is worth mentioning that the average healing time is 21 days[5].

Regarding the pathophysiology of DFU healing in the early stages of treatment (debridement and antibiotic therapy), healing is mediated by platelet-derived growth factors (PDGFs) and transforming growth factors-β (TGF-β) and then macrophages release the main growth mediators for healing[5].

Ozone is a gas composed of three oxygen particles, which are arranged in a cyclical way, its use began around the nineteenth century, is known to have several beneficial effects on wound healing, such as antioxidant and antibiotic effects. Because it stimulates antioxidant enzymes and has an antibacterial effect, it can be used in chronic wound infections caused by multidrug-resistant pathogens[5,11].

The use of ozone therapy brought improvements to conventional treatment, that is, the association of conventional treatment and complementary treatment (ozone therapy) brought benefits to cure DFU. This is because of ozone on the body; ozone stimulates the

expression of VEGF, TGF-β and PDGF from wounds at the initial stage after treatment, which leads to faster and more efficient healing.

It is noteworthy that the systemic effects that the use of ozone therapy can bring, increasing antioxidant enzymes, preventing atherosclerosis, increasing tolerance to free radicals and preventing free radical-mediated damage. Ozone has no genotoxic effect, which gives it safety in its use according to the recommended measures[7-9].

It turns out, in a study[5], that ozone therapy has improved in patients with coronary artery disease, increasing prothrombin time, decreased biomarkers of lipid and protein oxidation, and generally increased antioxidant enzymes. In diabetic patients with peripheral arterial diseases and with DFU, there was a significant improvement in blood glucose, reduction in lesion sizes, amputation numbers and there was a decrease in oxidative stress in patients who were treated with rectal ozone for 20 days, compared to the control group.

Depending on the study[8], there was a reduction in infection and amputation in the case group compared to the control group. The clinical trial conducted by Duarte et al, demonstrated that the use of ozone therapy associated with conventional treatment has attested improvement in relation to the following outcomes: number of amputations and extension of the lesion, when compared to the control group.

The experimental study analyzed[9] showed that when conventional treatment was done without the use of ozone, the glucose concentration did not change. However, when associated, glucose levels reached optimal reference values. This effect is due to the properties of ozone, which increases the sensitivity of insulin receptors, as this molecule generates an antioxidant effect.

Final Considerations

Therefore, improper treatment of DM can lead to serious complications such as diabetic foot which, in turn, can cause ulcers and tissue damage. Conventional therapy for DFU is done with antibiotics, debridement of the lesions, or even the amputation of parts of the limb or the limb.

On the other hand, ozone has shown promise in the treatment of these lesions. This gas has bactericidal action and stimulates antioxidant enzymes, thus contributing to wound healing. In addition, ozone improves the circulatory state, activates the immune system and has no toxic effects. Thus, ozone therapy has shown many results in the treatment of diabetic foot ulcers.

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