UNIVERSIDADE DE PASSO FUNDO

Leonardo Saraiva

EFEITO DA ÁGUA OZONIZADA NA HALITOSE E NOS PARÂMETROS CLÍNICOS DE PACIENTES COM PERIODONTITE: UM ENSAIO CLÍNICO RANDOMIZADO

Passo Fundo

2024

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EFEITO DA ÁGUA OZONIZADA NA HALITOSE E NOS PARÂMETROS CLÍNICOS DE PACIENTES COM PERIODONTITE: UM ENSAIO CLÍNICO RANDOMIZADO

Tese apresentada ao Programa de Pós-Graduação em Odontologia da Faculdade de Odontologia da UPF, para obtenção do título de Doutor em Odontologia – Área de Concentração em Clínica Odontológica, sob orientação do prof. Dr. **Fernando Fornari** e co-orientação do profa. Dra. **Micheline Sandini Trentin**.

Passo Fundo

2024

ATA CPG 177/2024 DA DEFESA DE TESE DE DOUTORADO DA CANDIDATA, LEONARDO SARAIVA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA – ÁREA DE CONCENTRAÇÃO EM CLÍNICA ODONTOLÓGICA DA UNIVERSIDADE DE PASSO FUNDO.

Aos vinte e três dias do mês de abril de dois mil e vinte e quatro, as 14h, no formato on-line, com o suporte do Curso de Odontologia da Universidade de Passo Fundo, sob a Presidência do Dr. Fernando Fomari em Sessão Pública, reuniu-se a Comissão Examinadora da Defesa de Tese de Doutorado do (a) candidato (a) **Leonardo Saraiva**, do Programa de Pós-Graduação em Odontologia - área de Concentração em Clínica Odontológica, constituída pelos Doutores: Fernando Fomari (presidente), Charise Dallazem Bertol (PPGEH), Leticia Stefenon (ATITUS), João Paulo De Carli (UPF), homologada pelo Conselho do Programa de Pós-Graduação em Odontologia. Iniciados os trabalhos, a presidência deu conhecimento aos membros da Comissão e ao candidato (a) das normas que regem a defesa de tese de doutorado e definiu a ordem a ser seguida pelos examinadores para a argüição; a seguir, o (a) candidato (a) passou a apresentação e defesa de sua tese "Efeito da água ozonizada na halitose e nos parâmetros clínicos de pacientes com doença periodontal: um estudo clínico randomizado". Encerrada a defesa, a avaliação foi a seguinte: Dr. Fernando Fomari aprovado; Dr. Charise Dallazem Bertol aprovado; Dr. Leticia Stefenon aprovado; João Paul De Carli Dr. aprovado; tendo sido o (a) candidato (a) **APROVADO**, fazendo jus ao título de "Doutor em Odontologia - Área de Concentração em Clínica Odontológica". Para estar em dia com as obrigações perante o curso, o (a) doutorando (a) terá o prazo de quarenta e cinco dias, a partir desta data, para entregar a tese de doutorado, com as alterações sugeridas pelos membros da Comissão Examinadora. Nada mais havendo a tratar, lavrou-se a presente ata, que vai assinada pelos Membros da Comissão Examinadora e pelo Coordenador do Programa de Pós-Graduação em Odontologia da Universidade de Passo Fundo, Prof. Dr. Álvaro Della Bona.

Prof. Dr. Fernando Fomari
(orientador)

Profa. Dr. Charise Dallazem Bertol
(PPGEH)

Prof. Dr. Leticia Stefenon
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Prof. Dr. João Paulo De Carli
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BIOGRAFIA DO AUTOR

Leonardo Saraiva, nascido em 11 de abril de 1990 em Passo Fundo, RS. Graduado em Odontologia pela Faculdade Especializada na Área de Saúde do Rio Grande do Sul (FASURGS), em 04 de março de 2017. Especialista em Saúde Coletiva com ênfase em saúde da Família e Comunidade pela Universidade do Oeste do Paraná (UNOPAR), Passo Fundo, em 02 de dezembro de 2019. Mestre em Envelhecimento Humano, pela Universidade de Passo Fundo (UPF), Passo Fundo, em 13 de agosto de 2020. Especialista em Periodontia pelo Instituto HD, Passo Fundo, em 15 de novembro de 2023. Em março de 2021 foi selecionado para o curso de Doutorado em Odontologia com ênfase em Clínica Odontológica do Programa de Pós-Graduação em Odontologia da Universidade de Passo Fundo. Desenvolve atividades como bolsista e pesquisador pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), com ênfase em ensaios clínicos e pesquisas epidemiológicas.

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A Deus!

À minha família! pelo apoio em todos os momentos da minha vida.

Ao meu orientador, Fernando Fornari. Serei eternamente grato pela oportunidade que me concedeu, pela dedicação e por todos os ensinamentos e compreensão diante das adversidades acadêmicas. ´

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LISTA DE ABREVIATURAS

(CH3)2S	Sulfato de Dimetilo
CAL	Nível de fixação clínica
CH3SH	Metil Mercaptano
CHX	Clorexidina
CSVs	Compostos Sulforados Voláteis
H2S	Sulfeto de Hidrogênio
IPV	Índice de placa visível
ISG	Índice de sangramento gengival
NBW3	Nanobolhas de água ozinizada
NG	Nível gengival
NIC	Nível de inserção clínica
ppb	Partes por bilhão
PS	Profundidades de sondagem
PS4	Profundidade de sondagem maior que 4 milímetros
RAR	Raspagem e alisamento radicular
SRP	Raspagem e planejamento radicular
SS	Sangramento à sondagem
TCLE	Termo de Consentimento Livre e Esclarecido

RESUMO[1]

A água ozonizada combinada ao tratamento convencional de periodontite pode trazer benefícios aos pacientes com halitose relacionada à Periodontite. O objetivo deste estudo foi avaliar o efeito da água ozonizada na halitose relacionada à periodontite através de um ensaio clínico randomizado e cegado de fase 2. Além disso, uma revisão sistemática com metanálise sobre o efeito da água ozonizada na Periodontite foi realizada. Vinte pacientes com halitose oral e doença periodontal foram alocados em dois grupos de tratamento. No grupo ozônio utilizou-se tratamento convencional de raspagem e alisamento radicular (RAR) mais água ozonizada (n = 10). No grupo placebo utilizou-se tratamento convencional (RAR) mais água destilada (n = 10). Ambos os grupos foram tratados por 30 dias com auxílio de curetas e equipamento ultrassônico, aplicados duas vezes por semana. Os desfechos foram mensurados 7 dias depois da última sessão de tratamento. A halitose foi medida com teste organoléptico e halímetro. Os parâmetros da Periodontite foram analisados através do índice de placa visível (IPV, %); índice de sangramento gengival (ISG, %); profundidade de sondagem ≥4 mm (PS4, %),

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sangramento à sondagem (SS, %), nível gengival (NG, mm), profundidade de sondagem (PS, mm), e nível de inserção clínica (NIC, mm). Resultados: Os pacientes tratados com água ozonizada apresentaram redução da halitose medida por teste organoléptico em comparação ao placebo (P = 0,027) e no halímetro (< 75 ppb: 100% vs. 60) (P = 0,086). A maioria dos parâmetros de Periodontite apresentou melhora superior com ozônio do que com placebo: IPV (P < 0,001), ISG (P = 0,002), PS4 (P = 0,016), SS (P < 0,001), NG (P = 0,008), e NIC (P = 0,002). Conclusão: O uso da água ozonizada se mostrou eficaz no controle da halitose oral, bem como nos parâmetros clínicos da periodontite em comparação ao grupo placebo. Na revisão sistemática, o estudo sugere que o uso de água ozonizada como adjuvante no tratamento da doença periodontal não cirúrgica reduz os índices periodontais em comparação aos grupos controle (água, água destilada e clorexidina).

Palavras-chave: Halitose. Periodontite. Ozonioterapia.

ABSTRACT[2]

Ozonated water combined with conventional periodontitis treatment can bring benefits to patients with halitosis related to periodontal disease (PD). The aim of this study was to evaluate the effect of ozonated water on periodontitis-related halitosis through a randomized, blinded phase 2 clinical trial. In addition, a systematic review with meta-analysis on the effect of ozonated water on PD was carried out. Twenty patients with oral halitosis and periodontal disease were allocated to two treatment groups. The ozone group used conventional scaling and root planing (SRP) plus ozonized water (n = 10). The placebo group used conventional treatment (SRP) plus distilled water (n = 10). Both groups were treated for 30 days using curettes and ultrasonic equipment, applied twice a week. Outcomes were measured 7 days after the last treatment session. Halitosis was measured using an organoleptic test and a halimeter. PD parameters were analyzed using the visible plaque index (IPV, %); gingival bleeding index (ISG, %); probing depth ≥4 mm (PS4, %), bleeding on probing (SS, %), gingival level (NG, mm), probing depth (PS, mm), and clinical attachment level

[2] Effect of Ozonized Water on Halitosis And Clinical Parameters of Patients with Periodontal Disease: A Randomized Clinical Trial.

(CAL, mm). Results: Patients treated with ozonated water showed a reduction in halitosis measured by organoleptic test compared to placebo (P = 0.027) and in the halimeter (< 75 ppb: 100% vs. 60) (P = 0.086). Most PD parameters showed greater improvement with ozone than with placebo: IPV (P < 0.001), ISG (P = 0.002), PS4 (P = 0.016), SS (P < 0.001), NG (P = 0.008), and CAL (P = 0.002). Conclusion: The use of ozonated water proved to be effective in controlling oral halitosis, as well as in the clinical parameters of periodontitis compared to the placebo group. In the systematic review, the study suggests that the use of ozonated water as an adjuvant in the treatment of non-surgical periodontal disease reduces periodontal indices compared to control groups (water, distilled water and chlorhexidine).

Palavras-chave: Halitosis. Periodontitis. Ozone Therapy.

INTRODUÇÃO

A halitose é definida como um cheiro desagradável do ar exalado pela cavidade oral, e sentida por terceiros (ORTIZ; FILIPPI *et al*., 2021). O mau cheiro bucal é atribuído a uma alta concentração local de populações microbianas intraorais, particularmente aquelas do biofilme da língua, assim como os biofilmes associados aos dentes e aos tecidos periodontais (DE GEEST *et al*., 2016; LALEMAN *et al*., 2014; ORTIZ; FILIPPI *et al*., 2021).

Cerca de 22% a 50% da população mundial é afetada por essa condição (AKAJI; FOLARANMI; ASHIWAJU *et al*., 2014; AYLIKC; COLAK, 2013; YANG *et al*., 2013). Entretanto, 10 a 20% das causas restantes são causadas pela halitose extraoral, como por exemplo, doenças otorrinolaringológicas, respiratórias, gastrointestinais, hematológicas, endócrino-metabólicas, emocionais, e também pelo consumo de alimentos e uso de drogas (AYLIKCI; COLAK, 2013; RÖSING; LOESCHE, 2011).

Os compostos de enxofre voláteis (CSVs), compostos de nitrogênio voláteis e os ácidos graxos de cadeia curta são os principais responsáveis pelo odor bucal desagradável ou fétido (HUGHES; MCNAB, 2008; SHIBUYA, 2001). A intensidade do

cheiro desagradável está relacionada com o nível de CSVs, que têm um odor pútrido desagradável (SEERANGAIYAN; JUCK; WINKEL, 2018).

Os principais CSVs exalados pelo ar da boca são o sulfeto de hidrogênio [H2S], metil mercaptano [CH3SH] e sulfureto de dimetil [(CH3) 2S)], que são produzidos por várias espécies bacterianas orais que degradam e metabolizam aminoácidos contendo enxofre, como cisteína e metionina (HUGHES; MCNAB, 2008).

O mau odor oriundo da cavidade oral é motivado principalmente por presença de bactérias anaeróbicas gram-negativas (DE GEEST *et al*., 2016). Das bactérias orais cultiváveis, as três mais produtoras de sulfeto de hidrogênio in vitro são a *P. gingivalis*, *T. denticola* e *T. forsythia* (PERSSON *et al*., 1990). Esses microrganismos anaeróbios gram-negativos estão também relacionados à Periodontite. Vários estudos têm investigado uma possível relação entre a presença dessas bactérias e o desenvolvimento de mau hálito e doenças periodontais, esses estudos encontram uma clara associação entre elas (BOSY *et al*., 1994; DE BOEVER; LOESCHE, 1995; MORITA; WANG, 2001).

Uma das etapas da terapia periodontal básica, consiste na instrução de higiene oral e na remoção mecânica do biofilme através da raspagem e alisamento radicular (RAR). No entanto, nem sempre é possível a remoção completa do biofilme dental e

dos fatores retentivos de placa, devido à presença de áreas de difícil acesso à instrumentação, como bolsas muito profundas e áreas de furca (AHAD *et al*., 2016).

Algumas terapias alternativas às vezes são sugeridas nos casos em que apenas o tratamento convencional não é suficiente no controle do processo inflamatório, como o uso de antibióticos sistêmicos (BIRANG *et al*., 2015). Entretanto, o desequilíbrio causado na flora intestinal e o desenvolvimento de resistência bacteriana são as principais desvantagens do uso de antibióticos (AHAD *et al*., 2016).

Estudos clínicos demonstraram resultados satisfatórios envolvendo a ozonioterapia associado à RAR no tratamento da Periodontite (NARDI *et al*., 2020). As pesquisas apontaram que os benefícios dessa terapia incluem a promoção à proliferação celular, efeitos analgésicos, anti-inflamatórios e bactericidas, as quais podem inibir a progressão da gengivite e periodontite (NARDI *et al*., 2020).

Portanto, o objetivo deste estudo foi avaliar o efeito da água ozonizada na halitose relacionada à periodontite através de um ensaio clínico randomizado e cegado de fase 2. Além disso, uma revisão sistemática com metanálise sobre o efeito da água ozonizada na Periodontite foi realizada como segunda produção.

PROPOSIÇÃO

Objetivo Geral

Avaliar o efeito da água ozonizada na halitose relacionada à periodontite.

Objetivos específicos

Em pacientes com doença periodontal, avaliar:

- A eficácia da água ozonizada no controle da halitose;
- O efeito da água ozonizada nos demais desfechos relacionados à Periodontite.
- O efeito da água ozonizada no tratamento da Periodontite, através de uma revisão de literatura sistemática com metanálise de ensaios clínicos randomizados.

Hipóteses

Este estudo testou a hipótese de que o uso de água ozonizada aliado ao tratamento convencional pode atenuar de

forma relevante a halitose e os parâmetros clínicos de pacientes com Periodontite.

ARTIGO SUBMETIDO*

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ARTIGO I

Superiority of ozonated water in controlling periodontitis and halitosis: A phase 2 randomized clinical trial

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Short title: Ozonated water and periodontitis

ABSTRACT

Background and aims: Ozonated water is beneficial for treating periodontal disease. Its effect on periodontitis-related halitosis is unknown. We assessed whether ozonated water attenuates halitosis in patients with periodontitis. **Methods:** In this phase 2 randomized clinical trial, patients with halitosis due to periodontitis were randomly assigned to 30-day treatment with scaling and root planning (SRP) plus ozonized water or placebo, applied twice a week. We

measured the parameters of halitosis and periodontitis before and after the treatments. **Results:** Twenty patients completed the trial (56 ± 12 years old, 70% women, periodontitis stage III/IV: 65%/35%). Patients treated with ozonated water (n = 10) showed a higher reduction in halitosis measured by organoleptic test than placebo (median score before/after treatment: 3/1 vs 3/2; P = 0.027). Most periodontitis parameters improved more with ozone than with placebo (P < 0.05), including visible plaque index, gingival bleeding index, probing depth ≥4 mm, bleeding on probing, gingival level, and clinical attachment level. **Conclusions:** In patients with periodontitis-related halitosis treated with the conventional approach, the addition of ozonated water was superior to placebo in controlling halitosis. Such an effect was related to periodontitis amelioration induced by ozonated water.

REBEC identifier: UTN number: U1111-1286-4184.

Keywords: Halitosis; Ozone; Periodontal disease; Periodontitis.

INTRODUCTION

Halitosis is an unpleasant smell in the air exhaled from the mouth that is perceived by someone nearby (Ortiz & Filippi, 2021). This condition is prevalent and negatively affects psychological well-being, impairing the oral-health-related quality of life (Rosing & Loesche, 2011; Schertel Cassiano et al., 2021). The malodor is related to changes in oral microbiota, attributed to biofilms in the tongue, teeth, and periodontal tissues (De Geest, Laleman, Teughels, Dekeyser, & Quirynen, 2016; Yang et al., 2013). Most cases of halitosis are of oral origin, as a manifestation of conditions such as periodontal disease, poor oral hygiene, and tongue coating (Aylikci & Colak, 2013; Seerangaiyan,

Juch, & Winkel, 2018). Among these, periodontitis is considered a relevant etiological factor of halitosis (Memon et al., 2023).

Oral malodor is secondary to the exhalation of a range of volatile molecules such as sulfur compounds, short-chain fatty acids, and nitrogen compounds (Hughes & McNab, 2008). Such process results from the action of gram-negative anaerobic bacteria, especially in the presence of periodontitis and tongue coating (De Geest et al., 2016). Hydrogen sulfide is the odorant most perceived in tests of organoleptic intensity (Greenman et al., 2004). In patients evaluated for oral halitosis, the organoleptic test remains the gold-standard technique, whereas sulfur monitors such as Halimeter can provide odorant quantification with diagnostic validity (Laleman, Dadamio, De Geest, Dekeyser, & Quirynen, 2014).

Severe periodontitis affects 11% of the global population. It is a chronic multifactorial inflammatory condition associated with dental plaque accumulation (Kwon, Lamster, & Levin, 2021). It may lead to progressive destruction of the teeth-supporting apparatus, compromising the periodontal ligament and alveolar bone (Papapanou et al., 2018).

The conventional treatment of periodontitis aims to remove supra and subgingival biofilm, reducing inflammation and preventing the evolution of periodontal disease (Muller Campanile, Giannopoulou, Campanile, Cancela, & Mombelli, 2015). A widely employed technique consists of oral hygiene instruction and mechanical removal of biofilm through scaling and root planning (SRP) (Papapanou et al., 2018). However, alternative therapies have emerged for cases where conventional treatment does not control the inflammatory process (Birang, Shahaboui, Kiani, Shadmehr,

& Naghsh, 2015). Studies have demonstrated promising results with ozone therapy and SRP in treating periodontitis (Nardi et al., 2020). Its benefits include analgesic, anti-inflammatory, and bactericidal effects, which can help in the regression of gingivitis and periodontitis (Nardi et al., 2020). However, the effects of ozone therapy on periodontitis-related halitosis are still unknown.

This study aimed to evaluate the role of ozonated water on halitosis related to severe periodontitis through a phase 2 randomized double-blinded clinical trial. We hypothesized that oral irrigation with ozonated water during SRP is beneficial in controlling halitosis, due to its therapeutic effect on periodontitis.

MATERIALS AND METHODS

Design, setting, and ethics

This study was a randomized, controlled, double-blinded, parallel groups, phase 2 trial. Participants were selected from appointments at a local University dental clinic, where the study was conducted from July 2023 to January 2024.

The institutional ethics committee approved the study (CAAE: 56696822.5.0000.5342), which is registered at ReBEC (Brazilian Clinical Trials Registry), UTN number: U1111-1286-4184. All participants signed an informed consent before entering the study.

Participants

Adult patients with periodontitis-related halitosis were invited to participate. Halitosis was initially identified by oral examination for periodontitis diagnosis, followed by a halimeter test to confirm halitosis (>75 parts per billion).

Patients had to have severe generalized periodontitis (stages III or IV) with periodontal pockets. Exclusion criteria included coating on more than a third of the tongue, non-oral halitosis, acute oral lesions or necrotizing ulcerative periodontitis, use of antibiotics in the last 90 days, pregnancy, breastfeeding, and decompensated systemic diseases, such as diabetes mellitus, chronic renal failure, and liver cirrhosis. After entry into the study, patients were treated for thirty days with two weekly sessions of SRP combined with ozonated water or a placebo.

Sample size and randomization

Sample size calculation was based on halitosis measurement with a halimeter, according to a study involving patients with oral halitosis (Mousquer et al., 2020) (baseline halimeter data: 187 ± 132 ppb). It was estimated the need of 56 patients (28 per group) providing 80% power with 5%

alpha, expecting a reduction of at least 25% in halimeter values after placebo (+ SRP) and a 50% reduction after ozonated water (+ SRP). The Biostat 5.0 software with the paired t-test was used for two-sided independent samples. A preliminary analysis revealed that data from twenty patients was sufficient to present the results.

Halitosis assessment

Patients had their breath examined before and after the treatments using a halimeter and organoleptic test. The first halitosis assessment was with a halimeter (Halimeter®/ Interscan Corporation, Chatsworth, CA, USA), which is a portable device capable of measuring sulfur odorants. A cut-off ≥75 parts per billion (ppb) confirmed halitosis. An abnormal test result allowed the subject to participate in the trial. In such a case, an organoleptic evaluation was performed at the same day. Patients were evaluated in the

morning and asked to avoid ingesting garlic, onions, and condiments two days before the exams and to avoid smoking and drinking coffee and alcohol in the last 12 hours. On the exam day, the patients were not allowed to use candy or gum but were permitted a snack and brushing with water, without toothpaste (Mousquer et al., 2020).

During the organoleptic test, the patient closed the mouth for 1 minute to concentrate the odor, then opened it and exhaled the breath odor directly, with the clinical examiner located 10 cm from the patient's mouth. We classified the perceived breath between 0 and 5, where 0: no odor; 1: barely detectable odor; 2: slight odor; 3: moderate odor; 4: striking odor; and 5: strong odor (Renvert, Noack, Lequart, Roldan, & Laine, 2020).

Oral examination

A trained dentist (LS) examined the oral cavity before and after the treatments. Periodontal clinical parameters were assessed as published elsewhere (Seydanur Dengizek et al., 2019): plaque index (in %), gingival index (in %), bleeding on probing (in %), probing sites > 4 mm (in %), probing depth (in mm), gingival level (in mm), and clinical attachment loss (CAL) (in mm). The percentage of periodontal pockets deeper than 4 mm, the percentage of CAL ≥3 mm, and missing teeth were recorded. Oral examinations were repeated thirty days after the trial ended. All clinical measurements were performed from six sites on each tooth using a Williams millimeter-calibrated periodontal probe (PGF/W, Chicago, IL, USA).

Periodontitis was defined according to a current classification scheme (Caton et al., 2018), including CAL≥3 mm on ≥2 non-adjacent teeth or probing depth ≥5 mm with

bleeding on probing (Papapanou et al., 2018). Periodontitis stage III demanded interproximal attachment loss ≥5 mm at the worst site or radiographic bone loss extending to the apical half or third of the root. Tooth loss of five or more teeth indicated periodontitis stage IV.

Participants underwent examination to determine the probing depth in 6 dental sites (buccal-mesial, buccal, buccal-distal, lingual-distal, lingual, lingual-distal), including all dental elements. The oral cavity was examined under natural light using a clinical mouth mirror and a 0.5 to 10 mm Williams probe (Papapanou et al., 2018). The periodontal criteria, such as plaque and gingival index, were assessed on the four smooth surfaces of the tooth, with the scores divided by four for index calculation per tooth (Papapanou et al., 2018). Gingival recession was the distance from the cementoenamel junction to the beginning of the gingival margin in a line parallel to the long axis of

the tooth. CAL was the sum of the probing depth plus the gingival level (recession) (measurement +) or hyperplasia (measurement -) (Papapanou et al., 2018).

Ozonated water

Ozonated water was prepared by converting distilled water with ozone gas (75–85 µg/mL) for 10–15 min using a Philozon Medplus® ozone generator (Philozon, Balneário Camboriú, Santa Catarina, Brazil). The final ozone concentration in water was 20 µg/mL (Yilmaz et al., 2013), which has an antimicrobial effect but it is free from harmful effects on the host (Katti & Chava, 2013). The water was ozonized five minutes before the treatment sessions to preserve the ozone properties and conditioned into glasses connected to dental equipment.

Conventional periodontitis treatment

The participants received conventional non-surgical treatment, consisting of adapting the oral environment through prophylaxis, supragingival, and subgingival scaling in areas with increased probing depth, using local anesthesia. A dentist specializing in periodontics (LSD) treated all the patients using manual curettes and sonic devices. The treatment goal occurred when the tactile sensation was smooth on the crown and root surfaces. The recipients containing the ozonized water and the placebo were in the dental equipment, which also include a triple syringe and ultrasound. The treatment consisted of 8 sessions, twice a week, for 30 days.

Randomization and blinding

A web-based system (www.randomizer.org) was used to generate random numbers drawn by the patient from papers folded in a brown envelope containing two options: 1)

treatment with ozonized water and 2) treatment with placebo, in a 1:1 ratio. Patient randomization occurred after halitosis confirmation.

The ozonated water and the placebo (distilled water) were prepared with identical physical appearance, allowing the dentist who applied the treatments and the patients to be blinded. The principal investigator (LS) prepared the whole process and was responsible for ozonizing the water and supplying the equipment. A trained dentist (LSD) treated the patients blinded to treatment groups.

Study protocol

The participants reported demographic and clinical data, followed by a periodontal examination for periodontitis confirmation (Figure 1). Subjects included in the study returned on another day for a halitosis assessment, following the described rules.

A dentist examined the oral cavity under natural light using a trio (probe no. 5, mirror, tweezers), cotton, gauze, and Williams's periodontal probe. The included patients were randomly allocated using blocks of two patients 1:1 for SRP with placebo and SRP with ozonated water.

Supragingival scaling of the two arches was initially performed, followed by scaling by sextants. Sessions occurred twice a week, totaling eight treatment sessions during the clinical trial. The average duration of each session was 40 minutes. Thirty days after the last session, patients returned for the final assessment and oral hygiene reinforcement. Patients were instructed to perform regular oral hygiene at home.

Statistical analysis

Quantitative data were evaluated with the Shapiro-Wilk test. In the case of normal distribution, the mean and standard

deviation values were used. Otherwise, median and interquartile range of 25%-75% were used. Categorical data were described with absolute and percentage frequencies. Quantitative data were analyzed using Student's t-test or its non-parametric counterpart (Mann-Whitney), while categorical data were analyzed using Fisher's exact test. Statistical analysis was performed with Graph Prism 8.0 software, considering an alpha of 5% as an indication of statistical significance.

RESULTS

Participants

Forty-one patients were invited to participate (Figure 2): Fourteen were excluded after the first interview (three with diabetes mellitus, eight because of tobacco use, and three with an unrevealing halimeter test), and seven did not complete the study (missed sessions). A total of 20 patients finished the trial: ten treated with SRP and ozonated water, and ten treated with SRP and placebo (Table 1). Patient groups did not differ regarding age, sex, periodontitis stage, and halitosis scores. The participants were mostly in their 5^{th} decade, women with stage III periodontitis and moderate halitosis (organoleptic score 3 and halimeter > 120 ppb).

The baseline parameters of periodontitis did not differ significantly between patients treated with ozonated water and placebo, including plaque index (68.6 ± 20.6 % vs. 56.3 ± 13.1 %; P = 0.129), gingival index (45.4 ± 21.5 % vs. 43.7

± 9.0 %; P = 0.820), bleeding on probing (51.4 ± 20.1 % vs. 39.1 ± 7.4 %; P = 0.086), probing sites >4 mm (50.8 ± 13.7 % vs. 39.6 ± 12.6 %; P = 0.073); probing depth (3.7 ± 0.3 mm vs. 3.6 ± 0.3 mm; P = 0.227), gingival level (0.94 ± 0.18 mm vs. 0.93 ± 0.14 mm; P = 0.862), and CAL (4.7 ± 0.4 mm vs. 4.5 ± 0.3 mm; P = 0.211).

Ozonated water and halitosis

After treatments, both ozonated water and placebo reduced halitosis measured by the organoleptic test (Table 2). Based on the comparisons of deltas (differences between halitosis scores before and after treatments), halitosis reduction was significantly greater after using ozonated water than placebo. The halitosis resolution rate, characterized by halitosis class 0 and 1 after the treatments, was higher with ozonated water than with placebo.

After treatments, both groups showed reduced halitosis measured by the halimeter (before vs. after ozonated water: 125 ± 20 vs. 50 ± 17, $P < 0.001$; and before vs. after placebo: 132 ± 30 vs. 64 ± 16, $P < 0.001$). Despite of the deltas did not differ between ozonated water and placebo (76 ± 22 vs. 68 ± 24; $P = 0.480$), the rate of halitosis resolution was greater after treatment with ozonated water than with placebo (100% vs. 60%; $P = 0.086$), with borderline statistical significance.

Ozonated water and periodontitis

The effect of treatments on periodontitis was expressed by comparing the deltas, corresponding to the difference before and after each treatment. Ozonated water was superior to placebo in controlling periodontitis parameters, except for probing depth (Table 3).

DISCUSSION

Individuals with periodontitis have approximately three to four times higher odds of having halitosis (Nini, Chen, Jinmei, Lufei, & Jingmei, 2024; Silva et al., 2017). This study evaluated the effect of ozonated water on halitosis resulting from periodontitis. Patients with this condition received conventional management for periodontitis, combined with ozonated water or placebo, in a randomized phase 2 clinical trial. Additionally, it was explored the effectiveness of ozonated water in controlling the variables that characterize periodontitis.

To our best knowledge, this is the first clinical trial evaluating the effect of ozonated water on halitosis related to periodontitis. Combined with SRP, ozonated water was superior to placebo in controlling halitosis through the organoleptic test after a 30-day treatment with applications

twice a week. On a scale of 0 to 5 in the organoleptic test, there was a reduction of two levels after using ozonated water (3 to 1), compared to one level for placebo (3 to 2). The qualitative analysis between resolved vs. unresolved halitosis also showed the superiority of ozonated water to placebo. Nowadays, the organoleptic test is the gold standard in diagnosing halitosis, identified by the smell of a trained examiner (Kumbargere Nagraj et al., 2019). Halitosis reduction was demonstrated in a randomized clinical trial with complete mouth disinfection in four weekly sessions with amelioration on organoleptic scores in patients with severe chronic periodontitis (Silveira et al., 2017).

In addition to conventional treatments such as SRP and chlorhexidine-based oral antiseptics, alternative management has been studied in periodontal disease, including ozone therapy (Smith, Wilson, Gandhi, Vatsia, & Khan, 2017). In patients with this condition, the topical

application of ozone in different formulations and sometimes combined with SRP is a safe and beneficial approach (Hayakumo, Arakawa, Mano, & Izumi, 2013; Kshitish & Laxman, 2010; Nardi et al., 2020; Ranjith, Niranjana, & Baiju, 2022). In addition to the antibacterial effect on the oral microbiota, ozonated water can inhibit oxidative stress, favoring the healing of periodontal tissues (Hayakumo et al., 2013). Ozone can act as an alternative antiseptic agent, eliminating bacteria that colonize periodontal pockets and the gingival sulcus (Hayakumo et al., 2013; Kshitish & Laxman, 2010; Nardi et al., 2020; Patel, Patel, Kumar, & Holmes, 2012; Ranjith et al., 2022; Yilmaz et al., 2013). This antimicrobial effect is explained by damage to the bacterial plasma membrane related to phospholipids and lipoproteins oxidation (Elvis & Ekta, 2011).

In dental practice, ozonated water is an oral irrigant with favorable chemical and physical properties. In the aqueous phase, ozone is biocompatible with dental structures, exerting its antimicrobial effect quickly, free from mutagenicity or toxicity to oral cells (Boch et al., 2016; Kshitish & Laxman, 2010). In the present study, ozone was combined with distilled water using automated equipment, resulting in an effective and safe concentration (20 µg/mL). This solution was packaged in a dental apparatus that kept its original composition for oral cavity irrigation. Regarding safety, inhaling ozone gas (O_3) can be toxic to the body, resulting in a runny nose, cough, headache, dyspnea, and nausea, among other symptoms (Naik, K, Kohli, Zohabhasan, & Bhatia, 2016). When mixed with water, these effects are absent.

In the present study, the likely mechanism by which ozonated water reduced halitosis was its beneficial effect on

periodontitis resolution. The resulting decrease in bacterial biofilms potentially blocks the odorous production chains (Iatropoulos et al., 2016). Patients with periodontitis exhale higher amounts of volatile sulfur compounds and methyl mercaptan, resulting from the metabolism of gram-negative anaerobic bacteria in the mouth, such as *P. gingivalis, T. denticola and T. forsythia* (De Geest et al., 2016). Conventional management of this condition consists of adequate oral hygiene and mechanical removal of biofilms through SRP (Iatropoulos et al., 2016). However, failure to remove dental biofilm and plaque-retentive factors may occur in areas that are difficult to access with instrumentation, such as deep pockets and furcation areas (Ahad et al., 2016).

The combination of SRP and ozonated water here tested was superior to placebo in controlling most of the variables that characterize periodontitis. Even with a small sample size, it

was possible to observe a clear superiority of ozonated water over placebo in improving periodontal clinical parameters. Such benefits were reported by other studies, specifically in plaque and gingival indices (Hayakumo et al., 2013; Kshitish & Laxman, 2010), bleeding on probing (Hayakumo et al., 2013; Kshitish & Laxman, 2010; Ranjith et al., 2022), gingival level (Ranjith et al., 2022), and clinical attachment level (Hayakumo et al., 2013; Ranjith et al., 2022). In agreement, a clinical trial described a gain in clinical insertion after 4 and 8 weeks in the ozonated water group than in the placebo group (Hayakumo et al., 2013).

This study has limitations and strengths. Limitations included the apparent small sample of participants, the relatively high loss of participants (26%) for a short-term study, and the lack of analysis of the oral microbiota. Even so, the results showed the superiority of ozonated water over placebo in improving periodontitis and the resulting oral

halitosis. We believe such benefits are generalizable to other populations of patients with periodontitis. However, long-term management still depends on appropriate intervention studies.

In conclusion, in patients with periodontitis, conventional treatment with SRP combined with ozonated water was superior to placebo in controlling halitosis related to severe periodontitis. Amelioration of periodontitis parameters after ozonated water might explain halitosis control.

REFERENCES

Ahad, A., Lamba, A. K., Faraz, F., Tandon, S., Chawla, K., & Yadav, N. (2016). Effect of Antimicrobial Photodynamic Therapy as an Adjunct to Nonsurgical Treatment of Deep Periodontal Pockets: A Clinical Study. *J Lasers Med Sci*, *7*(4), 220-226. doi:10.15171/jlms.2016.39

Aylikci, B. U., & Colak, H. (2013). Halitosis: From diagnosis to management. *J Nat Sci Biol Med*, *4*(1), 14-23. doi:10.4103/0976-9668.107255

Birang, R., Shahaboui, M., Kiani, S., Shadmehr, E., & Naghsh, N. (2015). Effect of Nonsurgical Periodontal Treatment Combined With Diode Laser or Photodynamic Therapy on Chronic Periodontitis: A Randomized Controlled Split-Mouth Clinical Trial. *J Lasers Med Sci, 6*(3), 112-119. doi:10.15171/jlms.2015.04

Boch, T., Tennert, C., Vach, K., Al-Ahmad, A., Hellwig, E., & Polydorou, O. (2016). Effect of gaseous ozone on Enterococcus faecalis biofilm-an in vitro study. *Clin Oral Investig, 20*(7), 1733-1739. doi:10.1007/s00784-015-1667-1
Caton, J. G., Armitage, G., Berglundh, T., Chapple, I. L. C., Jepsen, S., Kornman, K. S., . . . Tonetti, M. S. (2018). A new classification scheme for periodontal and peri-implant diseases and conditions - Introduction and key changes from the 1999 classification. *J Clin Periodontol, 45 Suppl 20*, S1-S8. doi:10.1111/jcpe.12935

De Geest, S., Laleman, I., Teughels, W., Dekeyser, C., & Quirynen, M. (2016). Periodontal diseases as a source of halitosis: a review of the evidence and treatment approaches for dentists and dental hygienists. *Periodontol 2000, 71*(1), 213-227. doi:10.1111/prd.12111
Elvis, A. M., & Ekta, J. S. (2011). Ozone therapy: A clinical review. *J Nat Sci Biol Med, 2*(1), 66-70. doi:10.4103/0976-9668.82319

Greenman, J., Duffield, J., Spencer, P., Rosenberg, M., Corry, D., Saad, S., . . . El-Maaytah, M. (2004). Study on the organoleptic intensity scale for measuring oral malodor. *J Dent Res, 83*(1), 81-85. doi:10.1177/154405910408300116

Hayakumo, S., Arakawa, S., Mano, Y., & Izumi, Y. (2013). Clinical and microbiological effects of ozone nano-bubble water irrigation as an adjunct to mechanical subgingival debridement in periodontitis patients in a randomized controlled trial. *Clin Oral Investig, 17*(2), 379-388. doi:10.1007/s00784-012-0711-7

Hughes, F. J., & McNab, R. (2008). Oral malodour--a review. *Arch Oral Biol, 53 Suppl 1*, S1-7. doi:10.1016/S0003-9969(08)70002-5

Iatropoulos, A., Panis, V., Mela, E., Stefaniotis, T., Madianos, P. N., & Papaioannou, W. (2016). Changes of volatile sulphur compounds during therapy of a case series of patients with chronic periodontitis and halitosis. *J Clin Periodontol, 43*(4), 359-365. doi:10.1111/jcpe.12521

Katti, S. S., & Chava, V. K. (2013). Effect of Ozonised water on Chronic Periodontitis - A Clinical Study. *J Int Oral Health, 5*(5), 79-84.

Kshitish, D., & Laxman, V. K. (2010). The use of ozonated water and 0.2% chlorhexidine in the treatment of periodontitis patients: a clinical and microbiologic study. *Indian J Dent Res, 21*(3), 341-348. doi:10.4103/0970-9290.70796

Kumbargere Nagraj, S., Eachempati, P., Uma, E., Singh, V. P., Ismail, N. M., & Varghese, E. (2019). Interventions for managing halitosis. *Cochrane Database Syst Rev, 12*(12), CD012213. doi:10.1002/14651858.CD012213.pub2

Kwon, T., Lamster, I. B., & Levin, L. (2021). Current Concepts in the Management of Periodontitis. *Int Dent J, 71*(6), 462-476. doi:10.1111/idj.12630

Laleman, I., Dadamio, J., De Geest, S., Dekeyser, C., & Quirynen, M. (2014). Instrumental assessment of halitosis for the general dental practitioner. *J Breath Res, 8*(1), 017103. doi:10.1088/1752-7155/8/1/017103

Memon, M. A., Memon, H. A., Muhammad, F. E., Fahad, S., Siddiqui, A., Lee, K. Y., . . . Yousaf, Z. (2023). Aetiology and associations of halitosis: A systematic review. *Oral Dis, 29*(4), 1432-1438. doi:10.1111/odi.14172

Mousquer, C. R., Della Bona, A., Milani, D. C., Callegari-Jacques, S. M., Ishikawa, K., Mayer, M. P. A., . . . Fornari, F. (2020). Are Lactobacillus salivarius G60 and inulin more efficacious to treat patients with oral halitosis and tongue coating than the probiotic alone and placebo? A randomized clinical trial. *J Periodontol, 91*(6), 775-783. doi:10.1002/JPER.19-0089

Muller Campanile, V. S., Giannopoulou, C., Campanile, G., Cancela, J. A., & Mombelli, A. (2015). Single or repeated antimicrobial photodynamic therapy as adjunct to ultrasonic debridement in residual periodontal pockets: clinical, microbiological, and local biological effects. *Lasers Med Sci, 30*(1), 27-34. doi:10.1007/s10103-013-1337-y

Naik, S. V., K, R., Kohli, S., Zohabhasan, S., & Bhatia, S. (2016). Ozone- A Biological Therapy in Dentistry- Reality or Myth????? *Open Dent J, 10*, 196-206. doi:10.2174/1874210601610010196

Nardi, G. M., Cesarano, F., Papa, G., Chiavistelli, L., Ardan, R., Jedlinski, M., . . . Grassi, F. R. (2020). Evaluation of Salivary Matrix Metalloproteinase (MMP-8) in Periodontal Patients Undergoing Non-Surgical Periodontal Therapy and Mouthwash Based on Ozonated Olive Oil: A Randomized Clinical Trial. *Int J Environ Res Public Health, 17*(18). doi:10.3390/ijerph17186619

Nini, W., Chen, L., Jinmei, Z., Lufei, W., & Jingmei, Y. (2024). The association between halitosis and periodontitis: a systematic review and meta-analysis. *Clin Oral Investig, 28*(6), 341. doi:10.1007/s00784-024-05732-0

Ortiz, V., & Filippi, A. (2021). Halitosis. *Monogr Oral Sci, 29*, 195-200. doi:10.1159/000510192

Papapanou, P. N., Sanz, M., Buduneli, N., Dietrich, T., Feres, M., Fine, D. H., . . . Tonetti, M. S. (2018). Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Periodontol, 89 Suppl 1*, S173-S182. doi:10.1002/JPER.17-0721

Patel, P. V., Patel, A., Kumar, S., & Holmes, J. C. (2012). Effect of subgingival application of topical ozonated olive oil in the treatment of chronic periodontitis: a randomized, controlled, double blind, clinical and microbiological study. *Minerva Stomatol, 61*(9), 381-398.

Ranjith, A., Niranjana, J. M., & Baiju, K. V. (2022). Adjunctive benefit of ozonized water irrigation with mechanical debridement in the management of Stage III periodontitis: A randomized controlled clinical and

biochemical study. *Int J Dent Hyg, 20*(2), 364-370. doi:10.1111/idh.12547

Renvert, S., Noack, M. J., Lequart, C., Roldan, S., & Laine, M. L. (2020). The Underestimated Problem of Intra-Oral Halitosis in Dental Practice: An Expert Consensus Review. *Clin Cosmet Investig Dent, 12*, 251-262. doi:10.2147/CCIDE.S253765

Rosing, C. K., & Loesche, W. (2011). Halitosis: an overview of epidemiology, etiology and clinical management. *Braz Oral Res, 25*(5), 466-471. doi:10.1590/s1806-83242011000500015

Schertel Cassiano, L., Abdullahi, F., Leite, F. R. M., Lopez, R., Peres, M. A., & Nascimento, G. G. (2021). The association between halitosis and oral-health-related quality of life: A systematic review and meta-analysis. *J Clin Periodontol, 48*(11), 1458-1469. doi:10.1111/jcpe.13530

Seerangaiyan, K., Juch, F., & Winkel, E. G. (2018). Tongue coating: its characteristics and role in intra-oral halitosis and general health-a review. *J Breath Res, 12*(3), 034001. doi:10.1088/1752-7163/aaa3a1

Seydanur Dengizek, E., Serkan, D., Abubekir, E., Aysun Bay, K., Onder, O., & Arife, C. (2019). Evaluating clinical and laboratory effects of ozone in non-surgical periodontal treatment: a randomized controlled trial. *J Appl Oral Sci, 27*, e20180108. doi:10.1590/1678-7757-2018-0108

Silva, M. F., Cademartori, M. G., Leite, F. R. M., Lopez, R., Demarco, F. F., & Nascimento, G. G. (2017). Is periodontitis

associated with halitosis? A systematic review and meta-regression analysis. *J Clin Periodontol, 44*(10), 1003-1009. doi:10.1111/jcpe.12786

Silveira, J. O., Costa, F. O., Oliveira, P. A. D., Dutra, B. C., Cortelli, S. C., Cortelli, J. R., . . . Oliveira, A. (2017). Effect of non-surgical periodontal treatment by full-mouth disinfection or scaling and root planing per quadrant in halitosis-a randomized controlled clinical trial. *Clin Oral Investig, 21*(5), 1545-1552. doi:10.1007/s00784-016-1959-0

Smith, N. L., Wilson, A. L., Gandhi, J., Vatsia, S., & Khan, S. A. (2017). Ozone therapy: an overview of pharmacodynamics, current research, and clinical utility. *Med Gas Res, 7*(3), 212-219. doi:10.4103/2045-9912.215752

Yang, F., Huang, S., He, T., Catrenich, C., Teng, F., Bo, C., . . . Xu, J. (2013). Microbial basis of oral malodor development in humans. *J Dent Res, 92*(12), 1106-1112. doi:10.1177/0022034513507065

Yilmaz, S., Algan, S., Gursoy, H., Noyan, U., Kuru, B. E., & Kadir, T. (2013). Evaluation of the clinical and antimicrobial effects of the Er:YAG laser or topical gaseous ozone as adjuncts to initial periodontal therapy. *Photomed Laser Surg, 31*(6), 293-298. doi:10.1089/pho.2012.3379

TABLES

Table 1. Baseline characteristics of the participants (n = 20).

	Ozonated water (n = 10)	Placebo (n = 10)	P
Age in years, mean ± SD	58.6 ± 7.5	54.7 ± 15.7	0.488†
Women, n (%)	7 (70%)	7 (70%)	0.999††
Periodontitis, n (%)			0.350††
Stage III	5 (50%)	8 (80%)	
Stage IV	5 (50%)	2 (20%)	
Organoleptic score*, median (range)	3 (2-4)	3 (2-4)	0.635#
Halimeter (ppb**), mean (range)	125 (98-155)	132 (97-176)	0.545†

*Range between 0 (no odor) and 5 (maximal odor); **parts per billion; †Student's t-test; ††Exact Fisher test; #Mann-Whitney test.

Table 2. Effect of ozonated water and placebo on halitosis measured by the organoleptic test (n = 20).

Halitosis	Ozonated water (n = 10)	Placebo (n = 10)	P
Median score (IQR*25-75%)	3.0 (2.7-4.0)	3.0 (3.0-3.0)	0.634††
Before treatments	1.0 (1.0-1.0)		
After treatments	2.0 (1.7-3.0)	2.0 (1.0-2.0)	0.027†
Delta (before-after)		1.0 (1.0-2.0)	

Halitosis resolution			
After treatment**, n (%)	10 (100)	3 (30)	0.003#

*Interquartile range 25-75%; **Halitosis scores 0 or 1; †Mann-Whitney test; ††In paired analysis (Wilcoxon test), scores reduced with both ozone (P = 0.002) and placebo (P = 0.002); #Exact Fisher test.

Table 3. Effect of ozonated water and placebo on periodontitis (n = 20).

Periodontitis parameters	Ozonated water (n = 10)	Placebo (n = 10)	P
	mean ± SD of delta*		
Plaque index (%)	42 ± 14	17 ± 12	<0.001
Gingival index (%)	25 ± 18	4 ± 3	0.002
Bleeding on probing (%)	26 ± 15	3 ± 2	<0.001
Probing sites > 4 mm (%)	3 ± 2	1 ± 1	0.016
Probing depth (mm)	0.09 ± 0.05	0.05 ± 0.04	0.089
Gingival level (mm)	0.13 ± 0.06	0.06 ± 0.03	0.008

Clinical attachment level (mm)	0.27 ± 0.19	0.11 ± 0.04	0.002

*Delta: difference between before and after treatments (a higher delta means periodontitis improvement).

FIGURE LEGENDS

Figure 1. Study phases

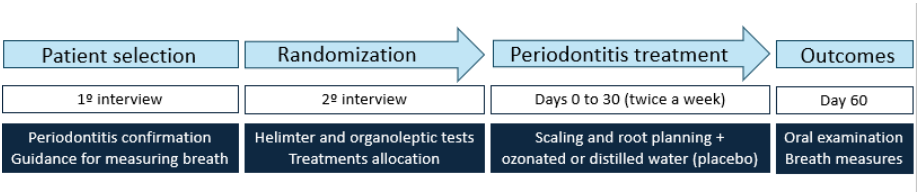
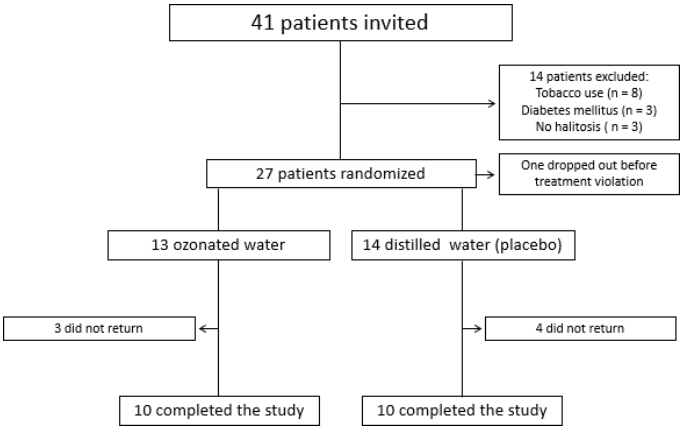


Figure 2. Participant flowchart



ARTIGO SUBMETIDO PARA REVISTA Ozone: Science & Engineering*

ARTIGO II

Effects of ozonized water on Periodontitis: systematic review with meta-analysis

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Summary

Objective: to verify the effects of ozonated water in the treatment of periodontitis, through a systematic literature review with meta-analysis of randomized clinical trials. **Materials and Methods:** Between the months of March 2023 and October 2023, articles were manually searched in the BIREME, PubMed and Web of Science databases. **Results:** 04 articles published in full text were included in this study. In the 4 studies, the ozonized water did better in reducing the bleeding rate. The heterogeneity index (I^2) was 47% with 95% CI. The result was: -6.29 [-12.06, - 0.53]

P=0.03. As for probing depth, the results indicate improvements in the reduction of this parameter, in the 3 studies that offered this data. However, there was no significant difference between them. The result was 0.41 [-0.09, 0.92] P=0.11. **Conclusion:** This study suggests that the use of ozonated water as an adjunct in the treatment of non-surgical periodontal disease reduces periodontal indices compared to control groups (water, distilled water and chlorhexidine). However, it is difficult to claim that it is statistically superior in all aspects studied, due to the heterogeneity of the protocols used and the short follow-up time of the clinical trials carried out.

Keywords: Periodontal Disease. Periodontitis. Ozone. Ozone therapy.

Introduction

According to the Report of the World *Workshop* on the Classification of Periodontal and Peri-implant Diseases and Conditions, carried out in 2017, periodontitis is a chronic multifactorial inflammatory disease characterized by the progressive destruction of the periodontium (1). The main observed characteristics of Periodontitis include loss of periodontal tissue support, manifested through loss of clinical attachment and alveolar bone loss assessed radiographically, presence of periodontal pockets and gingival bleeding (1). The inflammatory response is mainly related to the accumulation of microorganisms originating from dental biofilm, resulting in a dysbiotic bacterial community, which stimulates the host's immune system and generates exacerbated damage to periodontal tissue (2).

Therefore, non-surgical periodontal treatment aims to disorganize and remove the supra and subgingival biofilm, with the aim of reducing inflammation and the progression of Periodontitis (3). One of the steps of basic periodontal therapy consists of oral hygiene instruction and mechanical removal of biofilm through scaling and root planing (SRP). However, it is not always possible to completely remove dental biofilm and plaque-retentive factors, due to the presence of areas that are difficult to access for instrumentation, such as very deep pockets and furcation areas (4).

Alternative therapies are sometimes suggested in cases where conventional treatment alone is not sufficient to control the inflammatory process, such as the use of systemic antibiotics (5). However, the imbalance caused in the intestinal flora and the development of bacterial resistance are the main disadvantages of using antibiotics (4).

Recently, clinical studies demonstrated satisfactory results involving ozone therapy associated with RAR in the treatment of Periodontitis (6,7). Research has shown that the benefits of this therapy include promoting cell proliferation, analgesic, anti-inflammatory and bactericidal effects, which can inhibit the progression of gingivitis and periodontitis (6).

In addition to the well-established treatment alternatives to date, such as scaling and root planing (SRP), combined with the use of oral antiseptics based on chlorhexidine (CHX), Dentistry has been trying to innovate forms of treatment with the use of ozone therapy (8). Ozone therapy has been a promising area among researchers and clinicians in the most diverse areas.

According to several studies, ozone can act as an alternative antiseptic agent, which can reduce and control bacteria that colonize periodontal pockets and the gingival sulcus (9-11).

Ozone is a triatomic molecule, made up of three oxygen atoms, and its application in Medicine and Dentistry has been indicated for the treatment of numerous different pathologies, such as the elimination of bacteria, periodontal disinfection, prevention of tooth decay, endodontic treatment, sensitivity dentistry, and treatment of temporomandibular dysfunction (TMD) (8).

Ozone is already used worldwide in the treatment of many diseases as it is highly effective against viruses, bacteria and fungi, in addition to having anti-inflammatory action, it acts to increase the local supply of oxygen, promoting local homeostasis and inhibiting bacterial proliferation in numerous diseases (12).

Ozone's mechanism of action is damage to the cytoplasmic membrane of cells and, as a consequence, ozonolysis of double bonds, causing the modification of

intracellular contents due to the secondary oxidant that leads to oxidation and loss of function proteins. organelle (13).

In the aqueous phase, ozone has advantages, as it is biocompatible with dental structures, has an antimicrobial effect, lacks mutagenicity and rapid microbicidal effects, eliminating resistant oral microorganisms and, in addition, it is not toxic to oral cells (14). The objective of the present study was to verify the effects of ozonated water in the treatment of Periodontitis, through a systematic literature review of randomized clinical trials.

Materials and methods

Study Design

This study is characterized as a systematic literature review, registered in the International Prospective Registry of Systematic Reviews (PROSPERO, registration:

CRD42023473289), and conducted in accordance with the recommendations of the Statement of Preferred Items for Systematic Reviews and Meta-Analyses (PRISMA) (15).

Guiding Question and Search Strategy

Initially, the following guiding question was constructed: Is ozonated water effective, as an adjuvant therapy, in the treatment of Periodontitis?

To answer this question, between the months of March 2023 and October 2023, articles were manually searched in the BIREME, PubMed and Web of Science databases, using descriptors indexed in Decs (Descriptors in Health Sciences) and MeSH (*Medical Subject Headings*): “Ozone Therapy”, “Periodontal Diseases”, “Periodontitis”, “Ozone”. Boolean operators “AND” and “OR” were used for the search strategy. In the Pubmed database, the descriptors were used with the help of Mesh (Medical Subject Headings).

Study Selection

To select the studies, two independent reviewers (LS and FF) used the PICOS strategy, as follows: Population (P): permanent human teeth with Periodontitis. Intervention (I): application of aqueous ozone in periodontal treatment. Comparison (C): conventional periodontal treatment associated with ozone associated or not with the alternative use of chlorhexidine (positive control) or 0.9% saline solution (negative control). Outcome (O): effectiveness of ozone as an adjuvant therapy in periodontal treatment; and Study design(s): randomized clinical trials.

Inclusion Criteria

Randomized clinical trials comparing the results of aqueous ozone in patients with Periodontitis; Randomized

clinical trials with patients with probing depth (PS) greater than or equal to 4mm; articles in English.

Exclusion Criteria

Articles with different study designs, such as narrative reviews, systematic reviews, case reports, control groups, incomplete articles, case series and study protocols; studies that have treated other pathologies.

Methodological quality and risk of bias

To assess methodological quality and risk of bias, two independent reviewers checked the article data, discrepancies were resolved by discussion between the reviewers. The quality assessment using the modified Jadad scale and the risk of bias assessment proposed by the Cochrane Collaboration (Rev Man 5.4) were adopted. These instruments bring methodological aspects, such as masking,

randomization and sample loss, the higher the score the better the methodological quality (16).

Results

Articles published in full text, in the English language, published between 2010 and 2022 were included in this study. Articles that were not in accordance with the research subject were excluded. For exclusion, the included articles were selected by reading the title and abstract. The articles selected at this stage had their summaries read, when in doubt a third evaluator (MS) decided whether they were included or not. The articles selected by reading the abstract went to the full text reading stage and, from there, the final selection was made, as illustrated in the flowchart below (Figure 1).

Figure 1 – Article selection flowchart.

Figures 2 and 3 show the risk of bias of the selected studies.

Figure 3 – Summary of risk of bias per study.

The table below describes the characteristics of the selected studies (Table 1).

Table 2 below illustrates the main characteristics of these studies.

Data from the meta-analysis comparisons between bleeding and decreased probing depth from these studies can be seen in the figures below (Figures 4 and 5).

Figure 4- Meta-analysis of comparison between ozonated water group and control groups in bleeding after treatments.

It is noted that in the 4 studies the treatment group did better in reducing the bleeding rate, however, only 02 showed significant improvements (P=0.03). In two studies there were improvements in these indices, but without significant

differences between them (17, 18). The heterogeneity index (I^2) was 47% with 95% CI. The result was: -6.29 [-12.06, -0.53].

Figure 5 – Meta-analysis comparing the treatment group and the control group in reducing probing depth.

The results indicate improvements in the reduction of probing depth in the 3 studies that offered this data, however, there was no significant difference between them (P=0.11). The result was 0.41 [-0.09, 0.92]. The heterogeneity index (I^2) was 93% with 95% CI.

Discussion

Within periodontics, for the treatment of periodontal disease, the gold standard is scaling and root planing (SRP),

however, for long-term improvements, many substances are added, including the use of medical ozone.

However, studies using ozone therapy as an adjunct in the area of periodontics are still scarce. Therefore, this systematic review selected only 04 articles after careful evaluation and application of the inclusion and exclusion criteria (9,10,17, 18).

These results demonstrated effectiveness in reducing bleeding on probing, but in other clinical parameters, such as decreased probing depth, they are similar to other systematic literature reviews, as it can be noted that the use of ozone therapy with non-surgical periodontal treatment Conventional therapy, in addition to being scarce, is considered controversial and lacks effective results (19,20). But it is important to highlight that these results should not be considered definitive, due to the great heterogeneity of the

studies carried out, as well as the different vehicles used, such as gas, oil and water.

However, after applying filters and selecting the aqueous vehicle as an inclusion criterion for the present study, it was possible to obtain good homogeneity in the studies that used ozonated water. Proof of this was the bias scale of the selected studies, which mostly indicated a low risk of bias, according to the modified Jadad scale proposed by the Cochrane Collaboration (Figure 1 and 2) (16).

It can be argued that ozonated water is safer compared to ozone in gaseous form, due to numerous reasons, such as: epiphora, rhinitis, cough, headache, nausea, and vomiting, if inhaled through an open system (21,22). The present study points out that when ozonated water is compared with CHX 0.12% or a placebo (saline solution, water or distilled water), similar or superior results are found, demonstrating that this

therapy is as effective as CHX as an adjuvant. in periodontal treatment (9,10, 17, 18).

In the study by Hayakumo et al. (10) the clinical and microbiological effects of irrigation with nano bubbles of ozonized water was used as a complement to SRP for periodontal treatment, the authors found significant differences in all clinical aspects and parameters after 4 weeks in both groups. The reduction in probing depth and gain in clinical attachment after 4 and 8 weeks in the ozonated water group were significantly greater than those in the water-only group. Furthermore, only the ozonated water group showed statistically significant reductions in the mean total number of bacteria in subgingival plaque during the study period.

In the study by Ranthji et al. (18) the authors investigated the benefits of irrigation with ozonated water together with non-surgical periodontal therapy for the

treatment of periodontitis. According to the authors' analysis, irrigation with ozonized water resulted in a significant reduction in pocket depth in deep pockets (p=0.01) and in the number of sites with pocket depth ≥4 mm with bleeding on probing. Salivary interleukin 1 beta also significantly reduced in the test group after therapy.

Similar to the studies mentioned above, Al Habashneh et al. (17) determined the clinical and biological effects of the adjuvant use of ozone in non-surgical procedures in periodontal treatment. There was a statistically significant improvement in the study parameters in both groups between T0 and T1, except for the gingival index. However, this study, unlike the others, demonstrated that there were no significant differences in any study parameter between the test and control groups.

Regarding the results of the meta-analysis, they must be interpreted with caution, as when comparing the rate of

bleeding on probing when using distilled water with the control groups (water, distilled water and CHX), it is noted that in the 4 studies the treatment groups did better when reducing this parameter, however, only two showed significant improvements (I^2=47%; 95% CI). When comparing the difference in probing depth, all were statistically similar (I^2=93%; 95% CI), remembering that one study did not provide PS values and had to be left out (9).

Another point to be highlighted were the studies that analyzed the reduction of the microbiota. This was the major highlight for the ozonated water group over the control, as only this group showed statistically significant reductions in the average total number of bacteria in subgingival plaque during the study period (10). In line with these results, Kshitish and Laxman (9) claimed that there was a 25% reduction in Aa when using ozonated water, and CHX showed no difference.

CHX is known to be a gold standard antimicrobial for oral biofilm control, as it has a broad antiseptic spectrum and substantivity (9). However, this substance used as a mouthwash for long periods can cause damage to patients' mouths, such as a “metallic” taste, burning and staining of the teeth (9). This is why new substances are being studied, such as ozone, because in the aqueous phase it is biocompatible with dental structures, has an antimicrobial effect, has no mutagenicity and rapid microbicidal effects, eliminating resistant oral microorganisms, and is not toxic to cells. oral (14).

It is important to mention the disadvantage of aqueous ozone, as it has a short half-life at room temperature, but, if refrigerated, it can last for days (23). Finally, the limitations of this study must be mentioned, which include the small number of individuals selected for research, the short follow-up time, the absence of fixed protocols for randomized

clinical trials and the need for more clinical studies in this area.

Final Considerations

This study suggests that the use of ozonated water as an adjunct in the treatment of non-surgical periodontal disease reduces periodontal indices, compared to control groups (water, distilled water and CHX). However, it is difficult to claim that it is statistically superior in all aspects studied, due to the heterogeneity of the protocols used and the short follow-up time of the clinical trials carried out. Therefore, more clinical studies are needed.

References

1. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the

Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol 2018;89 Suppl 1:S173-S182.

2. Barin LM, Barcelos RCS, Vey LT, Pillusky FM, Palma VM, Kantorski KZ, Bürger ME, Maciel RM, Danesi CC. Role of the adjunctive antimicrobial photodynamic therapy to periodontal treatment at plasmatic oxidative stress and vascular behavior. J Photochem Photobiol B. 2017 Aug;173:538-544. doi: 10.1016/j.jphotobiol.2017.06.039. Epub 2017 Jun 28. PMID: 28692925.

3. Muller Campanile VS, Giannopoulou C, Campanile G, Cancela JA, Mombelli A. Single or repeated antimicrobial photodynamic therapy as adjunct to ultrasonic debridement in residual periodontal pockets: clinical, microbiological, and local biological effects. Lasers Med Sci 2015;30:27-34.

4. Ahad A, Lamba AK, Faraz F, Tandon S, Chawla K, Yadav N. Effect of Antimicrobial Photodynamic Therapy as an Adjunct to Nonsurgical Treatment of Deep Periodontal Pockets: A Clinical Study. J Lasers Med Sci 2016;7:220-226.

5. Birang R, Shahaboui M, Kiani S, Shadmehr E, Naghsh N. Effect of Nonsurgical Periodontal Treatment Combined With Diode Laser or Photodynamic Therapy on Chronic Periodontitis: A Randomized Controlled Split-Mouth Clinical Trial. J Lasers Med Sci 2015;6:112-119.

6. Nardi GM, Cesarano F, Papa G, Chiavistelli L, Ardan R, Jedlinski M, et al. Evaluation of Salivary Matrix Metalloproteinase (MMP-8) in Periodontal Patients Undergoing Non-Surgical Periodontal Therapy and

Mouthwash Based on Ozonated Olive Oil: A Randomized Clinical Trial. Int J Environ Res Public Health 2020;17

7. Rajeshwaran, N. e Malaippan, S. (2020) “Avaliação de microcolônias bacterianas presentes em amostras de tecido obtidas usando água ozonizada como agente de irrigação de pacientes com periodontite”, Journal of Pharmaceutical Research International , 32(19), pp. 90–97. doi: 10.9734/jpri/2020/v32i1930713.

8. Shete A V, Subramaniam A V, Sable D M, Patil S V, Chavan M S, Shete M V, Ozone Therapy: Healing Properties of the Blue Gas. Int J Oral Health Dent 2016;2(1):35-3

9. Kshitish D, Laxman VK. The use of ozonated water and 0.2% chlorhexidine in the treatment of periodontitis patients: a clinical and microbiologic study. Indian J Dent Res 2010;21:341-348.

10. Hayakumo S, Arakawa S, Mano Y, Izumi Y. Clinical and microbiological effects of ozone nano-bubble water irrigation as an adjunct to mechanical subgingival debridement in periodontitis patients in a randomized controlled trial. Clin Oral Investig 2013;17:379-388.

11. Patel PV, Patel A, Kumar S, Holmes JC. Effect of subgingival application of topical ozonated olive oil in the treatment of chronic periodontitis: a randomized, controlled, double blind, clinical and microbiological study. Minerva Stomatol. 2012 Sep;61(9):381-98. PMID: 22976566.

12. Giuliani G., Ricevuti G., Galoforo A., Franzini M. (2018). Aspectos microbiológicos do ozônio: atividade

bactericida e resistência a antibióticos/antimicrobianos em cepas bacterianas tratadas com ozônio . Ozone Ther. 3 :7971. doi: 10.4081/ozone.2018.7971

13. Elvis AM, Ekta JS. Ozone therapy: A clinical review. J Nat Sci Biol Med 2011;2:66-70

14. Boch T, Tennert C, Vach K, Al-Ahmad A, Hellwig E, Polydorou O. Effect of gaseous ozone on Enterococcus faecalis biofilm-an in vitro study. Clin Oral Investig 2016;20:1733-1739.

15. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, Moher D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med. 2009 Jul 21;6(7):e1000100. doi: 10.1371/journal.pmed.1000100. Epub 2009 Jul 21. PMID: 19621070; PMCID: PMC2707010.

16. Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, Savovic J, Schulz KF, Weeks L, Sterne JA; Cochrane Bias Methods Group; Cochrane Statistical Methods Group. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011 Oct 18;343:d5928. doi: 10.1136/bmj.d5928. PMID: 22008217; PMCID: PMC3196245.

17. Al Habashneh R, Alsalman W, Khader Y. Ozone as an adjunct to conventional nonsurgical therapy in chronic periodontitis: a randomized controlled clinical trial. J Periodontal Res 2015;50:37-43.

18. Ranjith A, Niranjana JM, Baiju KV. Adjunctive benefit of ozonized water irrigation with mechanical debridement in the management of Stage III periodontitis: A randomized controlled clinical and biochemical study. Int J Dent Hyg 2022;20:364-370.

19. D Ambrosio F, Caggiano M, Acerra A, Pisano M, Giordano F. Is Ozone a Valid Adjuvant Therapy for Periodontitis and Peri-Implantitis? A Systematic Review. J Pers Med. 2023 Apr 8;13(4):646. doi: 10.3390/jpm13040646. PMID: 37109032; PMCID: PMC10143404.

20. Moraschini V, Kischinhevsky ICC, Calasans-Maia MD, Shibli JA, Sartoretto SC, Figueredo CM, Granjeiro JM. Ineffectiveness of ozone therapy in nonsurgical periodontal treatment: a systematic review and metaanalysis of randomized clinical trials. Clin Oral Investig. 2020 Jun;24(6):1877-1888. doi: 10.1007/s00784-020-03289-2. Epub 2020 May 12. PMID: 32399735.

21. Dallapria LC, Cardoso V, Gambin DJ, Oliveira A De, Wagner MC, Carli JP De. Aqueous versus gaseous ozone , what is the most effective adjunct to periodontal treatment ? A systematic review. 2023;90–101.

22. Seydanur Dengizek E, Serkan D, Abubekir E, Aysun Bay K, Onder O, Arife C. Evaluating clinical and laboratory effects of ozone in non-surgical periodontal treatment: a randomized controlled trial. J Appl Oral Sci 2019;27:e20180108.

23. Galdeano MC, Wilhelm AE, Goulart IB, Tonon RV, Freitas-silva O, Germani R, et al. Effect of water temperature and pH on the concentration and time of ozone saturation. Braz. J. Food Technol.2018;1-7.

Table 1 – Results.

Title	Year	Type of study	Objective	Type of treatment	Conclusion
Clinical and microbiological effects of ozone nano-bubble water irrigation as an adjunct to mechanical subgingival debridement in periodontitis patients in a randomized controlled trial HAYAKUMO et al. (10)	2013	Randomized Clinical Trial	To evaluate the clinical and microbiological effects of NBW3 irrigation as a complement to subgingival debridement for periodontal treatment.	GT: RAR + Nano bubbles of ozonized water. GC:RAR + water.	The reductions in mean PPD from baseline to 4 and 8 weeks in the NBW3 (ozonated water) group were significantly greater than those in the water group. Just the The NBW3 group showed statistically significant reductions in the average total number of bacteria in subgingival plaque

					during the study period.
The use of ozonated water and 0.2% chlorhexidine in the treatment of periodontitis patients: a clinical and microbiological study (9)	2010	Randomized Clinical Trial	Evaluation and comparison of the effects of oral irrigation with ozonated water and 0.2% chlorhexidine on clinical parameters such as Plaque Index, Gingival Index and Gingival Bleeding Index.	GT: RAR + Ozonated water GC: RAR + CHX 0.2%	Ozone can be considered an alternative management strategy due to its powerful ability to inactivate microorganisms. A greater percentage reduction in plaque index (12%), gingival index (29%), and bleeding index (26%) was observed with ozone irrigation compared to chlorhexidine.

Ozone as an adjunct to conventional nonsurgical therapy in chronic periodontitis: a randomized controlled clinical trial. AL HABASHNEH et al. (17)	2015	Randomized Clinical Trial	Determine the clinical and biological effects of the adjuvant use of ozone in non-surgical procedures in periodontal treatment.	GT: RAR + ozonated water GC: RAR + distilled water.	Irrigation with ozonated water as an adjunctive therapy to ARR did not produce any statistically significant benefit compared to ARR with distilled water irrigation. There was statistically significant improvement in study parameters in both groups between T0 and T1, except gingival index.
Adjunctive benefit of ozonized	2022	Randomized	Discover the benefits of ozonated	GT: RAR +	The average clinical insertion gain

water irrigation with mechanical debridement in the management of Stage III periodontitis: A randomized controlled clinical and biochemical study. RANJITH et al.		Clinical Trial	water irrigation together with non-surgical periodontal therapy for the treatment of periodontitis.	ozonated water GC: RAR + distilled water.	in moderate and deep pockets was significantly greater in the test group (p <0.01).

Source: By the author himself, 2023. Caption: CG: control group; GT: test group; RAR= scaling and root planing; NBW3= ozonized water nano bubbles; PPD= probing depth; CHX= Chlorhexidine.

Table 2 – Characteristics of the selected studies.

Reference	**Year**	**Number of participants**	**Middle Ages**	**Diagnosis**	**Probing depth**	**Most effective agent**
HAYAKUMO et al. (10)	2013	N= 21 GT n=10 GC n= 11	45.9 ± 14.8 years	Chronic periodontitis	≥ 4mm	Ozonated water
KSHITISH; LAXMAN (9)	2010	N = 16 GT n= 8GC n= 8	(20-60 years)	Chronic periodontitis	Did not provide	Ozonated water
AL HABASHNEH et al. (17)	2015	N= 41 GT: n=20GC: n=21	39.7 ± 13.7 years (GT) 39.0 ± 10.2 Years (GC)	Chronic periodontitis	> 5mm	Both, with no statistical difference.
RANJITH et al.	2022	N= 50 GT: n=25GC: n=25	49.23 ± 8.87 (GT) 47.55 ±	Stage III periodontitis	≥4mm	Ozonated water

			9.69 (GC)			

Source: From the author himself, 2023. Legend: N= population; n= sample; ±= standard deviation; CG: control group; GT: test group.

Figure 1 – Article selection flowchart.

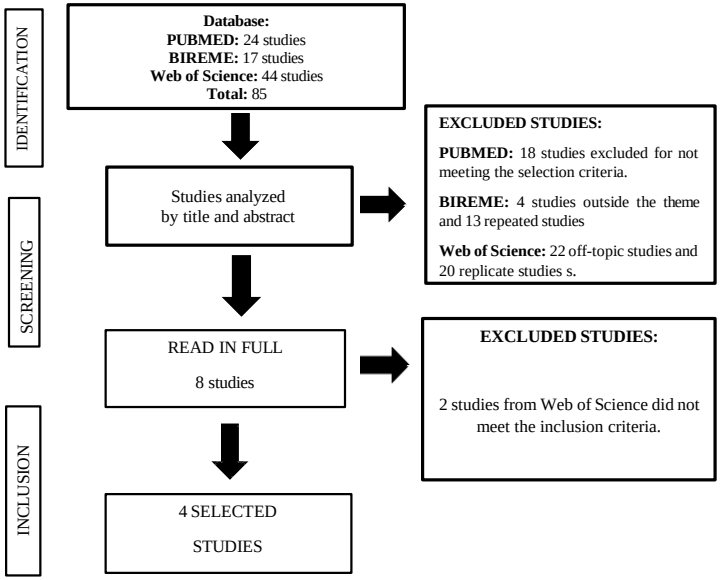


Figure 2 – Total risk of bias of selected articles.

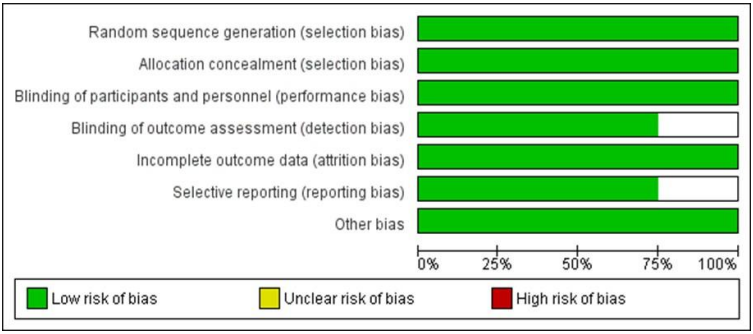


Figure 3 – Summary of risk of bias per study.

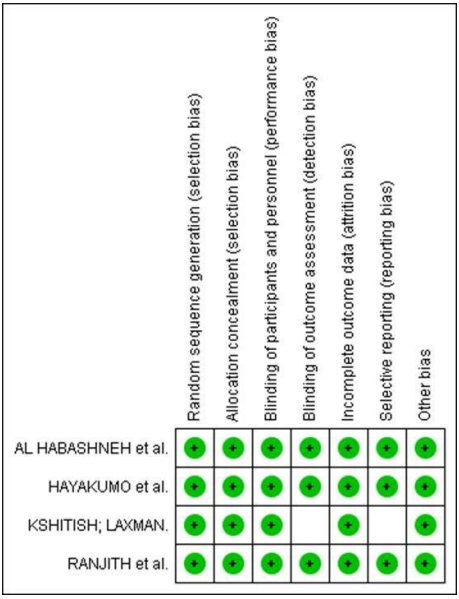


Figure 4- Meta-analysis of comparison between ozonated water group and control groups in bleeding after treatments.

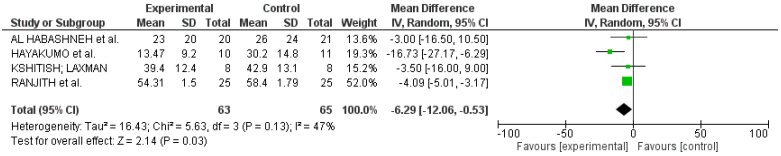
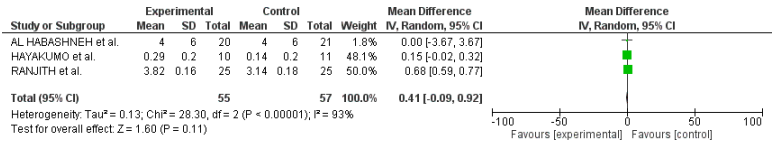


Figure 5 – Meta-analysis comparing the treatment group and the control group in reducing probing depth.



CONSIDERAÇÕES FINAIS

Nesse estudo, o uso da água ozonizada se mostrou eficaz no controle da halitose oral, bem como nos parâmetros clínicos da periodontite em comparação ao grupo placebo. No entanto, mais ensaios clínicos randomizados devem ser realizados, com protocolos bem definidos e maior tempo de acompanhamento.

REFERÊNCIAS

AHAD, A. *et al*. Effect of antimicrobial photodynamic therapy as an adjunct to nonsurgical treatment of deep periodontal pockets: a clinical study. *Journal of lasers in medical sciences*, v. 7, n. 4, p. 220-26, 2016.

AKAJI, E. A.; FOLARANMI, N.; ASHIWAJU, O. Halitosis: a review of the literature on its prevalence, impact and control. *Oral Health Prev. Dent*, v. 12, n. 4, p. 297-304, 2014.

ALSHEHRI, F. A. Knowledge and attitude of Saudi individuals toward self-perceived halitosis. *The Saudi Journal for Dental Research*, v. 7, n. 2, p. 91-95, 2016.

ANAGHA, V. S; SUBRAMANIAM, A. V.; SABLE, D. M.; PATIL, S. V.; CHAVAN M. S.; SHETE, M. V.; DIWAN, N. N. Ozone therapy: healing properties of the blue gas. I*nternational Journal of Dentistry*, v. 2, n. 1, p. 35-8, 2016.

ARMITAGE, G. C. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol*, v. 4, n. 1, p. 1-6, 1999.

AYLIKCI, B.U.; COLAK, H. Halitosis: From diagnosis to management. *J Nat Sci Biol Med*. v. 4, n. 1, p. 14-23, 2013.

BARIN, L.M. *et al*. Role of the adjunctive antimicrobial photodynamic therapy to periodontal treatment at plasmatic

oxidative stress and vascular behavior. *Journal of Photochemistry & Photobiology*, 173: 538–544, 2017.

BELEGOTE, I. S.; PENEDO, G. D. S.; SILVA, I. C. B. D.; BARBOSA, A. A.; BELO, M. T. D. N.; NETO, O. I. Tratamento de doença periodontal com ozônio. *Braz. J. Surg. Clin. Res*., v. 23, n. 2, p.101-104, 2018.

BIRANG, R. *et al*. Effect of Nonsurgical Periodontal Treatment Combined With Diode Laser or Photodynamic Therapy on Chronic Periodontitis: A Randomized Controlled Split-Mouth Clinical Trial. *J Lasers Med Sci*, v.6, p.112-119, 2015.

BOCH, T.; TENNERT, C.; VACH, K.; AL-AHMAD, A.; HELLWIG, E.; POLYDOROU, O. Effect of gaseous ozone on Enterococcus faecalis biofilm-an in vitro study. *Clin Oral Investig*, v. 20, n. 7, p. 1733-1739, 2016. Disponível em: https://doi.org/10.1007/S00784-015-1667-1. Acesso em 07 out 2021.

BOSY, A.; KULKARNI, G. V.; ROSENBERG, M.; MCCULLOCH, C. A. Relationship of oral malodor to periodontitis: evidence of independence in discrete subpopulations. *J. Periodontol*. v. 65, n. 1, p. 37–46, 1994.

CAMPANILE, V. *et al*. Single or repeated antimicrobial photodynamic therapy as adjunct to ultrasonic debridement in residual periodontal pockets: clinical, microbiological, and local biological effects. *Lasers in medical science*, v. 30, n. 1, p. 27-34, 2015

CATON, J.; ARMITAGE, G.; BERGLUNDH, T.; CHAPPLE, I. L. C.; JEPSEN, S.; KORNMAN, K. S.; MEALEY B. L.; PAPAPANOU, P. N.; SANZ, M.; TONETTI, M. S. A new classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification. *J Periodontol*. v. 45, (Suppl 20:S1-S8), 2018. Disponível em: https://onlinelibrary.wiley.com/doi/10.1111/jcpe.12935 Acesso em 01 ago 2021.

CASTILLO, A.; MESA, F.; LIE´BANA, J.; GARCI´A-MARTINEZ, O.; RUIZ, S.; GARCI´A-VALDECASAS, J.; O'VALLE, F. Periodontal and oral microbiological status of an adult population undergoing hemodialysis: a cross-sectional study. *Oral Diseases*, v. 13, n. 2, p. 198-205, 2007.

CONSELHO FEDERAL DE ODONTOLOGIA. Resolução CFO-166/2015: reconhece e regulamenta o uso pelo cirurgião-dentista da prática da Ozonioterapia. Rio de Janeiro: Diário

Oficial da União, 2015. P. 95. Disponível em: http://www.crors.org.br/legislacao/%5Bwww.crors.org.br%5Dresolucao_cfo_166-2015_-reconh_e_regula_uso_do_cd_ozonioterapia.pdf.

DANSER, M. M.; GÓMEZ, S. M.; VAN DER WEIJDEN, G. A. Tongue coating and tongue brushing: a literature review Int. *J. Dent. Hyg*. v. 1, n. 3, p. 151-8, 2003.

DE BOEVER, E. H.; LOESCHE, W. J. Assessing the contribution of anaerobic microflora of the tongue to oral malodor. *J Am Dent Assoc*. v. 126, n. 10, p. 1384-93, 1995.

DE GEEST, S., LALEMAN, I.; TEUGHELS, W.; DEKEYSER, C.; QUIRYNEN, M. Periodontal diseases as a source of halitosis: a review of the evidence and treatment approaches for dentists and dental hygienists. *Periodontology 2000*. v. 71, n. 1, p. 213-227, 2016.

DENGIZEK, S. E.; SERKAN, D.; ABUBEKIR, E.; BAY, K. A.; ONDER, O.; ARIFE, C. Evaluating clinical and laboratory effects of ozone in non-surgical periodontal treatment: a randomized controlled trial. *J Appl Oral Sci*. v. 27, s. n, p. e20180108, 2019. Disponível em: https://www.scielo.br/j/jaos/a/SxzHjRBZCt4JnPCYZWX8x8D/?lang=en Acesso em 05 ago 2023.

ELVIS, A. M.; EKTA, J. S. Ozone Therapy: A Clinical Review. *J Nat Sci Biol Med*, v. 2, n. 1, p. 66–70, 2017. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3312702/. Acesso em 07 out 2021.

FERNANDES, L. A. *et al*. Experimental periodontal disease treatment by subgingival
irrigation with tetracycline hydrochloride in rats. *Journal of Applied Oral Science*, v.
18, n. 6, p. 635-640, maio 2010.

GÓMEZ, S. M.; DANSER, M. M.; SIPOS, P. M.; ROWSHANI, B.; VAN DER VELDEN, U.; VAN DER WEIJDEN, G. A. Tongue coating and salivary bacterial counts in healthy/gingivitis subjects and periodontitis patients; *J. Clin. Periodontol*. v. 28, n. 10, p. 970–8, 2001.

HABASHNEH, A.; ALSALMAN, W.; KHADER, Y. Ozone as an adjunct to conventional nonsurgical therapy in chronic periodontitis: a randomized controlled clinical trial. *Journal of Periodontal Research*. v. 50, n. 1, p. 37-43, 2014.

HAYAKUMO, S.; ARAKAWA, S.; MANO, Y.; IZUMI, Y. Clinical and microbiological effects of ozone nano-bubble water irrigation as an adjunct to mechanical subgingival debridement in periodontitis patients in a randomized controlled trial. *Clinical Oral Investigations*. v. 17, n. 2, p. 379-88, 2013.

HUGHES, F. J.; MCNAB, R. Oral malodour - a review. *Arch Oral Biol* v. 53, (Suppl 1:S1-7), 2008. Disponível em: https://pubmed.ncbi.nlm.nih.gov/18460398/ Acesso em 25 julho 2021.

IWAUCHI, M.; HORIGOME, A.; ISHIKAWA, K.; MIKUNI, A.; NAKANO, M.; XIAO, J. Z.; ODAMAKI, T.; HIRONAKA, S. Relationship between oral and gut microbiota in elderly people. *Immun Inflamm Dis*. v. 7, n. 3, p. 229-36, 2019.

KATTI, S. S,; CHAVA,V. K. Effect of ozonised water on chronic periodontitis – a clinical study. *J Int Oral Health.* V. 5, n. 5, p. 79-84, 2013.

KSHITISH, D.; LAXMAN, V. K. The use of ozonated water and 0.2% chlorhexidine in the treatment of periodontitis patients: a clinical and microbiologic study. *Indian J Dent Res*. v. 21, n. 3, p. 341-8, 2010.

KUMAR, A., BHAGAWATI, S., TYAGI, P., & KUMAR, P. Current interpretations and scientific rationale of the ozone usage in dentistry: A systematic review of literature. *European Journal Of General Dentistry*, v. 3, n. 3, p. 175-180, 2014.

LALEMAN, I.; DADAMIO, J.; DE GEEST, S.; DEKEYSER, C.; QUIRYNEN, M. Instrumental assessment of halitosis for the general dental practitioner. *J Breath Res*. v. 8, n. 1, (s.p), 2014.

Disponível em: https://pubmed.ncbi.nlm.nih.gov/24566293/ Acesso em 24 julho 2021.

LANG, N. P.; BARTOLD, P. M. Periodontal health. *J Periodontol*. v. 89, Suppl 1:S9-S16, 2018. Disponível em: https://pubmed.ncbi.nlm.nih.gov/29926938/ Acesso em: 30 julho 2021.

LINDHE J. *Tratado de Periodontia Clínica e Implantologia Oral*. 5ed. Rio de Janeiro: Guanabara Koogan, 2010.

MOREO G.; MUCCHI, D.; CARINCI F. Efficacy ozone therapy in reducing oral infection of periodontal disease: a randomized clinical trial. *Biol Regul Homeost Agents*. v. 34, n. 4, p. 31-36, 2020.

MORITA, M.; WANG, H. L. Relationship between sulcular sulfide level and oral malodor in subjects with periodontal disease. *J Periodontol*. v. 72, n. 1, p. 79-84, 2001.

MOUSQUER, C. R.; BONA, A. D; MILANI, D.C.; FORNARI, F. *et al*. Are Lactobacillus salivarius G60 and inulin more efficacious to treat patients with oral halitosis and tongue coating than the probiotic alone and placebo? A randomized clinical trial. *Journal of Periodontology*, Chicago, v. 91, n. 6, p. 775-783, 2020.

NAGRAJ, S. K.; EACHEMPATI, P.; UMA, E.; SINGH, V. P.; ISMAIL, N. P.; VARGHESE, E. Interventions for managing halitosis. *Cochrane Database Syst Rev*. v. 11, n. 12, (s. p), 2019. Disponível em: https://pubmed.ncbi.nlm.nih.gov/31825092/ Acesso em 01 ago 2021.

NAIK, S. V.; K, R.; KOHLI, S.; ZOHABHASAN, S.; BHATIA, S. Ozone- A Biological Therapy in Dentistry- Reality or Myth? Open Dent J. v. 11, n. 10, p. 196-206, 2016.

NARDI, G. M.; CESARANO, F.; PAPA, G.; CHIAVISTELLI, L.; ARDAN, R.; JEDLINSKI, M. MAZUR, M.; GRASSI, R.; GRASSI, F. R. Evaluation of Salivary Matrix Metalloproteinase (MMP-8) in Periodontal Patients Undergoing Non-Surgical Periodontal Therapy and Mouthwash Based on Ozonated Olive Oil: A Randomized Clinical Trial. *International Journal of Environmental Research and Public Health*. v. 17, n. 18, (s. p), 2020.

ORTIZ, V.; FILIPPI, A. Halitosis. *Monogr Oral Sci*. v. 29, (s. n), p. 195-200, 2021.

PAPAPANOU, P. N.; SANZ, M.; BUDUNELI, N.; DIETRICH. T.; FERES, M.; FINE, D.H. *et al*. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification

of Periodontal and Peri-Implant Diseases and Conditions. *J Clin Periodontol*. 2018;45(Suppl 20):S162-70

PATEL, P. V.; PATEL, A.; KUMAR, S.; HOLMES, J. C. Effect of subgingival application of topical ozonated olive oil in the treatment of chronic periodontitis: a randomized, controlled, double blind, clinical and microbiological study. *Minerva Stomatol*. V. 61, n. 9, p. 381-98, 2012.

PERSSON, S.; EDLUND, M. B.; CLAESSON, R.; CARLSSON, J. The formation of hydrogen sulfide and methyl mercaptan by oral bacteria. *Oral Microbiol Immunol.* v. 5, n. 4, p. 195-201, 1990.

PIVA, A.; AVANTAGGIATO, P.; CANDOTTO, V.; PELLATI, A.; MOREO, G. The use of ozone therapy for treatment of periodontal disease: a split-mouth, randomized, controlled clinical trial. *J Biol Regul Homeost Agents*, v. 34, n. 3, p. 91-98, 2020.

RAHIMI-MOVAGHAR, V.; ESLAMI, V. The Major Efficient Mechanisms of Ozone Therapy are Obtained in Intradiscal Procedures. *Pain Physician*, v. 15, n. 6, p. 1007-8, 2012. Disponível em: https://pubmed.ncbi.nlm.nih.gov/23159972/ Acesso em 05 ago 2021.

RAJESHWARAN, N.; MALAIPPAN, S. Assessment of Bacterial Micro Colonies Present in Tissue Samples Obtained Using Ozonated Water as an Irrigating Agent from Patients with

Periodontitis. *Journal of Pharmaceutical Research International*. v. 32, n. 19, p. 90-97, 2020.

RAPONE, B.; FERRARA, E.; CORSALINI, M.; CONVERTI, I.; GRASSI, F. R.; SANTACROCE, L.; TOPI, S.; GNONI, A.; SCACCO, S.; SCARANO, A.; DELVECCHIO, M. The Effect of Gaseous Ozone Therapy in Conjunction with Periodontal Treatment on Glycated Hemoglobin Level in Subjects with Type 2 Diabetes Mellitus: An Unmasked Randomized Controlled Trial. Int. *J. Environ. Res. Public Health.* v. 17, n. 15, (s. p), 2020. Disponível em: https://pubmed.ncbi.nlm.nih.gov/32751340/ Acesso em 05 ago 2021.

RENVERT, S.; NOACK, M. J.; LEQUART, C.; ROLDÁN, S.; LAINE, M. L. The Underestimated Problem of Intra-Oral Halitosis in Dental Practice: An Expert Consensus Review. *Clin Cosmet Investig Dent*. v. 12, s. n, p. 251-262, 2020.

RIZZO, A. A. The possible role of hydrogen sulfide in human periodontal disease. 1. Hydrogen sulfide production in periodontal pockets. *Periodontics.* v. 5, n. 5, p. 233–236, 1967.

ROSENBERG, M.; MCCULLOCH, C. A. Measurement of oral malodor: current methods and future prospects. *Journal of Periodontology*. v. 63, n. 9, p.776- 82, 1992.

RÖSING, C.K.; LOESCHE, W. Halitosis: an overview of epidemiology, etiology and clinical management. *Braz Oral Res.* v. 25, n. 5, p. 466-71, 2011.

SCHIMIDT, J.; KRAUSE, F.; HAAK, R. Halitosis: measurement in daily practice. *Quintessence Int*. v. 46, n. 7, p. 633–641, 2015.

SEEMANN, R.; BIZHANG, M.; DJAMCHIDI, C.; KAGE, A.; NACHNANI, S. The proportion of pseudo-halitosis patients in a multidisciplinary breath malodour consultation. *Int Dent J*. v. 56, n. 2, p. 77-81, 2006.

SEEMANN, R.; CONCEICAO, M. D.; FILIPPI, A.; GREENMAN, J.; LENTON, P.; NACHNANI, S. Halitosis management by the general dental practitioner--results of na international consensus workshop. *J. Breath Res*. v. 8, n. 1, s. p, 2014. Disponível em: https://pubmed.ncbi.nlm.nih.gov/24566222/ Acesso em 05 ago 2021.

SEERANGAIYAN, K.; JÜCH, F.; WINKEL, E. G. Tongue coating: its characteristics and role in intra-oral halitosis and general health-a review. *J Breath Res*. v. 12, n. 3, p. 034001, 2018.

SHIBUYA, K. Constituents and origins of physiological malodor. *J Dent Hlth.* v. 51, n. 5, p. 778-92, 2001.

SKURSKA, A.; PIETRUSKA, M.; PANICZKO-DRĘŻEK, A.; DOLIŃSKA, E.; ŻELAZOWSKA-RUTKOWSKA, B.; ŻAK, J.; WYSOCKA, J. Evaluation of the influence of ozonotherapy on the clinical parameters and MMP levels in patients with chronic and aggressive periodontitis. *Advances in Medical Sciences*. v. 55, n. 2, p. 297-307, 2010.

SOMBIÈ, R.; TIENDRÉBÉOGO, A. J. F.; GUIGUIMDÉ, W. P. L.; GUINGANÉ, A.; TIENDRÉBÉOGO, S.; OUOBA, K.; BOUGOUMA, A. [Halitosis: multidisciplinary diagnostic and therapeutic approaches]. *Pan Afr Med J*. v. 30, n. 201, s. p, 2018. Disponível em: https://www.panafrican-med-journal.com/content/article/30/201/full/ Acesso em 30 julho 2021.

SOUZA, D. C.; COSTA, M. D. M. A.; NASCIMENTO, F.; MARTINS, V. M.; DIETRICH, L. Ozonioterapia em odontologia: E suas aplicabilidades. *Research, Society and Development*, v. 10, n. 6, p. e11410615517, 2021. Disponível em: https://rsdjournal.org/index.php/rsd/article/view/15517 Acesso em 07 ago 2021.

TASDEMIR, Z.; OSKAYBAS M. N.; ALKAN, A. B.; CAKMAK, O. The effects of ozone therapy on periodontal therapy: A randomized placebo-controlled clinical trial. *Oral Diseases*. V. 25, n. 4, p. 1195-1202, 2019.

YAEGAKI K., SANADA, K. Biochemical and clinical factors influencing oral malodor in periodontal patients. *J. Periodontol*. v. 63, n. 9, p. 783–9, 1992.

YANG, F.; HUANG, S.; HE, T.; CATRENICH, C.; TENG, F.; BO, C.; CHEN, J.; LIU, J.; LI, J.; SONG, Y.; LI, R.; XU, J. Microbial basis of Oral Malodor Development in humans. *J Dent Res*. v. 92, n. 12, p. 1106-12, 2013.

YILMAZ, S.; ALGAN, S.; GURSOY, H.; NOYAN, U.; KURU, B. E.; KADIR, T. Evaluation of the Clinical and Antimicrobial Effects of the Er:YAG Laser or Topical Gaseous Ozone as Adjuncts to Initial Periodontal Therapy. *Photomedicine and Laser Surgery*. V. 31, n. 6, p. 293-98, 2013.

YOUNG, A.; JONSKI, G.; ROLLA, G. Inhibition of orally produced volatile sulfur compounds by zinc, chlorhexidine or cetylpyridinium chloride: effect of concentration. *Eur J Oral Sci.* v. 111, n. 5, p. 400-404, 2003.

WINKEL, E. G.; ROLDÁN, S.; VAN WINKELHOFF, A. J.; HERRERA, D.; SANZ, M. Clinical effects of a new mouthrinse containing chlorhexidine, cetylpyridinium chloride and zinc-

lactate on oral halitosis. a dual-center, double-blind placebo-controlled study. *J Clin Periodontol*. v. 30, n. 4, p. 300-306, 2003.

APÊNDICES

APÊNDICE I - AVALIAÇÃO DE HALITOSE

Você está participando da pesquisa “EFEITO DA ÁGUA OZONIZADA NA HALITOSE DE PACIENTES COM DOENÇA PERIODONTAL: UM ENSAIO CLÍNICO RANDOMIZADO”. Você será examinado (a) pela parte da manhã, por favor, evite a ingestão de alho, cebola e condimentos nos 2 dias que antecedem o exame. Evite fumar e ingerir café e álcool nas últimas 12 horas que antecedem o exame. Na manhã do exame, você não poderá utilizar balas ou chicletes, mas será permitido um lanche e escovação com água, sem creme dental.

APÊNDICE II – TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

UNIVERSIDADE DE PASSO FUNDO

FACULDADE DE ODONTOLOGIA

Campus I – Bairro São José
CEP 99052-900 - Passo Fundo – RS

Fone: (54)3316-8395 (54)3316-8554

e-mail: ppgodonto@upf.br

Você está sendo convidado(a) a participar da pesquisa intitulada "**EFEITO DA ÁGUA OZONIZADA NA HALITOSE E NOS PARÂMETROS CLÍNICOS DE PACIENTES COM DOENÇA PERIODONTAL: UM ENSAIO CLÍNICO RANDOMIZADO**", de responsabilidade do pesquisador Me. Leonardo Saraiva. Caso não queira participar, não há problema algum. Você não precisa me explicar porque, e não haverá nenhum tipo de punição por isso.

A) JUSTIFICATIVA: Pacientes com doença periodontal têm prejuízos clínicos e sociais por apresentarem inflamação nos tecidos periodontais (gengiva), perda de dentes, comprometimento estético e funcional, e halitose (mau hálito). Há poucos estudos testando remédios antimicrobianos no controle do mau hálito decorrente desta doença. O tratamento hoje utilizado é a raspagem e o alisamento da raiz dos dentes afetados. O acréscimo de remédios antimicrobianos poderia auxiliar na

redução do mau hálito do paciente. Entre tais remédios, aquele à base de água ozonizada é um candidato, considerando-se seu benefício em doenças orais demonstrado em ensaios clínicos, além do bom perfil de segurança com aumento da cicatrização tecidual.

B) OBJETIVO: avaliar o efeito da água ozonizada comparada ao placebo combinados ao tratamento convencional durante 30 dias, no controle da halitose e nos parâmetros clínicos da doença periodontal.

C) PROCEDIMENTOS, LOCAL, DIA, HORA DA PESQUISA: Você vai fazer uma consulta com o Leonardo para avaliação da sua boca quanto à doença periodontal. Se for aprovado para o estudo, iniciará o tratamento convencional (raspagem e alisamento radicular) e será sorteado para receber água ozonizada ou placebo, que serão idênticos para evitar que você e o Leonardo saibam o que está usando (será revelado após o fim do estudo). As consultas serão duas por semana durante 30 dias. Os dias e horários das consultas seguirão a disponibilidade de agenda da instituição. A duração aproximada de cada consulta será de 1 hora e 30 minutos, aproximadamente.

D) POSSÍVEIS DESCONFORTOS E RISCOS: Durante os tratamentos você poderá sentir dor leve, pequenos sangramentos da gengiva e algum gosto ruim na boca, próprios do tratamento convencional. Poderá ter desconforto psicológico ao responder o questionário e analisar a halitose. No caso de manifestação de cansaço ou indisposição a entrevista ou as sessões serão interrompidas imediatamente. O pesquisador estará sempre disponível para controlar esses riscos. Em função da pandemia da COVID-19, todas as consultas seguirão normas de segurança para reduzir o risco de eventual contaminação.

E) BENEFÍCIOS: O tratamento irá melhorar sua saúde bucal e de todo o seu corpo, pois diminui a inflamação causada por essa. Acreditamos que a sua participação nesse estudo traz benefícios, pois permitirá aos pesquisadores conhecer mais sobre as condições clínicas e saúde bucal das pessoas que possuem mau hálito por doença periodontal e que irão se submeter ao tratamento. Você estará indiretamente auxiliando o possível desenvolvimento de estratégias para as necessidades em saúde bucal em indivíduos com mau hálito, e estará contribuindo para a tese de doutorado do Leonardo. Caso seja diagnosticado alguma injúria durante o exame clínico, você receberá orientações sobre como proceder.

F) ESCLARECIMENTOS: Você terá a garantia de receber esclarecimentos sobre qualquer dúvida relacionada à pesquisa e poderá ter acesso aos seus dados em qualquer etapa do estudo.

G) LIBERDADE: Sua participação nessa pesquisa não é obrigatória e você pode desistir a qualquer momento, retirando seu consentimento. A sua participação é voluntária e a recusa em participar não irá acarretar qualquer penalidade ou perda de benefícios.

H) SEM GASTOS E REMUNERAÇÃO: Caso tenha alguma despesa relacionada à pesquisa, você terá o direito de ser ressarcido (a). Você não receberá pagamento pela sua participação no estudo.

I) DIREITO A INDENIZAÇÃO: Caso ocorra eventual dano comprovadamente decorrente da sua participação na pesquisa, você tem o direito de buscar indenização.

J) SIGILO E DA PRIVACIDADE: Os dados serão registrados no próprio formulário de pesquisa e mantido sob sigilo. Seu nome ou qualquer dado de identificação não aparecerá em lugar nenhum, preservando sua identidade. Todas as amostras

biológicas e os resultados provenientes delas serão usados apenas para as finalidades descritas no protocolo de pesquisa e no termo de consentimento livre e esclarecido. Após a conclusão do estudo, qualquer material remanescente será destruído de acordo com as boas práticas clínicas.

K) DIVULGAÇÃO DOS RESULTADOS: Os resultados serão divulgados através de resumos apresentados em congressos e na forma de artigo científico, publicado em revista da área correspondente à doença periodontal. Seu nome não aparecerá nas publicações.

L) DÚVIDAS: Caso você tenha dúvidas sobre o comportamento dos pesquisadores ou sobre as mudanças ocorridas na pesquisa que não constam no TCLE, e caso se considera prejudicado (a) na sua dignidade e autonomia, você pode entrar em contato com o (a) pesquisador (a) telefone (54) 999999911, ou com o Programa de Pós-Graduação em Odontologia da Universidade de Passo Fundo (54) 3316-8395, ou também pode consultar o Comitê de Ética em Pesquisa da UPF, pelo telefone (54) 3316-8157, que funciona de segunda a sexta das 08h às 12h e das 13h30 às 17h30min.

Os pesquisadores garantem e se comprometem com o sigilo e a confidencialidade de todas as informações fornecidas por você para este estudo. Da mesma forma, o tratamento dos dados coletados seguirá as determinações da Lei Geral de Proteção de Dados (LGPD – Lei 13.709/18).

É garantido a você o direito a ressarcimento em caso de despesas comprovadamente relacionadas à sua participação no estudo, bem como, ao direito a indenização em caso de danos nos termos da lei. O operador dos dados será o pesquisador Leonardo Saraiva e o guardião desses dados será o prof. Dr. Fernando Fornari. Seus

dados serão mantidos sob segredo (gravados num computador com senha).

Dessa forma, se senhor (a) concorda em participar da pesquisa como consta nas explicações e orientações acima, coloque se nome no local indicado abaixo.

Desde já, agradecemos a sua colaboração e solicitamos a sua assinatura de autorização neste termo, que será também assinado pelo pesquisador responsável em duas vias, sendo que uma ficará com você e outra com o (a) pesquisador (a).

Passo Fundo, de _____de 202_.

Nome do (a) participante:
Assinatura:

Nome do (a)s pesquisador (a)s:

Me. Leonardo Saraiva Dr. Fernando Fornari (Orientador)

_____ _____

Assinatura Assinatura

Apêndice III – Questionários e Ficha Clínica

BLOCO A- PERFIL DEMOGRÁFICO E SOCIOECONÔMICO

Nome:_____

Telefone: ( ) _____( ) _____

Endereço: _____

Data da entrevista: _____/_____/_____

Entrevistador:_____ Examinador:_____

A01) Data de nascimento:____/____/____(_____idade)
A02) Sexo:

Masculino (1)
Feminino (2)

A03) Cor da Pele

Branca (0)
Preta (1)
Parda (2)
Amarela (3)
Indígena (4)

A04) Qual é o seu estado civil?

Solteiro (a) (0)
Casado (a) (1)
Divorciado/Separado (a) (2)
Viúvo (a) (3)
União estável (4)

A05) O(a) sr.(a) trabalha ou trabalhou alguma vez na vida?

Não, nunca (Pule para a questão A06) (0)
Trabalhou, mas não está trabalhando (1)
Sim, está trabalhando (2)
IGN (9)

O que o(a) Sr.(a) está fazendo atualmente?

Trabalhando (0)
Aposentado (1)
Aposentado, mas trabalhando (2)

Encostado (3) Do lar (4) Desempregado (5) NSA (8) IGN (9) Qual é (ou era) sua ocupação profissional? _____
A06) Até que série o(a) sr.(a) estudou? Nenhuma (0) 1ª até 3ª série (primário incompleto) (1) 4ª série (primário completo) ou 1º grau (ginasial) incompleto (2) 1º grau (ginasial) completo ou 2º grau (colegial) incompleto (3) 2º grau (colegial) completo ou nível superior incompleto (4) Nível superior completo (5) NSA (8) IGN (9)
A07) No mês passado, quanto (em reais) receberam as pessoas que moram na sua casa em seu trabalho principal, incluindo salários, bolsa família, pensão, aluguel, aposentadoria e outros rendimentos? Pessoa 1: R$ _____por mês Pessoa 2: R$ _____por mês Pessoa 3: R$ _____por mês Pessoa 4: R$ _____por mês Pessoa 5: R$ _____por mês (00000) Não recebeu (88888) NSA (99999) IGN

Critérios de exclusão Analfabetismo?

( )Sim ( )Não

Usou antibiótico nos últimos 90 dias?

( )Sim ( )Não

Fuma? ( )Sim ( )Não

Uso de Álcool? ( )Sim ( )Não

Gravidez/Amamentação?

( )Sim ( )Não

Doenças sistêmicas

Diabetes melitus?

( )Sim ( )Não

Insuficiência renal?

( )Sim ( )Não

Cirrose hepática?

( )Sim ( )Não

Periodontite:

( )Sim ( )Não

Halitose por Doença periodontal: ( )Sim ( )Não

Presença de saburra lingual: ( )Sim ( )Não

Se sim:

( ) Saburra fina em até 1/3 do dorso da língua (1)

( ) Saburra fina em mais de 1/3 do dorso da língua ou espessa em até 1/3 (2)

( ) Saburra espessa em mais de 1/3 do dorso da língua (3)

1ª Avaliação Data do exame:

Teste organoléptico:

( ) Ausência de cheiro (0) ( )Mau cheiro quase imperceptível (1)

( )Mau cheiro leve (2) ( )Mau cheiro moderado (3)

( )Mau cheiro forte (4) ( )Mau cheiro grave (5) Halímetro:

2ª Avaliação Data do exame:

Teste organoléptico:

( )Ausência de cheiro (0) ( )Mau cheiro quase imperceptível (1) ( )Mau cheiro leve (2) ( )Mau cheiro moderado (3)

( )Mau cheiro forte (4) ( )Mau cheiro grave (5)

Halímetro:

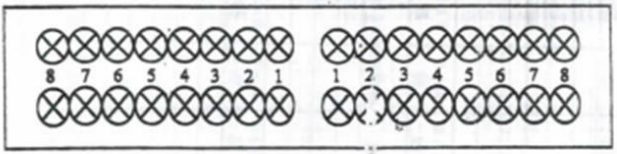
ANEXOS

ANEXO I – Exames Periodontais

UPF Universidade de Passo Fundo | FO - Faculdade de Odontologia

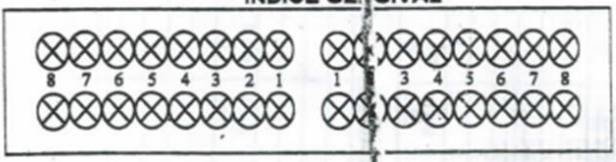
PERIOGRAMA

ÍNDICE DE BIOFILME



Data:____/_____/_____ Porcentagem:_____%

ÍNDICE GENGIVAL



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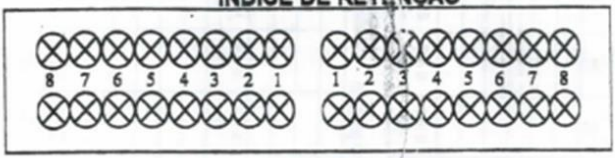
ÍNDICE DE RETENÇÃO



Data:____/_____/_____ Porcentagem:_____%

Diagnóstico Clínico Periodontal:_____

Prognóstico: _____

UPF Universidade de Passo Fundo | FO - Faculdade de Odontologia

PERIOGRAMA

V	18 D V M	17 D V M	16 D V M	15 D V M	14 D V M	13 D V M	12 D V M	11 D V M	21 M V D	22 M V D	23 M V D	24 M V D	25 M V D	26 M V D	27 M V D	28 M V D
Sang																
NG																
PS																
NI																
Mob																
Fur																
P	18 D P M	17 D P M	16 D P M	15 D P M	14 D P M	13 D P M	12 D P M	11 D P M	21 M P D	22 M P D	23 M P D	24 M P D	25 M P D	26 M P D	27 M P D	28 M P D
Sang																
NG																
PS																
NI																

Sang: **Sangramento à sondagem** NG: **Nível gengival** PS: **Profundidade de sondagem** NI: **Nível de inserção** Mob: **Mobilidade** Fur: **Furca**

V	48 D V M	47 D V M	46 D V M	45 D V M	44 D V M	43 D V M	42 D V M	41 D V M	31 M V D	32 M V D	33 M V D	34 M V D	35 M V D	36 M V D	37 M V D	38 M V D
Sang																
NG																
PS																
NI																
Mob																
Fur																
L	48 D L M	47 D L M	46 D L M	45 D L M	44 D L M	43 D L M	42 D L M	41 D L M	31 M L D	32 M L D	33 M L D	34 M L D	35 M L D	36 M L D	37 M L D	38 M L D
Sang																
NG																
PS																
NI																

Data do exame: __/__/____ **Assinatura do paciente:**_____ **Prontuário:**_____

ANEXO II - PARECER CONSUBSTANCIADO DO CEP

UNIVERSIDADE DE PASSO FUNDO/ VICE-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO - VRPPG/ UPF

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITO DA ÁGUA OZONIZADA NA HALITOSE E NOS PARÂMETROS CLÍNICOS DE PACIENTES COM DOENÇA PERIODONTAL: UM ENSAIO CLÍNICO RANDOMIZADO

Pesquisador: LEONARDO SARAIVA

Área Temática:

Versão: 1

CAAE: 56696822.5.0000.5342

Instituição Proponente: FUNDACAO UNIVERSIDADE DE PASSO FUNDO

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 5.324.346

Apresentação do Projeto:

A halitose é definida como a exteriorização de odores fétidos pela boca e reconhecida por pessoas que fazem parte do convívio do paciente. A etiologia deve-se principalmente as condições orais dos pacientes (80 a 90%), como a doença periodontal (DP) e saburra lingual, ou ainda por condições sistêmicas (10%). O uso da água ozonizada tem se mostrado promissor para diversas doenças orais, atuando como um agente antisséptico alternativo, que pode diminuir e controlar as bactérias que colonizam as bolsas periodontais e o sulco gengival.

Objetivo da Pesquisa:

Avaliar, em pacientes com doença periodontal tratados convencionalmente, o efeito da água ozonizada versus placebo no controle da halitose e nos demais parâmetros clínicos da doença periodontal.

Avaliação dos Riscos e Benefícios:

Segundo os pesquisadores, os riscos são dor leve, sangramentos controlados, gosto ruim na boca, ou desconforto psicológico ao responder o questionário e analisar a halitose. No caso de manifestação de cansaço ou indisposição a entrevista ou as sessões serão interrompidas imediatamente. O pesquisador estará sempre disponível para controlar esses riscos. Como

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benefício o tratamento irá melhorar a saúde bucal e de todo corpo, pois diminui a inflamação causada pela doença periodontal.

Comentários e Considerações sobre a Pesquisa:

Trata-se de um ensaio clínico randomizado, duplo-cego, com grupos paralelos, que terá duração de 4 semanas. Sessenta e dois pacientes com doença periodontal moderada a grave, identificados nos ambulatórios da Faculdade de Odontologia da UPF, farão parte da amostra. Os voluntários serão randomizados em 2 grupos de tratamento (n=31): G1 – tratamento convencional (RAR) e placebo; G2 – tratamento convencional (RAR) e água ozonizada. Os tratamentos serão realizados duas vezes por semana e os desfechos serão mensurados antes e depois do tratamento, que serão: halitose (teste organoléptico e halimetro); desfechos clínicos da DP e microbiota oral, por meio de reação em cadeia da polimerase (PCR) para diferenciar e quantificar espécies bacterianas periodontopatogênicas.

Considerações sobre os Termos de apresentação obrigatória:

O protocolo foi instruído e apresentado de maneira completa e adequada. Os compromissos do pesquisador e das instituições estavam presentes. O projeto foi considerado claro em seus aspectos científicos e metodológicos.

Recomendações:

Após o término da pesquisa, o CEP UPF solicita: a) A devolução dos resultados do estudo aos sujeitos da pesquisa ou a instituição que forneceu os dados; b) Enviar o relatório final da pesquisa, pela plataforma, utilizando a opção, no final da página "Enviar Notificação"+ relatório final.

Recomendações:

1) Adicionar ao TCLE, ao lado dos dados do CEP o texto: "O Comitê está localizado no Campus I da Universidade de Passo Fundo, na BR 285, Bairro São José, Passo Fundo/RS. O Comitê de Ética em pesquisa exerce papel consultivo e, em especial, educativo, para assegurar a formação continuada dos pesquisadores e promover a discussão dos aspectos éticos das pesquisas em seres humanos na comunidade."

2) Deixar o TCLE como um texto corrido, não em itens.

Conclusões ou Pendências e Lista de Inadequações:

Diante do exposto, este Comitê, de acordo com as atribuições definidas na Resolução n. 466/12, do Conselho Nacional da Saúde, Ministério da Saúde, Brasil, manifesta-se pela aprovação do

projeto de pesquisa na forma como foi proposto.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1909823.pdf	11/03/2022 17:51:27		Aceito
Outros	declaracaopesqnaoiniciada.doc	11/03/2022 17:50:52	LEONARDO SARAIVA	Aceito
Brochura Pesquisa	brochurapesquisa.doc	09/03/2022 20:36:30	LEONARDO SARAIVA	Aceito
Projeto Detalhado / Brochura Investigador	Projetodetalhado0903.pdf	09/03/2022 20:34:58	LEONARDO SARAIVA	Aceito
Outros	termousodadosconfid.pdf	09/03/2022 20:32:53	LEONARDO SARAIVA	Aceito
Orçamento	orcamentopesq.pdf	09/03/2022 20:26:47	LEONARDO SARAIVA	Aceito
Declaração de Pesquisadores	declaracaopesquisad.pdf	09/03/2022 20:25:27	LEONARDO SARAIVA	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	declaracaobio.pdf	09/03/2022 20:23:39	LEONARDO SARAIVA	Aceito
Cronograma	Cronograma.pdf	09/03/2022 20:22:03	LEONARDO SARAIVA	Aceito
Declaração de Instituição e Infraestrutura	TERMOINFRA.pdf	09/03/2022 20:16:38	LEONARDO SARAIVA	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE090322.pdf	09/03/2022 20:15:57	LEONARDO SARAIVA	Aceito
Folha de Rosto	FolhadeRostoCEP.pdf	09/03/2022 20:11:14	LEONARDO SARAIVA	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:

Não

PASSO FUNDO, 31 de Março de 2022

Assinado por:
Felipe Cittolin Abal
(Coordenador(a))

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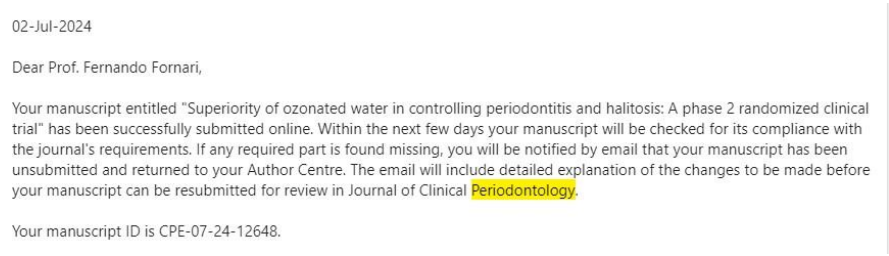
ANEXO III- NORMAS REVISTA Journal Of Clinical Periodontology

https://onlinelibrary.wiley.com/page/journal/1600051x/homepage/forauthors.html

Comprovante de Submissão à Revista:


02-Jul-2024

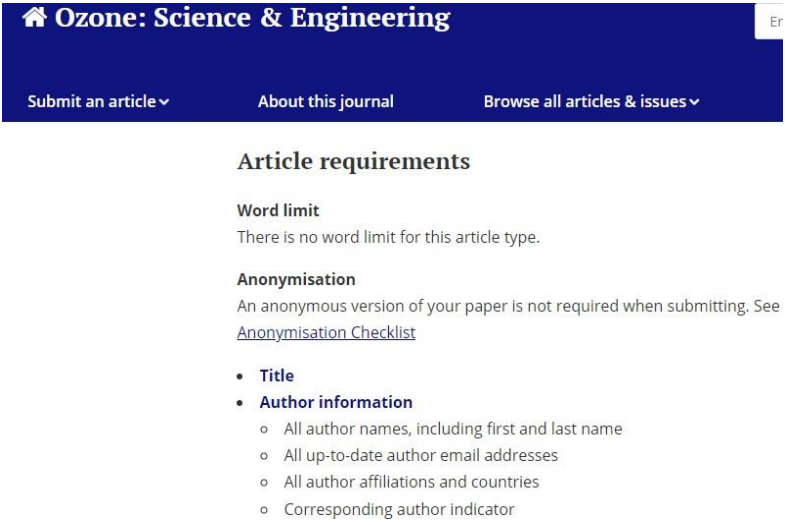
Dear Prof. Fernando Fornari,

Your manuscript entitled "Superiority of ozonated water in controlling periodontitis and halitosis: A phase 2 randomized clinical trial" has been successfully submitted online. Within the next few days your manuscript will be checked for its compliance with the journal's requirements. If any required part is found missing, you will be notified by email that your manuscript has been unsubmitted and returned to your Author Centre. The email will include detailed explanation of the changes to be made before your manuscript can be resubmitted for review in Journal of Clinical Periodontology.

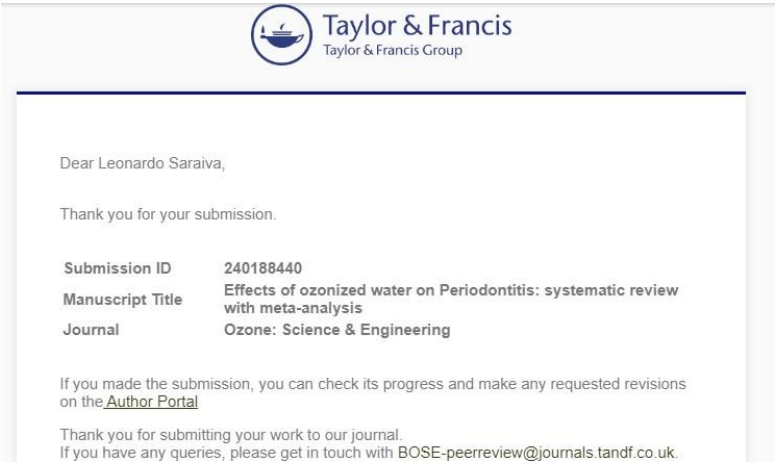
Your manuscript ID is CPE-07-24-12648.

ANEXO IV- NORMAS REVISTA Ozone: Science & Engineering

https://www.tandfonline.com/action/authorSubmission?show=instructions&journalCode=bose20



Comprovante de submissão à Revista:



Taylor & Francis
Taylor & Francis Group

Dear Leonardo Saraiva,

Thank you for your submission.

Submission ID	**240188440**
Manuscript Title	**Effects of ozonized water on Periodontitis: systematic review with meta-analysis**
Journal	**Ozone: Science & Engineering**

If you made the submission, you can check its progress and make any requested revisions on the Author Portal

Thank you for submitting your work to our journal.
If you have any queries, please get in touch with BOSE-peerreview@journals.tandf.co.uk.