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Original Article

Oxygen-ozone therapy for management of oral mucositis after hematopoietic stem cell transplantation: a quasi-experimental study*

Terapia oxigênio-ozônio para manejo da mucosite oral pós transplante de células-tronco hematopoéticas: estudo quase-experimental

Terapia oxigênio-ozônio para manejo da mucosite oral pós transplante de células-tronco hematopoéticas: estudo quase-experimental

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Abstract

Objective: to investigate the effect of Oxygen-Ozone Therapy (OOT) in the management of oral mucositis (OM) after Hematopoietic Stem Cell Transplantation (HSCT). **Method:** quasi-experimental study. Inclusion criteria: age over 7 years and OM Grade I. Seventeen patients participated and were allocated to the Control Group (n=8) or Intervention Group (n=9). The protocol consisted of mouthwashes with Water for Ozonated Injection, ozonation concentration of 56 µg/L and medicinal oxygen flow of 1/4L, for 15 minutes, twice a day. The OM grade, pain scores, positive blood cultures and length of hospital stay were assessed. Analysis using the Mann-Whitney U test. **Results:** in the Intervention Group, a predominance of Grade II OM was observed; the evolution of the OM grade and pain score was statistically lower with $p<0.05$, between days D+6 to D+11; it was also observed an average of 7.3 days less of length of stay. **Conclusion:** the effects of OOT on OM after HSCT are positive.
Descriptors: Stomatitis; Complementary Therapies; Hematopoietic Stem Cell Transplantation; Nursing; Oncology Nursing

Resumo

Objetivo: investigar o efeito da Terapia Oxigênio-Ozônio (TOO) no manejo da mucosite oral (MO) pós Transplante de Células-Tronco Hematopoéticas (TCTH). **Método:** estudo quase-experimental**.** Critérios de inclusão: idade superior a 7 anos e MO Grau I. Participaram 17 pacientes alocados no Grupo Controle (n=8) ou Grupo de Intervenção (n=9). O protocolo consistiu em bochechos com Água para Injeção Ozonizada, concentração de ozonização de 56 µg/L e fluxo de Oxigênio medicinal de 1/4L, durante 15 minutos, duas vezes ao dia. Avaliou-se o grau de MO, escores de dor, hemoculturas positivas e tempo de internamento. Análise com teste U de Mann-Whitney. **Resultados:** no Grupo Intervenção observou-se predomínio de MO Grau II; a evolução do grau

da MO e escore de dor foi estatisticamente menor com p<0,05, entre os dias D+6 ao D+11; média de 7,3 dias a menos de internação. **Conclusão:** os efeitos da TOO na MO no pós TCTH são positivos.
Descritores: Estomatite; Terapias Complementares; Transplante de Células-Tronco Hematopoéticas; Enfermagem; Enfermagem Oncológica

Resumen

Objetivo: investigar el efecto de la Terapia de Oxígeno-Ozono (TOO) en el manejo de la mucositis oral (MO) post Trasplante de Células Madre Hematopoyéticas (TCMH). **Método:** estudio cuasiexperimental. Criterios de inclusión: edad superior a 7 años y MO Grado I. Participaron 17 pacientes asignados al Grupo Control (n=8) o Grupo de Intervención (n=9). El protocolo consistió en enjuagues con Agua para Inyección Ozonizada, concentración de ozonización de 56 µg/L y flujo de Oxígeno medicinal de 1/4L, durante 15 minutos, dos veces al día. Se evaluó el grado de la MO, puntuaciones de dolor, hemocultivos positivos y tiempo de internación. Análisis con prueba U de Mann-Whitney. **Resultados:** en el Grupo Intervención se observó predominio de la MO Grado II; la evolución del grado de la MO y la puntuación del dolor fue estadísticamente menor con p<0,05, entre los días D+6 hasta D+11; media de 7,3 días menos de internación. **Conclusión:** los efectos de la TOO en la MO en el post TCMH son positivos.
Descriptores: Estomatitis; Terapias Complementarias; Transplante de Células Madres Hematopoyéticas; Enfermería; Enfermería Oncológica

Introduction

Oral mucositis (OM) is a highly frequent stomatological event that has a strong impact on the quality of life of patients undergoing Hematopoietic Stem Cell Transplantation (HSCT). It affects between 42% to 98% of individuals who receive high doses of cytostatic agents in the pre-transplant conditioning period.[1]

OM is characterized by damage mediated by Reactive Oxygen Species (ROS) and is the result of high oxidative stress caused by chemotherapeutic and radiotherapeutic agents. OM begins with a burning sensation in the oral cavity and, later, the lesion evolves into erythematous, erosive and inflammatory conditions with the formation of ulcers.[2] Extensive damage to the mucosa can be intensely painful, causing refractoriness to aggressive analgesic management. In severe OM, the patient suffers from confluent ulcers, which cause extreme pain and prevents oral intake, increasing the risk of local and systemic infection, as well as the cost of health resources.[1]

In HSCT, OM develops approximately three to four days after hematopoietic stem cell infusion. Between two and four days after the onset of symptoms, OM can progress to ulceration that lasts for seven to ten days and, as the neutrophilic increase occurs, which is progressive in the patient, OM presents spontaneous regression.[3]

For the prophylaxis and treatment of OM, the Guidelines of the Multinational Association of Supportive Care in Cancer (MASCC) and the International Society of Oral Oncology (ISOO), recommend: photobiomodulation (PBM) or intraoral low-level laser; cryotherapy; and the use of Keratinocyte growth factor (KGF-1) intravenously in patients with hematologic cancer who underwent the HSCT protocol with Total Body Irradiation (TBI).[4]

As a multifactorial event in its etiology, the prophylaxis and management of OM are still not clearly defined and there are variations in approaches between reference centers. Among the reasons for the limited preventive and therapeutic approaches are included: the availability of numerous accessible oral care products that interfere with the standardization of techniques; difficulty in implementing guidelines (especially in the pediatric setting); lack of consistency between the various existing guidelines; as well as the preference for outdated views that overlap with recent scientific evidence. Treatment is mostly palliative and supportive, with the objective of relieving symptoms, accelerating tissue repair and controlling any infections of oral origin. It also uses anesthetics, anti-inflammatory drugs, antiseptic mouthwashes and antimicrobial agents.[1,5]

There is a range of highly evidence-based scientific studies, particularly in the area of Dentistry, which reinforce the relevance of Oxygen (O_2) - Ozone (O_3) Therapy (OOT), also known as Ozone Therapy. This is a moderate pro-oxidative therapy, efficient for periodontal and oral treatments.[6-8] Regarding the applicability of OOT, in certain diseases, it is recorded that it can be combined with conventional therapies in a synergistic way, with great therapeutic potential, since in the pathophysiology oxidative, inflammatory and infectious processes are involved.[2,6-7]

Recently, it was discovered that expression loss of Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) upon radiation exposure sensitized the tongue epithelium of mice.[2,9] This result demonstrates that Nrf2 activation has the potential to prevent the development of OM. Experimental results demonstrated that Ozone (O_3) *ex vivo* or *in vivo* can activate Nrf2. Thus, this mechanism may explain the genomic target of O_3, including ozonated water, which induces the transcription of enzymatic

proteins such as Dismutase (SOD) and Catalase (Cat), actively providing protection against the harmful effects of free radicals in the pathological process of OM.[2,9]

It is worth highlighting that Ozone Therapy is increasingly being adopted and regulated worldwide. In 2020, according to the Madrid Declaration,[10] an international consensus document on good practices with the therapy, regulates its application routes and dosimetry, indications and contraindications, it is also regulated in other 13 countries. Approximately seven countries emphasized the institution of the practice for pain treatment, within the scope of public health, that is, made available by the government. It is worth mentioning, as a theoretical-scientific basis, that the *Declaración de Madrid sobre la Ozonioterapia*[10] is an official document of the International Scientific Committee on Ozone Therapy (ISCO3) and a global consensus, which unifies the application criteria in addition to standardizing the administration protocols of Ozone Therapy through different routes, including local and systemic, with demonstrated applicability for a significant number of diseases.

Currently, it is discussed in more than 50 countries, with an increase in the scope of the public sphere. In Brazil, Ozone Therapy has been part of the list of Integrative and Complementary Practices since 2018, through the Ordinance No. 702, of March 21, 2018,[11] which includes more practices in the *Política Nacional de Práticas Integrativas* (PNPIC), and it is important to develop research that proves its effects and contributions to the health status of individuals. The objective of the study was to investigate the effect of OOT on the management of OM after HSCT.

Method

This is a quasi-experimental study with allocation of participants into two groups, the Control Group, which received Standard Therapy (ST), and the Intervention Group that received OOT therapy. The clinical intervention was carried out in an open manner, without masking or blinding aimed at the researcher or the research participants, since the ozonated water has a characteristic odor, thus making it difficult to apply any masking strategy.

Quasi-experimental studies do not include all the characteristics of a true experiment and occur in the event of some impossibility of complete experimental

control. It is known that experimental and quasi-experimental study designs are the most suitable when the objective is to analyze a relation of cause and effect.[12] We highlight that it was not possible to develop a randomized clinical study due to the complexities of patients undergoing HSCT, such as the use of different drug classes (antibiotics, antifungals, corticosteroids, vitamins, among others); heterogeneous sampling; and due to the multifactorial etiology of OM.

OOT is widely used in Veterinary Medicine and Dentistry. In the latter, successes have been demonstrated with the management of wound healing, dental caries, oral lichen planus, gingivitis and periodontitis, halitosis, osteonecrosis of the jaw, postsurgical pain, plaque and biofilms, root canals, dentin hypersensitivity, temporomandibular joint disorders and tooth whitening.[13-15] Therefore, the articles that support water ozonation are supported by such professional scenarios.

A pre-clinical stage was established, in which the procedures were carried out in August 2020 in the industrial environment of the ozone generator used, in the city of São José dos Campos, São Paulo, Brazil. The set of resources organized to obtain ozonated solutions in the necessary concentrations were as follows: medical oxygen cylinder; flow control valve with pressure gauge; ozone generating device (ANVISA Registration No. 8150910000, OZONE & LIFE®, model O&L1.5 RM); liquid ozonizing tower (Ozone & Life®); Kit-7423 (*Vacu-vials*®); and Spectrophotometer. The following solutions were tested: sterile water injection (SWI); non-sterile monodistilled water; non-sterile double-distilled water; and 0.9% saline solution (SS). Respecting the solubilization of ozone gas in an aqueous medium through the low flow of oxygen (¼ flow).[16]

To standardize the procedure, a volume of 500 mL was established, ensuring that it was compatible with different brands of ozonizing tower, thus ensuring the reproducibility of the process. The ozonation period of all tested solutions was measured with a spectrophotometer at different times (05, 10, 15, 20, 25 and 30 minutes). From the standardized volume of 500 mL of SWI, ozonation for the quasi-

experimental study applied an Ozone (O_3) concentration of 56 µg/mL, at ¼ flow of Medical Oxygen (O_2), for 15 minutes. It should be noted that the location did not allow the control of ambient temperature, which, during all tests, varied between 19°C and 30°C. Under these conditions, it was reached a final concentration of 9.94 µg/mL of saturated Ozone in SWI.[16]

This research was conducted in the hospitalization unit of the Serviço de Transplante de Medula Óssea (STMO), of the Hospital de Clínicas Complex, at the Universidade Federal do Paraná (CHC/UFPR), which is an international reference for this type of treatment.

The project was approved by the Research Ethics Committee of the aforementioned Hospital, on May 10 of 2020, under Opinion No. 4,018,509. As for the research participants, they were approached at the time of admission or in the days that followed. The adult Informed Consent Form (TCLE), the legal guardian TCLE, and the Informed Assent Form (TALE) were read together with the patients and their respective legal representatives. The TALE was adopted with accessible language for minors or legally incompetent patients. After reading the informed consent form, adult patients and thee legal guardians for children participating in the study signed the terms. All patients admitted to the STMO of CHC/UFPR for HSCT who were eligible and agreed to participate were randomized.

The data collection period was from September 17 of 2020 to April 8 of 2021.

Daily oral assessment was performed from D+1 for all patients, and the application of the mouthwash with OOT began only when OM Grade I was present. This conduct is justified by the fact that the onset of OM is multifactorial, and each patient may manifest it on different days. In addition to the fact that the study was based on the assessment of OM management, the presence of OM was necessary for the application of OOT. Therefore, justifying the standardization of the start of OOT from OM Grade I.

The inclusion of patients was developed according to the following criteria: age over 7; undergoing HSCT; and with established OM (Grade I). The exclusion

criteria were patients with glucose-6-phosphate dehydrogenase (G-6-PD) deficiency; toxic hyperthyroidism; severe coagulation disorders; acute and massive hemorrhage; severe cardiovascular instability; acute alcohol poisoning; acute myocardial infarction; during a convulsive state; hemochromatosis; patients with recent treatment with iron and copper; and participants in another ongoing research study at the service.

Patients who met the criteria for inclusion in the study and signed the TCLE and/or TALE form proceeded to the allocation stage. Thus, participants were allocated, according to the order of admission date, to the Intervention Group (IG) or Control Group (CG). The allocation sequence, enrollment of research participants, and assignment of the OOT protocol to study participants were performed by the main researcher.

Regarding the treatment received by patients, in the CG, the Standard Treatment (ST), offered by the hospital service was maintained exclusively, which consists of oral hygiene (using toothpaste and alcoholic chlorhexidine) and photobiomodulation (PBM). The application of PBM, in accordance with the local protocol, was performed by the unit's dentist and/or residents of Oncology Dentistry. A wavelength of 660 nm, 1 joule/cm^2 was applied for 5 minutes, once a day, for five days each week, from Monday to Friday. The ST began on the day after the hematopoietic stem cell infusion (D+1) and continued from the onset of OM until its complete regression.

The oral mucosa in the IG was evaluated from the day after the hematopoietic stem cell infusion (D+1). In view of the finding OM, the participants received ST, as part of the service routine, and mouthwash or rinsing with ozonated Water for Injection (WFI), called OOT, was added twice a day, until complete regression of the lesion. Regarding the volume of the rinse, it is empirically clear that younger children have difficulty rinsing with a volume greater than 50ml; and that rinsing lasting 1 minute and 30 seconds (1'30"), performed with 100ml, is not

easily accepted by adults. Thus, the volume of the mouthwash was standardized at 80 ml for patients aged 13 and over, and 50 ml for patients up to 12 years of age. The time taken to perform the mouthwash was timed at 1'30" for all applications. After the mouthwashes began, they were performed twice a day, every day during the period of presence of the OM.

The WFI was ozonated in the data collection field, and as soon as the ozonation time was completed, it was immediately offered to the research participants. A tolerance of 20 minutes of WFI availability was adopted. If it was not used, a new WFI was ozonated. The WFI was purchased by the principal investigator in sealed boxes and stored in a locked cabinet, together with the Ozone Generator and Ozonizing Tower, both owned by the researcher, and remained in the hospital throughout the data collection period. At each round of ozonation, a new WFI unit was opened after the sterile packaging was removed.

Data collection was performed daily for all participants in the IG and CG, from the day after transplantation (D+1...) until complete regression of OM. In this sense, conditioning for HSCT begins between 3 and 7 days before the day of hematopoietic stem cell infusion, depending on the chemotherapy protocol. The days prior to the day of infusion are recorded as negative in decreasing order, D-7 to D-1, for example. The day of the infusion, of the transplant itself, is considered day zero (D0). And, from the day after the cell infusion, the counting of days in time is positive and increasing (D+1, D+2, D+3, ...).

A rigorous and systematic evaluation of the oral cavity was performed daily in order to observe the sequence of inflammatory and biological events related to OM, namely: the palate, the buccal mucosa, the side of the tongue, the tongue and the floor of the mouth. All information was duly recorded during the duration of the research, in a specific instrument for data collection.

For assessing the OM grade, the scale recommended by the World Health Organization (WHO)[17] was used, which is widely used and easy to understand, namely: Grade I - there is desquamation associated or not with erythema and pain; Grade II - there are ulcerations with or without erythema and the patient is able to ingest solid foods; Grade III - there is a predominance of ulcerations with or without

extensive erythema and the patient is able to ingest only liquids; Grade IV - confluent ulcerations and oral feeding is impossible. Daily assessments and records of the presence, severity and duration of OM were developed.

In order to help research participants identify, express and measure pain, as well as take steps regarding clinical pharmacological measures for its relief, complaints were measured using the Visual Analogue Scale (VAS)[18] and the Visual Numeric Scale (VNS).[18] A question was added to this assessment to clarify whether the pain was located in the oral cavity and whether or not dysphagia was present.

Data on blood cultures were collected from the electronic medical records of research participants, since it is routine for the research sector to investigate microorganisms when a patient has a fever. Blood cultures are valid for 48 hours and, if the fever persists, a new blood culture is collected.

The study population consisted of 23 patients hospitalized for HSCT. There were six losses to follow-up, the reasons were: referral to the Intensive Care Unit (ICU) followed by death (n=1); hemodynamic instability (n=1); insufficient amount of mouthwash (n=1); and non-adaptation to the 'taste' of ozonated water (n=03). Nine patients were allocated to the Intervention Group (IG) and eight patients to the Control Group (CG) (Figure 1).

It should be noted that the following formula was used to estimate the sample calculation: $n = N.Z2.p.(1-p) \,/\, Z2.p.(1-p) + e2.N-1$. Where: n - calculated sample; N - population; Z - normal variable; p - real probability of the event; e - sampling error. To estimate the calculation of the study population, data from 2017 to 2019 were used, which obtained an annual average of 51 treated patients, in the age range of 8 to 24 years, with a sampling error of 0.05%, 95% confidence level and maximum frequency of 3%, thus concluding that each group (study and control) would be composed of 25 patients, for a Randomized Clinical Trial.

However, the course of the Coronavirus Disease – 2019 (COVID-19) pandemic during the data collection period was decisive for the reduction of the sample size of this study. Having performed an analysis of the effect size in the main variables, using the formula $r = z \div \sqrt{N}$, where "r" is the observed effect size, "z" is the parameter calculated by the Mann-Whitney test and "N" is the analyzed sample quantity, totaling 17 patients in total, IG + CG, the method became a quasi-experimental study due to the reduction in the sample quantity.

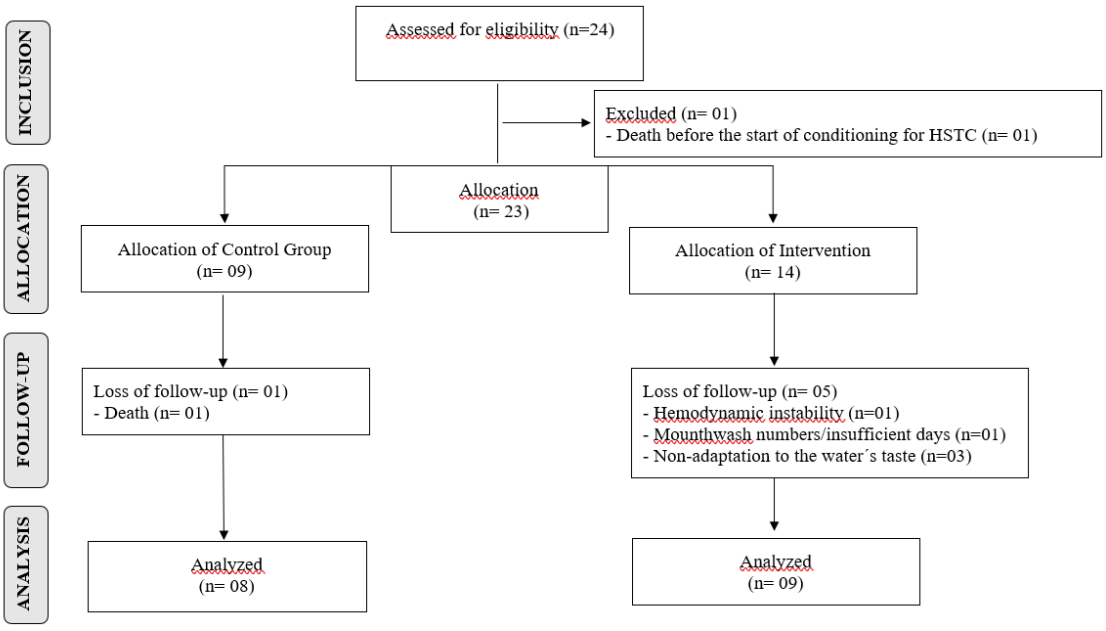
The data resulting from the research were tabulated in Microsoft Excel® spreadsheets. Descriptive and statistical analysis was then performed with the support of the *SPSS Statistics 25 IBM®* program.[19] For descriptive analysis, absolute and proportional frequencies of the qualitative variables were determined.

Continuous variables with symmetrical distribution were subjected to measures of central tendency and dispersion, which were expressed as means and standard deviation (mean + SD). For variables with asymmetrical distribution, medians were used, assigning minimum and maximum values.

In the univariate analysis, the t-test was applied to continuous variables. Variables with asymmetric distribution and ordinal variables were analyzed with the nonparametric Mann-Whitney U test. To reinforce the Mann-Whitney U test data, the effect size was calculated.[20]

This research was based on and used the flow diagram and checklist described in the guidelines recommended by the Consolidated Standards of Reporting Trials (CONSORT).[21]

Figure 1 – Flow of inclusion, randomization and analysis of the Intervention and Control Groups, Curitiba, Paraná, Brazil, 2023



Source: Adaptation of Consolidated Standards of Reporting Trials Group.[21]

The main analyzed outcome was the resolution of OM. This was verified by the reduction of symptoms (in particular, the reduction of pain scores) and, with the faster recovery time, indicating lower grades of OM after HSCT, considering the OM classification criteria recommended by the WHO.[17]

Results

The flowchart (Figure 1) shows the inclusions (n=23), as well as the losses (n=6) due to follow-up, discontinuities - five (5) of which were in the IG, as well as the total number of final participants in each group. It is worth noting that one loss was due to the patient's hemodynamic instability, caused by severe sepsis, associated with torpor, which made it impossible to follow the research protocol for more than three consecutive days.

In the CG, there was one loss to follow-up due to referral to the ICU, followed by death. Only one eligible participant was excluded before randomization due to death from Invasive Aspergillosis, even before the start of conditioning for HSCT. Thus, the study population consisted of 23 patients hospitalized for HSCT, of which there were six (6) losses to follow-up. In this quasi-experimental research, 17 participants were analyzed, nine (9) allocated to the IG and eight (8) allocated to the CG.

Males were predominant in both the CG, with n=6 (75%), and in the IG, with n=7 (77.7%), totaling 76.4% of the total sample (n=17). The predominant age group was young adults in the CG and adults in the IG. As for the race/ethnicity variable, there was a higher frequency of whites (n=9) in both groups (Table 1).

Table 1 – Characterization of the sample of participants (CG+IG), Curitiba, Paraná, Brazil, 2023

Variables	CG (n=8)		IG (n=9)		Total (n=17)	
	n	(%)	n	(%)	n	(%)
Sex						
Male	6	75	7	77.8	13	76.4
Female	2	25	2	22.2	4	23.6
Age						
Children (≤12)	0	-	1	11.2	1	5.9
Teenagers (>12 to ≤18)	1	12.5	0	-	1	5.9
Young adults (>18 to ≤24)	4	50	2	22.2	6	35.3
Adults (>24 to ≤60)	2	25	4	44.4	6	35.3
Seniors (>60)	1	12.5	2	22.2	3	17.6
Race/Ethnicity						
Yellow	0	-	0	-	0	-
White	5	62.5	4	44.4	9	53
Indigenous	0	-	0	-	0	-
Mixed	1	12.5	4	44.4	5	29.4
Black	2	25	1	11.2	3	17.6
Diagnosis						
Congenital disease	0	-	2	22.2	2	11.8
Non-malignant hematologic disease	2	25	1	11.1	3	17.7
Malignant hematologic disease	6	75	6	66.7	12	70.5
Type of HSCT						
Autologous	2	25	4	44.4	6	35.3
Allogeneic						
• Matched Related	1	12.5	1	11.1	2	11.7
• Haploidentical	2	25	3	33.4	5	29.5
• Unrelated	3	37.5	1	11.1	4	23.5
Comorbidities						
Yes	2	25	3	33.3	5	29.5
No	6	75	6	66.7	12	70.5

Caption: IG – Intervention group; CG – Control group

There was a predominance of malignant hematologic diseases in both groups, CG (75.0%) and IG (66.7%), respectively. Regarding the type of transplant, autologous was the most frequent (35.2%), followed in decreasing order by haploidentical transplant (29.4%), unrelated transplant (23.5%) and compatible related transplant (11.7%).

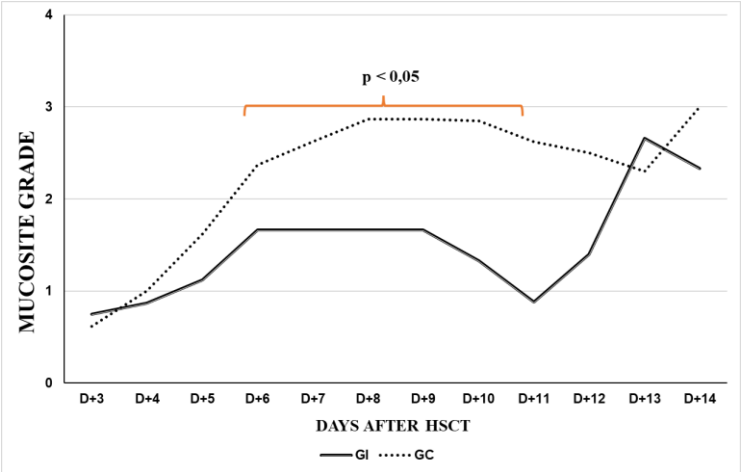
The related comorbidities were: Systemic Arterial Hypertension (n=2); Chronic Obstructive Pulmonary Disease (n=1); Smoking (n=1); and Kallmann Syndrome (n=1). Comorbidities were present in 29.5% of the total sample, being frequent in 25.0% of the CG sample and 33.3% of the IG.

The most severe grades of OM, Grade III and Grade IV, were more frequent in the CG. Grade III was present in 3 participants (37.5%) and Grade IV was also present in 3 participants (37.5%). While in the IG, there was a predominance of Grade II OM (n=6; 66.6%), according to the WHO classification.[13]

On average, 20 days of the CG and 20 days of the IG were analyzed, which referred to the period related to the concentration validity of the OM, in both groups. After the 20th day, few participants showed persistence of OM, not demonstrating that there is a viability of statistical analysis of the data after D+20.

The Mann-Whitney U test showed that there was a statistically significant difference (p= 0.042) between the CG and the IG as for the OM grade (Figure 2), between days D+6 to D+11, in which the IG showed less OM evolution, when compared to the CG.

Figure 2 – IG and CG participants according to the average grade of OM from D+3 to D+14 post-HSCT, Curitiba, Paraná, Brazil, 2023

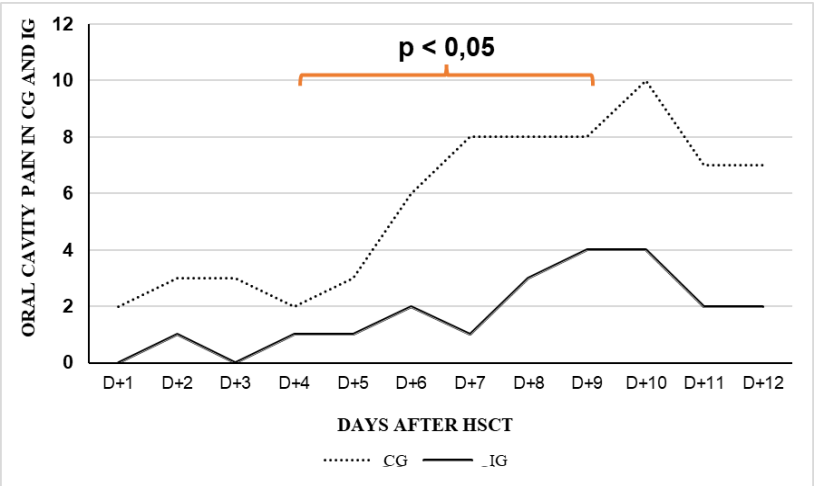


Caption: OM – Oral Mucositis; IG – Intervention Group; CG - Control Group; HSCT – Hematopoietic Stem Cell Transplantation

The Mann-Whitney U test showed that there was a statistically significant difference between the CG and IG, with p = 0.005, related to the pain assessment by VAS or VNS, only on D+11.

The variable pain in the oral cavity presented similar behavior to the OM grade (Figure 3), with statistically significant results, between the 6th and 11th day, after HSCT: D+6 (p = 0.010); D+7 (p < 0.001); D+8 (p= 0.003); D+9 (p= 0.005); D+10 (p= 0.028); and D+11 (p= 0.025).

Figure 3 - Frequency of participants in IG and CG according to complaints of oral cavity pain, Curitiba, Paraná, Brazil, 2023



Caption: IG – Intervention Group; CG - Control Group; HSCT – Hematopoietic Stem Cell Transplantation

Based on Choen's criteria,[19] the effect size (Table 2) observed for the variables OM grade, pain score (VNS/VAS) and pain in the oral cavity was calculated, corroborating the statistical significance demonstrated in the p value.

Table 2 – Significance values (p-value) and effect size (r) of the intervention in the analyzed variables, Curitiba, Paraná, Brazil, 2023

Variable	P Value	Parameter: Mann-Whitney Test (z)	Size of the observed effect (z)
OM grade			
D+6	0.039	-2,062	0.50
D+7	0.009	-2,602	0.63
D+8	0.007	-2,676	0.65
D+9	0.010	-2,578	0.63
D+10	0.018	-2,367	0.57
D+11	0.042	-2,032	0.49
Pain *Score* (VNS/VAS)			
D+11	0.005	-2,817	0.68
Oral Cavity Pain			
D+6	0.010	-2,592	0.49
D+7	< 0.001	-4,000	0.97
D+8	0.003	-3,000	0.73
D+9	0.005	-2,785	0.68
D+10	0.028	-2,197	0.53
D+11	0.025	-2,245	0.54

Caption: r= 0.1 (small effect), r= 0.3 (medium effect) and r= 0.5 (large effect), r is the size of the observed effect, z is the parameter calculated by the Mann-Whitney test

For the analysis of bacterial translocation, data were collected from blood cultures and the bacteria identified were: *Klebsiella Pneumoniae Carbapenemase* (KPC) (n=1); *Cutibacterium acnes* (n=1); *Staphylococcus epidermidis* (n=1); *Pseudomonas aeruginosa* (n=1); Gram-positive cocci of unidentified species (n=1); Bacillus *cereus* (n=1); and *Streptococcus viridans* (n=1).

The CG (n=8) presented a relative frequency of 37.5% of positive blood cultures, while in the IG (n=9) this percentage was 55.5%. Although the frequency of positive blood cultures was higher in the IG, among the identified bacteria, was the *Streptococcus viridans*, which is part of the indigenous oral and gastrointestinal tract microbiota and was identified in 12.5% of the CG participants, which suggested the breakdown of the oral mucosa barrier with the occurrence of bacterial translocation into the bloodstream.

The variable related to length of stay days showed that the IG had on average 7.3 fewer days in total hospitalization, when compared to the CG. Regarding the median hospitalization days, the CG had 5 more days, totaling 32 days, when compared to the IG (27 days).

Discussion

The OM grade between days D+6 to D+11 (p= 0.042), in which the IG showed less OM evolution, when compared to the CG, corroborates a study in which rats that had physical lesions in the oral mucosa were treated with OOT, with a volume of 10 ml and a concentration of 56 µg/mL per session. Immediately after the surgical procedure, the CG and IG showed open and bloody wounds, with less bleeding in the OOT group. On D+3, the wound in the IG was almost completely healed, while in the CG the wound still showed bleeding points. After seven days, the wounds in both groups were completely healed and a better macroscopic appearance was presented by the IG, whereas in the CG there was still an area with erythema in the scar.[22]

Similarly, in a Japanese study with three arms in an animal model, after the induction of OM, the mouths of the animals in the IG were rinsed four times a day (every 6 h) with saline solution in the first group, and with ozonated saline solution (with a final concentration of 1.5 ug/mL and applied with a pressure of 4.0 kgf/cm2) in the second group, while the mouths of the CG were left untouched. A significant difference was observed in relation to the severity of OM between the CG (without treatment) and the other Groups, on days D+11 and D+16; the group treated with ozonated saline solution presented less severe lesions when compared to the group that received 0.9% saline solution and the CG.[23]

An Italian Randomized Clinical Trial, in which the efficacy of ozonated water for the treatment of erosive Oral Lichen Planus was assessed, patients received a mouth rinse twice a week for 1 minute and repeated four times a day for four consecutive weeks, totaling eight applications. It was concluded that patients treated with OOT had lower pain scores ($p < 0.05$) and a better treatment efficacy index at all observed times.[24]

An Iranian study that assessed the use of ozonated water in patients with OM and pain induced by head and neck radiotherapy indicated that the group not treated with OOT presented higher degrees of OM and greater intensity of pain related to OM.[25]

It is suggested that the high incidence of microbial infections may also be related to OM during the severe neutropenia phase, since the depletion of circulating neutrophils favors bacterial multiplication. The discontinuity of the oral mucosa is a gateway for several pathogens, so that the immune system becomes vulnerable to failure in protecting against the action of microorganisms.[26]

The presence of *Streptococcus viridans* in a blood culture from a central catheter in a post-HSCT patient is in line with the consulted literature. It is indicated that, in recent decades, gram-positive microorganisms have become the dominant etiologies in the scenario of bacteremia, responsible for up to 70% of bacteremias in patients with severe neutropenia (characterized by neutrophils with values below 500/μL). The translocation of these bacteria from the oral mucosa to the bloodstream and to the heart muscles can lead to septic shock and infective endocarditis, respectively.[27]

The high incidence of microbial infections during the severe neutropenia phase may also be related to OM, since the depletion of circulating neutrophils favors bacterial multiplication. And, with the discontinuity of the oral mucosa, an entry point for several pathogens is established and the organism becomes susceptible, as it is unable to protect itself from these pathological microorganisms.[28]

The critical colonization in OM, Chemotherapy-induced, occurs for two reasons. The first is the presence of a large number of bacteria in the mouth, in addition to the fact that the mucosa is constantly covered by mucus. The second is that, in OM, the surfaces of ulcerated lesions are covered by necrotic material, providing fertile ground

for bacterial proliferation. It is believed due to the fact that the ulcer surface is covered by mucus and necrotic material, it hinders the action of pharmaceutical agents in the treatment of OM.[23]

Convergent with this line of reasoning, mouthwash with ozonated WFI, which has bactericidal action, can provide conditions to minimize the number of bacterial colony-forming units as well as the mechanical removal of necrotic material.[23] The research demonstrated that 12.5% of patients who did not rinse their mouths with ozonated WFI had positive blood cultures for *Streptococcus viridans*.

It is pertinent to emphasize that, according to evidence produced by scientific research, bacterial bloodstream infections continue to be a significant cause of mortality and morbidity in patients with neutropenia. Approximately 58% of septic episodes in these patients are caused by gram-positive bacteria. High-dose chemotherapy, such as that used in HSCT, followed by OM, and gastrointestinal mucositis are predictive factors for severe infections by *Streptococcus viridans*.[28]

In a Japanese study, it was concluded that the reduction in bacterial count was more pronounced in the group of rats that received oral cavity rinsing with ozonated water, as it reduced the bacterial count and encouraged healing of the OM.[23]

OM is associated with increased use of various resources such as additional consultations and prolonged hospitalizations, resulting in a substantial increase in health care expenditures. It is worth noting that the patient/family binomial and the health system experience economic pressures during the treatment period.[29]

Thus, the budget allocated for the management of individuals with OM generally includes expenditures for prolonged hospitalization, emergency department visits, hospital admission, increased number of clinical and nutritional consultations, use of total parenteral nutrition, use of feeding tube or gastrostomy tube, use of analgesics and opioids, and use of oral or intravenous antibiotics.[29]

During the pandemic, the increased flow of rare genetic diseases in children to the research setting service impacted its sampling, considering in particular that the majority of children undergoing HSCT were under 2 years of age and, therefore, not

eligible for the research. These children remained hospitalized for long periods due to complications related to comorbidities and the HSCT itself, with a consequent outcome of reduced bed flow.

Furthermore, considering the characteristics of the study site, which treats rare and serious cases of genetic and hematological diseases, and because it is an international reference transplant center, the sample of this study was heterogeneous in terms of age, diagnosis, conditioning and type of transplant performed. Finally, blinding the study was not possible for either the researcher or the research participants, since ozonated water has a characteristic odor and taste, which makes the masking technique unfavorable.

Conclusion

The objective of the research was achieved, demonstrating in the investigation process that OOT, with Ozonized WFI, according to the presented protocol, added to the standard treatment of the service (oral hygiene with toothpaste and alcoholic chlorhexidine and PBM) presented positive and faster effects in the management of OM in the IG, which was composed of people in the post-hematopoietic stem cell transplant period, of a reference service, in the capital of the Paraná state, in the southern region of Brazil.

In general, in the IG there was less intensity of symptoms related to pain scores; lower OM grades; and faster recovery when compared to the CG. The length of hospital stay after HSCT was, on average, 7.3 days shorter in the IG.

With the application of OOT added to standard treatment, it is possible to achieve a lesser length of stay of patients, a fact that points both to a reduction in health costs and to the protection of various aspects of family stress, and also favors greater turnover of beds available for HSCT, avoiding long queues that generate greater suffering for the patient and family, in addition to pressure on the sphere of service management.

The antimicrobial action of ozonated water is suggested for the management of OM, showing that there was a positive blood culture for *Streptococci viridans*

only in the CG.

The findings confirm the legitimacy of ozonated WFI for the treatment of OM as an assistive technology and encourage the development of new technologies for the management of this condition. Finally, it is essential that, increasingly, through robust and compelling research, the scientific community recognizes OOT as proven scientific knowledge with strong evidence.

Contributing to this desired scenario, it is recommended to conduct studies with methodological refinement applied to OOT, with results that promote demystification before the scientific society, as well as its consolidation as applied science from the perspective of integrative and complementary care, especially in the Brazilian scenario.

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