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Ozone Therapy in the Adjuvant Treatment of Burn Wounds: A Case Study Series

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Abstract

The study aimed to describe the use and analyze the action of ozone therapy in the adjuvant treatment of burn injuries in adults. This is a series of case studies, with a descriptive and exploratory approach, carried out in a public teaching hospital in the southern region of Brazil, from May 2021 to February 2022. The patients recruited were referred by the institution's plastic surgery team and the final sample consisted of three participants, who were followed up weekly on an outpatient basis. Three instruments were used for data collection, as well as wound measurement and photographic records. All the participants had partial thickness lesions and were men aged between 26 and 58. After seven days of using ozone therapy, all three participants showed an improvement in their pain, and after 14 days there was no pain. With regard to the reduction in the affected area, after 14 days all the patients showed a significant reduction. In addition, none of the patients had sequelae, infections, or adverse events. Ozone therapy proved to be beneficial as an adjuvant in reducing pain and in the healing process f wounds caused by burns. However, further research is encouraged due to the lack of care protocols and clinical studies on this subject.

Keywords: Stomatherapy, Nursing, Healing, Burns, Ozone

Introduction

Injuries resulting from burns are recurrent worldwide and are responsible for high morbidity and mortality rates. According to the World Health Organization, 180,000 people die every year, and non-fatal injuries have a greater impact on morbidity rates [1].

The most common outcome in burn patients is infection of the lesions, which causes the majority of deaths. Due to the damage to the integumentary system, the tissue becomes devitalized and a cascade of biochemical effects ensues, reducing tissue perfusion and the chemotaxis of immune system cells, allowing microorganisms to multiply, resulting in delayed healing [2, 3]

In addition, there is a greater susceptibility to sequelae, functional restrictions, aesthetic and psychological damage, making it difficult for people to return to their work activities and integrate into society. It is an aggravation in the biopsychosocial and economic areas, directly impacting on quality of life [2, 4, 5].

In view of the problems caused by burns, it is necessary to subsidize health care and less expensive care technologies that are ef-

fective and cost-effective. In this sense, ozone therapy is a viable alternative for reducing complications and infections [6-8]. It is therefore essential that researchers promote scientific evidence in this area, as there is low adherence to its use, linked to a lack of care protocols and few clinical studies, making it difficult to validate and apply [9, 10].

Ozone therapy stands out for its powerful bactericidal, virustatic, and fungicidal action, without causing selectivity of the most resistant pathogens; and it corroborates in the elimination of biofilm [7, 11, 12]. Its mechanism of action is due to the formation of its by- products: immediate reaction Reactive Oxygen Species (ROS) and late reaction Lipid Oxidation Products (LOPs), which inactivate microorganisms by oxidatively breaking down the phospholipids and lipoproteinase present in their membranes, causing damage to the bacterial cell envelope and the cell wall of fungi. In viruses, it interferes with their reproduction, as lipoproteins, proteins, and glycoproteins are oxidized, damaging the viral capsids [7, 13, 14]. In addition to its antimicrobial properties, ozone also stimulates wound healing through controlled oxidative stress, which leads to increased production and migration of growth factors and increased oxygen levels at the wound site [15].

The moment the gas comes into contact with organic fluids, it immediately reacts by ceasing to exist, simultaneously forming reactive oxygen species and lipid oxidation products. Lipid oxidation products cause vasodilation by acting on the endothelium, releasing prostacyclin, interleukin-8, and nitric oxide. Reactive oxygen species generate platelet aggregation and the release of platelet-derived growth factors, transforming growth factor β and interleukin-8, which play an important role in rapid wound healing [16].

The stimulation of oxygen metabolism is caused by an increase in the rate of glycolysis in erythrocytes and consequently results in the stimulation of 2,3- diphosphoglycerate, which leads to an increase in the amount of oxygen released into the tissues. Ozone activates the Krebs cycle, increasing the oxidative carboxylation of pyruvate and stimulating energy production [17]. Immune action is directed at monocytes and lymphocytes which, once stimulated, release small quantities of cytokines in an endogenous and controlled manner. This regulation acts as a potentiator of the immune system, activating neutrophils and stimulating the synthesis of some of these cytokines [18]. Currently, in many countries, this therapy is applied as an efficient and low-cost alternative, helping to treat wounds that are difficult to heal, especially in diabetic patients, and to disinfect contaminated wounds. In Brazil, as in other underdeveloped countries, the search for alternatives to help treat burns is a social requirement, since the majority of the population uses the public health system. Therefore, the financial resources for acquiring new technologies for the treatment of burns are not a feasible reality for the majority of patients [19].

Thus, ozone therapy has been proposed as one of the treatments that can bring health and quality of life benefits to patients by accelerating the wound healing process [20]. It is noteworthy that since 2018, ozone therapy has been included in the list of treatment modalities included by the National Policy for Integrative and Complementary Practices (PNPIC) in Brazil's Unified Health System (SUS). It is therefore a possibility for sustainable use in these treatments.

The aim of this study was to describe the use of ozone therapy as an adjuvant treatment for adults suffering from burns.

Methods

This is an exploratory, descriptive, case series study carried out between May 2021 and February 2022, following the method recommended by the Joanna Briggs Institute (2020). The research was carried out at a teaching hospital located in Santa Catarina, Brazil.

All patients with burns seen by the institution's Plastic Surgery department were invited to take part. After being discharged from the hospital, the patients were referred for outpatient follow-up. Once they had accepted the invitation, they signed the Informed Consent Form (ICF) and the image authorization form.

- **Inclusion criteria:** people over the age of 18 with burn wounds; agreeing to undergo procedures and dressings indicated by the medical and nursing team; attending outpatient appointments once a week.
- **Exclusion criteria:** having one of the following conditions: pregnancy, cancer, glaucoma, G6PD (glucose 6-phosphate) enzyme deficiency, decompensated hyperthyroidism, severe anemia; not attending scheduled appointments to continue treatment; people with adverse events due to the use of the products and therapies used.
- **Discontinuation criteria:** using other products or therapies that were not prescribed or presented in the study; reporting an adverse event; not attending outpatient appointments for more than a week. During the period mentioned above, five participants were eligible, but two were eliminated due to the discontinuation criteria.

Three instruments were used to collect the data: 1) a clinical assessment instrument prepared by the researchers; 2) a visual analog scale (VAS); 3) a follow-up instrument: the Bates-Jensen Wound Assessment Tool (BJWAT) adapted with the Nerds and Stones mnemonic. Instrument 1 was applied during the first clinical assessment and includes the following variables: socio-demographic and economic data, etiology of the injury and previous treatments carried out, associated risk factors, clinical date, assessment of the injury, and physical examination. Tool 2 was used to measure pain intensity using a numerical scale from 0 (no pain) to 10 (worst pain experienced in a lifetime), classified as mild (1-2), moderate (3-7), and severe (8-10).

Instrument 3 was used to assess and monitor the patient and the evolution of the wound, which is made up of the following domains: size, depth, detachment, type and quantity of necrotic tissue, type and quantity of exudate, granulation tissue, epithelialization, the color of the perilesional area, edema and hardening of the peripheral tissue. To calculate the total score on the instrument, the points for each domain were added up, giving a finishing score that can vary from 9 (healed wound) to 65 (wound degeneration). To assess the risk of infection, the mnemonic Nerds (corresponds to five signs of local infection of superficial tissues) and Stones (corresponds to seven signs related to infection of deep tissues) was used, which requires at

least three of the signs in one of the levels assessed to establish infection [21].

The primary outcome variable was the regression of the burn area in cm² or until complete healing, which was assessed weekly. The lesion was photographed using a device with a triple camera, each with 12, 12 and 8 megapixels respectively, without flash, and a resolution of 4000 x 3000 pixels. Secondary outcomes were the regression of the instrument 3 score and the VAS scale, measured over the course of follow-up until complete repair.

The study complied with the precepts established by Resolution 466/12 of December 2012 of the National Health Council. To this end, it was approved by the Human Research Ethics Committee (CEPSH) with a substantiated opinion CAAE 65869817.4.0000.0121.

Case Report 1

C1, Brazilian, male, white, 46 years old, high school graduate, Catholic, truck driver, lives in the municipality of Palhoça/SC, married, three children, family income of approximately five minimum wages. Burn resulting from a domestic accident with cooking oil on 05/09/2021. His first visit was to an Emergency Care Unit on May 10, 2021, in which there was no improvement and he went to the school hospital's emergency service on May 12, 2021, after which he was referred for outpatient follow-up. At the time of the consultation, he was being treated with silver sulphadiazine at the Basic Health Unit. Habits: water intake of two liters per day (water from treatment company), consuming protein (chicken/meat) at the main meals (lunch º0Íło9ló0póo0and dinner); having three main meals: breakfast, lunch, and dinner; including in these vegetables and legumes, carbohydrates and proteins. Vesico-intestinal eliminations are present every day, without alterations. He reported pain measured at 9, according to the VAS (severe pain), which wakes him up continuously at night, taking painkillers on average every 4 to 6 hours (paracetamol). He says he is afraid of having his leg amputated. Denies smoking and drinking (only socially). Denies comorbidities and continuous medication. No previous allergies.

The patient arrived at the clinic with an easy expression of severe pain (VAS 9), walking with the aid of crutches, lucid, oriented in time and space, and communicative.

Skin and mucous membranes are normal, hydrated, anicteric, and acyanotic. Breathing room air without respiratory effort, eupneic. He had a superficial partial or superficial second-degree burn, according to the classification of the Burned Body Surface (BBS), classified as a minor burn, on the left lower limb (LLL) with friable granulation tissue almost entirely in the wound bed, with small points of eschar located in the anterior tibial region, extending to the foot, reaching the toes and expanding to the lateral and calf region, with a large amount of odorless serosanguinolent exudate, measuring 770 cm² in the area (55x14). Margins are regular, adhered, and delimited. The perilesional area is mostly intact, but in some areas is macerated with the presence of edema. The monitoring of the patient lasted 6 weeks, and there was a total cure in 35 days.

Case Report 2

C2, Brazilian, male, brown, 26 years old, healthy, complete first degree, non- practicing Catholic, sofa upholsterer, lives in São José/SC, single, no children, family income of approximately three minimum wages. Burn resulting from an accident with fire flames on both hands on 05/28/2021. His first visit was to the UPA, where he was prescribed silver sulfadiazine. After the lesions worsened, he went to the emergency department of the teaching hospital on June 3, 2021, where instrumental debridement of the lesions was carried out by a plastic surgeon and he was then referred for outpatient follow-up. Habits: water intake of 1.5 liters per day (water from treatment company), consuming predominantly carbohydrates at meals throughout the day, eating an average of five to six meals a day, including snacks, soft drinks, and fatty meats. Vesico-intestinal eliminations are present every day, without alterations. He reported pain measured at 10, according to AVA (severe pain), and was taking an opioid analgesic (codeine). He said he was concerned about the functionality of his hands after treatment. He denied any previous allergies, smoking, alcoholism, comorbidities, or use of continuous medication. The patient arrived at the clinic with an easy expression of severe pain, walking unaided, lucid, oriented in time and space, and communicative. Skin and mucous membranes are normal, hydrated, anicteric, and acyanotic. Breathing room air without respiratory effort, eupneic. He had a superficial partial or superficial second-degree burn on both hands, measuring 5.25 cm² in area (10.5 x 0.5) on the left hand; and 5.5 cm² in area (11 x 0.5) on the right hand, according to the SCQ classified as a minor burn. In the left palmar region and in the proximal phalanges of the middle and ring fingers, points of epithelialization; phalanges of the index, middle, and ring fingers with the presence of granulation tissue, sphacelus, and a moderate amount of purulent exudate with an odor; plantar region of the hand in the process of epithelialization, starting from the proximal phalanges. In the right palmar region, there was fibrinous sphacelate tissue throughout the lesion, a large amount of serous exudate with an odor; extensive and numerous ruptured flictenas, and two flictenas ruptured during conservative instrumental debridement; absence of granulation tissue, perilesional maceration. The patient was followed up for three weeks and achieved complete repair in 21 days. There were no sequelae in terms of limb functionality.

Case Report 3

C3, Brazilian, male, white, 58 years old, healthy, completed high school, non- practicing Catholic, businessman, lives in the municipality of Palhoça-SC, married, 1 daughter, family income of more than 10 minimum wages. Burn resulting from an accident at work involving electricity, after coming into contact with a supermarket's power generator, which he was treated at the school hospital in the emergency department on 10/12/2021. After discharge, he was referred for outpatient follow-up. At the time of the consultation, he was receiving daily dressings in the Emergency Department with silver sulfadiazine on his left upper limb (LUL) and collagenase on his face, and was taking paracetamol + codeine. He reported being distressed by his appearance, due to the involvement of his face and upper limbs. Habits: drinking 1.5 liters of water a day (from the treatment company), consuming protein (chicken/beef) at the main meals (lunch and dinner); eating three main meals: breakfast, lunch, and dinner; including vegetables and legumes, carbohydrates, and proteins.

Vesico-intestinal eliminations are present every day, without alterations. He reported pain measured at 10, according to AVA (severe pain). No comorbidities, no medication in continuous use, no previous allergies. No smoking or social drinking.

The patient arrived at the clinic walking unaided, lucid, oriented in time and space, and communicative. Skin and mucous membranes are pale, hydrated, anicteric, and acyanotic. Breathing room air without respiratory effort, eupneic. Hemodynamically stable. He had a superficial partial burn or superficial second-degree burn according to the SCQ classified as a minor burn; a lesion all over the direct face, the scalp in the healing phase, undefined margins, a large amount of sphacelus and de-epithelialization, and a medium amount of serous exudate. Lesion on left arm, forearm, and hand; it was not possible to measure the lesion due to the patient's pain, self-reported at 10. Lesions with regular, adhered, and flat margins, the bed covered in sphacelus, and areas of de- epithelialization with large amounts of serous-purulent exudation, slight odor, and edema. Perilesional skin with dots of pale tissue and edema. Adjacent skin is epithelialized and intact. The patient was followed up for four weeks and achieved complete repair in 21 days.

At each session, the patients were seen in a nursing consultation, where they were assessed on their reactions and perceptions of the last session, doubts about home care, evaluation of the therapy, evolution records, and photos. At home, dressings were changed by the family member after guidance, as the secondary dressing was changed after saturation, which lasted an average of four days. The team remained available via the WhatsApp chat application to answer questions about the treatment, during which telemonitoring was carried out.

Patients C1 and C3 did not use antibiotics before, during, or after ozone therapy, except for C2, who used them before starting ozone therapy. All the patients in the study had an adequate vaccination schedule for the adult acellular bacterial triple vaccine. In addition, none of the patients had any complaints or symptoms after the therapy.

With regard to wound hygiene, the following procedures were carried out at each visit: cleaning the perilesional area with degerming chlorhexidine followed by extensive removal with saline solution; cleaning the wound bed with ozonated distilled water (60 mcg) for 10 minutes (used immediately after preparation); application of transcutaneous ozone via bag for 10 minutes and 20 minutes of dispersion and then application of primary and secondary coverage.

Results

After the first ozone therapy session, patients C1 and C2 showed a reduction in the injured area. C1 showed total epithelialization in 35 days (after the fifth session); C2 showed total epithelialization of the left hand after seven days afterward the first ozone therapy session and total epithelialization of the right hand happened after 14 days, afterward the second session. The patient C3 reduces significantly the injured lesion and total epithelialization occurred in 21 days. The cicatrization evolution along the time progress for each case can be observed in Figure 1.

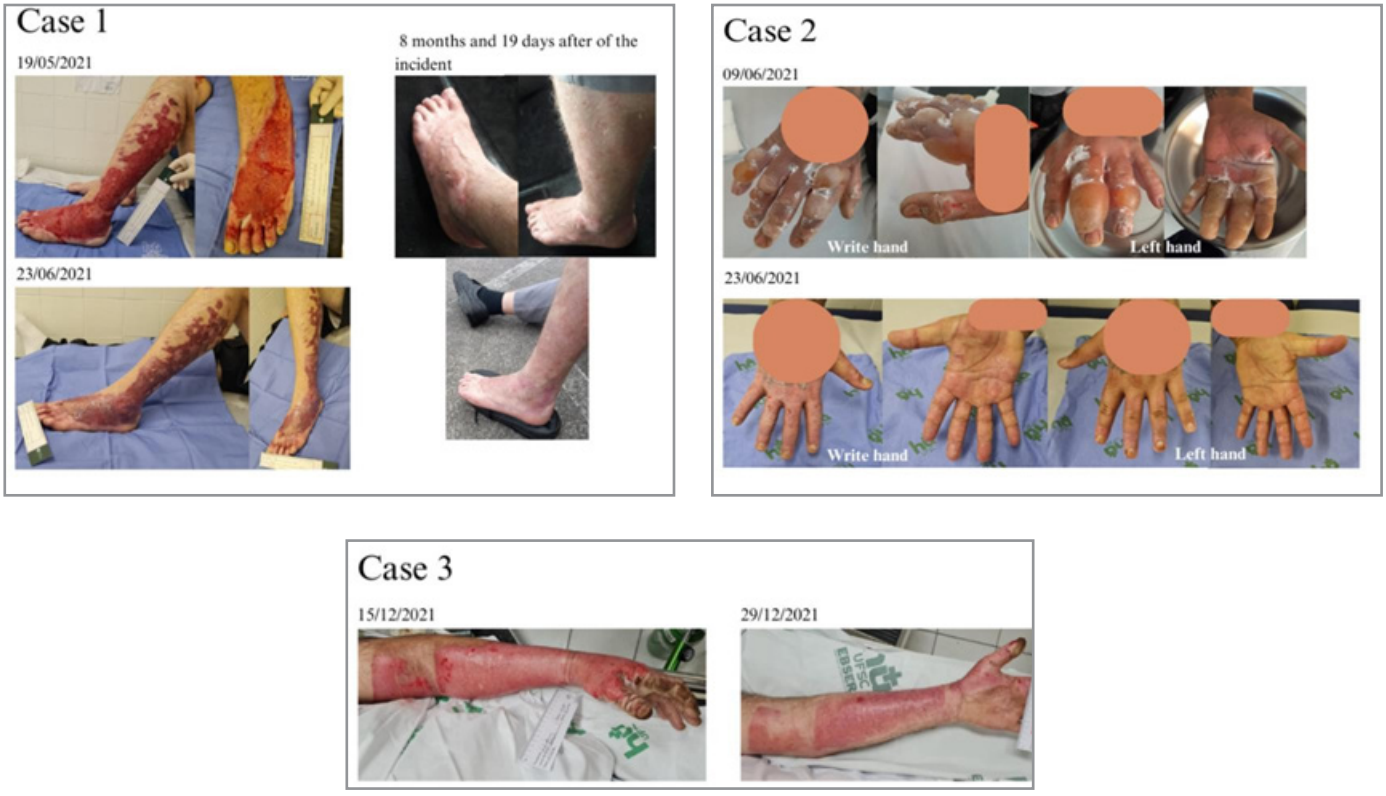

Figure 1: Cicatrization evolution after ozone therapy

After the first application of ozone, the patients showed a significant reduction in pain: case 1- VAS from 9 to 4; case 2-VAS from 10 to 4; case 3-VAS from 10 to 3. After the second session, none of the patients complained of pain, so they stopped taking painkillers.

When instrument 3 was applied, the scores regressed and the lesions epithelialized in all patients. According to the Nerds and Stones scale, only C2 showed superficial involvement indicative of local infection, evidenced by increased exudate, presence of debris, and a foul odor; this ceased 14 days after the second session.

Chart 1: Evolution of Nerds and Stones values in D1, D2, D3 and D4.

Patient	D1	D2	D3	D4
C1	39	37	32	15
C2	32	23	12	---
C3	37	32	23	12

In none of the patients was there any hyperpigmentation of the post-injury tissue or any kind of sequelae.

Among the participants, patient C1 kept in touch with the team after being discharged from outpatient appointments and sent a photo recorded on his phone of how his scarring looked eight months after the accident. He expressed his gratitude to the team and shared a message about how ozone therapy had transformed his life; it contributed to his well-being and helped with pain control and the healing aspect. At the beginning of the treatment, after explaining the applicability of the therapy and clearing up his doubts, he didn't believe in its benefits and was surprised by the results obtained, the evolution of the wound, and the speed of healing.

The flowchart (Figure 2) shows the ozone treatment, including the evaluation performed by the mnemonic Nerds and Stones (Appendix A).

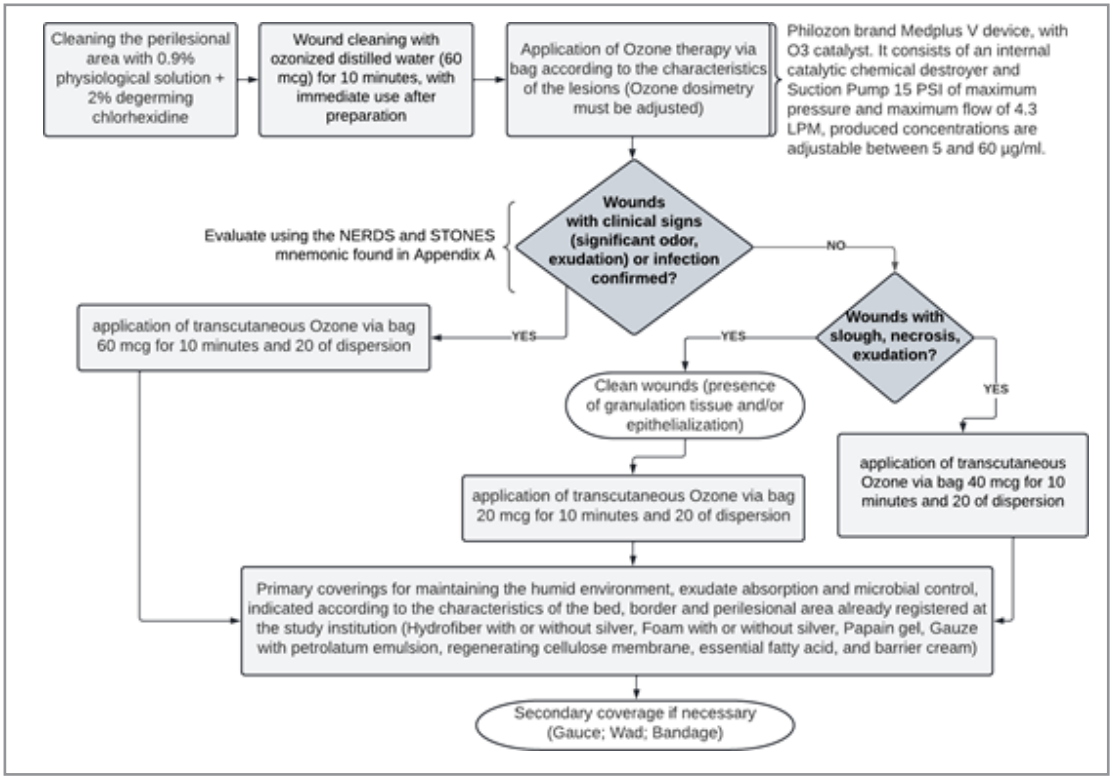

Figure 2: Ozone treatment flowchart.

Also, the covering used for treatment according to chronological order was elaborated for case 1 (Chart 2).

Chart 2: Covering used according to chronological order for case 1 treatment

Date	O3 dose	Primary covering	Secondary Covering	Perilesional area	Change frequency
19/05/21	60 mcg	Hydrofiber Dressing with Silver (AQUACEL® Ag)	Wad and bandage	Barrier cream	Every 3 days (secondary covering) or according to saturation
26/05/21	60 mcg	Hydrofiber Dressing with Silver (AQUACEL® Ag)	Wad and bandage	Barrier cream	Every 3 days (secondary covering) or according to saturation
02/06/21	60 mcg	Hydrofiber Dressing with Silver (AQUACEL® Ag) in malleolar region, other areas gauze with petrolatum	Gauze and bandage	Barrier cream	Every 3 days (secondary covering) or according to saturation
09/06/21	20 mcg	Foam with silver and adhesive border in the malleolar region; other areas gauze with petrolatum	Gauze and bandage	Barrier cream	Weekly
16/06/21	20 mcg	Moisturizer with almond oil in the epithelized area; other areas gauze with petrolatum	Gauze and bandage	-	Weekly
23/06/21	-	Moisturizer with almond oil in the epithelized area	-	-	Daily

The covering used for treatment according to chronological order was elaborated for case 2 (Chart 3).

Chart 3: Covering used according to chronological order for case 2 treatment

Date	O3 dose	Primary covering	Secondary Covering	Perilesional area	Change frequency
09/06/21	60 mcg	Left hand: PHMB gel and petrolatum gauze. Right hand: Hydrofiber Dressing with Silver (AQUACEL® Ag)	Gauze and bandage	Barrier cream	Left hand: Every 2 days or according to saturation. Right hand: Every 3 days (secondary covering) or according to saturation
16/06/21	40 mcg	Left hand: PHMB gel and petrolatum gauze. Right hand: Hydrofiber Dressing with Silver (AQUACEL® Ag)	Gauze and bandage	Barrier cream	Left hand: Every 2 days or according to saturation. Right hand: Every 3 days (secondary covering) or according to saturation
23/06/21	-	Moisturizer with almond oil in the epithelized area, in both hands	-	-	Daily

In addition, the covering used for treatment according to chronological order was elaborated for case 3 (Chart 4).

Chart 4: Covering used according to chronological order for case 3 treatment

Date	O3 dose	Primary Covering	Secondary Covering	Perilesional Area	Change Frequency
15/12/21	60 mcg	Face: Essential fatty acids. Left upper limb: Hydrofiber Dressing with Silver (AQUACEL® Ag)	Gauze, non adherent gauze, wad and bandage	Barrier cream; moisturizer cream with almond oil on intact areas	Face: Daily. Left upper limb: Every 3 days (secondary covering) or according to saturation
22/12/21	40 mcg	Face: Moisturizer cream with almond oil. Left upper limb: PHMB gel and petrolatum gauze.	Gauze and bandage	Barrier cream; moisturizer cream with almond oil on intact areas	Face: Daily. Left upper limb: Every 2 days (secondary covering) or according to saturation
29/12/21	-	Moisturizing cream	-	-	Daily
44682	-	Moisturizing cream	-	-	Daily

Discussion

The literature researched for this study showed that there are no studies on the use of ozone therapy via bag in burns in humans, which makes it difficult to compare and discuss the findings of this research.

Although it is not the same mechanism of action, there are studies on the use of ozonized oil in second-degree burns that show assertive results. In a non-randomized clinical trial, ozonated oil was used versus hyaluronic gel for 12 weeks, both of which showed a reduction in erythema, tension, itching, and burning sensation. There was no significant difference between the treatments, but the oil was more effective in preventing the appearance of tissue hyperpigmentation after injury [22]. A literature review study showed that ozonized oil prevents hyperpigmentation and reduces patients' pain complaints, demonstrating a low-cost alternative [23]. The results found are similar to those of the present study.

The inflammatory process is expected and necessary in the healing stage, but an exacerbated response, as occurs in burn victims, can interfere with tissue repair through the production of COX 2, prostaglandin E2, and pro-inflammatory cytokines. Tumor necrosis factor alpha (TNFα) acts on the nuclear transcription factor kappa B (NF-κB), which causes a cascade of biochemical effects leading to chronic inflammation, which contributes to the manifestation of pain since pain is multifactorial. In this sense, ozone has an anti-inflammatory action as it increases the production of Adenosine Triphosphate (ATP) in the mitochondria, improving oxidation and cell metabolism, increasing the production of nuclear factor erythroid 2-related factor 2 (Nrf2), acting to reduce TNFα, resulting in an analgesic effect and promoting more comfort and well-being for the patient [11,13, 24, 25].

Infection is one of the most recurrent complications and is considered responsible for the majority of deaths in burn patients. Due to the tissue damage caused by heat production, proteins are denatured, causing tissue devitalization. In the management of local treatment for burns, priority is given to cleaning the wound as soon as possible, as it is a potentially contaminated injury since the presence of devitalized tissue provides opportunities for the proliferation of microorganisms. It is advantageous to invest in therapies, such as medical ozone, which act in the prophylaxis and/or treatment of infections without causing microorganism resistance [7, 26, 27].

In the search for studies with high levels of scientific evidence, the first meta- analysis on ozone therapy for the management of chronic ulcers was published in China in 2010. It consisted of three randomized clinical trials with 212 participants comparing ozone therapy with any other intervention. One of them, with 101 participants, compared ozone treatment vs. antibiotic therapy over a period of 20 days, and it was observed that gas therapy was associated with a greater reduction in ulcer area over a shorter hospital stay [28].

Similarly, in a systematic review carried out in Australia, the authors included randomized clinical trials whose objective was to evaluate the benefits and harms of ozone therapy in managing chronic wounds. Nine studies with 453 patients were included, and it was found that in most of the studies analyzed, there was a significant improvement in wound healing. These results indicate the importance of ozone as a therapeutic option in the treatment of chronic wounds with high rates of improvement in shorter times [29].

Regarding the applicability of transcutaneous ozone by bag and nursing care, there are few clinical studies and care protocols for wound treatment, especially for burn injuries, which interferes with professional adherence and safety 8,10,26. The lack of scientific production may be multifactorial, but there is evidence that ozone therapy is a low-cost, easy-to-maintain, presumable, and conservative alternative, which has few side effects when used correctly; in addition to reflecting on patient recovery, it consequently reduces intervention rates, resulting in a reduction in public spending [11, 27].

Conclusion

Transcutaneous ozone therapy was effective as an adjuvant treatment in pain control, a significant reduction in wound size, and in the quality of healing of burn injuries.

There were no adverse events during treatment, and none of the participants progressed to infection; all had favorable outcomes. Furthermore, there were no sequelae which are common in burns, such as skin contractures, itching, and hypertrophic and/or keloid scars, which interfere with the patient's functional, aesthetic, and quality of life.

In view of this report, we encourage new research with high scientific evidence, which describes and analyzes the benefits and cost-effectiveness of ozone therapy in the treatment of people with burn wounds; also focus on protocols and the rehabilitation process that contribute to these findings, as there are gaps in knowledge that need to be better explored.

Highlights

- Ozone therapy could be a great adjuvant treatment for wounds caused by burns;
- Improvement of the pain relief after ozone therapy;
- Ozone therapy improves the healing, through the healing process and reducing pain.

Authorship Contributions

- The conception and design of the study, or acquisition of data, or analysis and interpretation of data: Girondi JBR, de Bem JG, Soares CF, Duarte FO.
- Drafting the article or revising it critically for important intellectual content: Girondi JBR, de Bem JG, Soares CF, Duarte FO, de Liz GM, Grespi LO, Lino MM, Castro LSEPW.
- Final approval of the version to be submitted: Girondi JBR, de Bem JG, Soares CF, Duarte FO, de Liz GM, Grespi LO, Lino MM, Castro LSEPW.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Nil.

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