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Research Protocol

Dentistry Section

Evaluation of Ozonated Water and Ozonated Olive Oil as an Adjunct to Non Surgical Periodontal Therapy in Chronic Periodontitis: A Research Protocol

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ABSTRACT

Introduction: Periodontitis is characterised by inflammation of the supporting tissues of the teeth, leading to progressive destruction of the periodontal ligament and alveolar bone, which results in the formation of periodontal pockets and gingival recession. The primary goal of periodontal therapy is to eliminate these pathological features. While Scaling and Root Planing (SRP) is a widely accepted treatment, it has limitations, such as difficulty accessing deeper pockets and root concavities.

Need of the study: Ozone therapy, known for its antimicrobial and anti-inflammatory properties, has gained attention as a potential adjunctive treatment in the management of Chronic Periodontitis (CP). Despite growing interest in these treatments, limited research has directly compared the effectiveness of ozonated water and ozonated olive oil in combination with SRP for treating CP. The present study seeks to fill the gap in the literature by assessing and comparing the clinical outcomes when ozonated water or ozonated olive oil is applied as an adjunct to SRP.

Aim: This study aims to assess the efficacy of ozonated water and ozonated olive oil as adjuncts to non surgical periodontal therapy, specifically SRP, in treating CP.

Materials and Methods: A double-blind, randomised controlled clinical trial will be conducted in the Outpatient Department of Periodontics at Datta Meghe Institute of Higher Education and Research, Wardha, Maharashtra, India. from June 2025 to June 2026. A total of 30 participants will be divided into two groups: Group 1 will receive SRP followed by ozonated water application, while Group 2 will receive SRP followed by ozonated olive oil application. The participants will be assigned to each group by coin-flip randomisation. Pocket probing depth, plaque index, papillary bleeding index, and Clinical Attachment Level (CAL) will be assessed at each follow-up interval. Unpaired and paired t-tests will be used to evaluate data from baseline, 1-month, and 3-months for each group. A p-value of less than 0.05 will be deemed significant.

Keywords: Oral prophylaxis, Oxygen, Periodontal disease, Pocket probing depth, Sub-gingiva

INTRODUCTION

Periodontitis is an inflammatory condition affecting the periodontium, caused by specific microbial groups. It leads to the gradual deterioration of the periodontal ligament and alveolar bone, which can result in gingival recession, periodontal pockets, or both [1]. Common indicators of periodontitis include gingival inflammation, clinical attachment loss, radiographic evidence of alveolar bone loss, deep probing sites, mobility, bleeding on probing, and pathologic migration [2].

The SRP is the most widely used and clinically recognised treatment for periodontitis. However, SRP has limitations, such as difficulties in reaching deep pockets, root concavities, and furcation areas. Traditionally, mechanical debridement has been combined with chemotherapeutic agents for more effective periodontal management [3]. Ozone, composed of three oxygen atoms, is naturally present in the stratosphere in concentrations ranging from 1 to 10 parts per million [4]. It is continuously formed and decomposed into oxygen molecules. In dentistry, ozone is being explored as an antiseptic due to its strong antibacterial properties and lack of drug resistance [5]. Among available antiseptic agents, ozone exhibits the highest level of biocompatibility. Its additional oxygen atom enhances its antibacterial efficacy while also improving tissue blood circulation and immune response [6]. Ozone has demonstrated effectiveness against both Gram-positive and Gram-negative oral bacteria, supporting its long-standing use in medicine [7].

Ozone therapy can be administered in various forms, with gaseous and aqueous applications being the most common [8]. In-vitro studies have confirmed the antibacterial efficacy of ozonated water against both Gram-positive and Gram-negative oral bacteria [9,10]. Multiple clinical investigations have reported its effectiveness against significant periodontal pathogens such as *Tannerella forsythia, Porphyromonas gingivalis*, and *Actinobacillus actinomycetemcomitans* [9-11].

Ozonated olive oil, produced by infusing ozone into olive oil to a concentration of about 5% ozone in oil, transforms it from a greenish liquid into a white gel. Research has demonstrated its antimicrobial activity against Gram-positive and Gram-negative bacteria, viruses, and fungi. In periodontology, ozonated olive oil is primarily used for the prevention and management of gingivitis, periodontitis, peri-implantitis, and wound healing [12].

Thus, the present study aims to evaluate and compare the effectiveness of ozonated water and ozonated olive oil as adjuncts to non surgical treatment for CP.

Primary objectives:

1. To evaluate the effect of ozonated olive oil as an adjunct to non surgical management of CP.
2. To evaluate the effect of ozonated water as an adjunct to non surgical management of CP.

Secondary objectives: To compare the effect of ozonated olive oil versus ozonated water as adjuncts to non surgical management of CP in terms of PPD reduction and CAL gain.

Null hypothesis: There will be no significant difference in the efficacy of ozonated olive oil versus ozonated water as adjuncts to non

surgical management of CP in terms of PPD reduction and CAL gain.

Alternate Hypothesis: There will be a significant difference in the efficacy of ozonated olive oil versus ozonated water as adjuncts to non surgical management of CP in terms of PPD reduction and CAL gain.

REVIEW OF LITERATURE

Periodontal disease is a complex infectious condition resulting from bacterial accumulation and the host's immune response to infection. It is influenced by environmental factors, acquired risk elements, and genetic predisposition [13,14]. Anaerobic bacteria play a significant role in periodontal tissue destruction, making bacterial reduction a primary objective of periodontal treatment. SRP, performed manually or ultrasonically, is generally effective in reducing bacterial load and preventing tissue damage. However, deep periodontal pockets, anatomical variations, and inaccessible sites pose challenges to complete bacterial elimination, highlighting the necessity for antimicrobial adjuncts to SRP [15]. Adjunctive antibiotics can be administered systemically or locally, though systemic antibiotics have drawbacks such as bacterial resistance and strain development [16].

Kwon T et al., emphasised that effective periodontitis prevention and treatment require precise diagnosis, elimination of underlying causes, and mitigation of risk factors. Following initial non surgical therapy, which includes SRP and home-care evaluation, residual areas with active periodontitis may be addressed using traditional reconstructive surgery or contemporary regenerative procedures [17].

Ameyaroy DK et al., conducted a split-mouth longitudinal study comparing the effectiveness of photodynamic therapy and ozone therapy in the non surgical treatment of CP. Although there was no statistically significant difference in clinical parameters between the two therapies at two, four, or six months, ozone therapy produced slightly better clinical outcomes. These findings suggest that ozone therapy and photodynamic therapy are both effective non surgical treatments for CP, with ozone therapy consistently yielding superior results [18].

In a microbiological and clinical investigation, Kshitish D and Laxman VK. examined the use of ozonated water and 0.2% chlorhexidine in periodontitis treatment. They concluded that ozone's potent bactericidal properties make it a viable alternative treatment method. There is growing evidence supporting its effectiveness against bacterial, viral, and fungal pathogens, for both professional and home-care applications. Ozone therapy's unique properties and lack of adverse effects indicate its potential for CP treatment [19].

Therefore, this study aims to assess and compare the clinical efficacy of two distinct antimicrobial adjuncts ozonated water and ozonated olive oil when used as local adjuncts to SRP in the management of CP.

MATERIALS AND METHODS

A double-blind, randomised controlled clinical trial will be conducted at Datta Meghe Institute of Higher Education and Research, Wardha, Maharashtra, India from June 2025 to June 2026. Permission from the Institutional Ethics Committee (IEC). has been obtained prior to conducting the study, with IEC number: DMIHER(DU)/IEC/2024/254, and the trial registration has been completed on the Clinical Trials Registry India (CTRI) portal with registration CTRI/2024/06/068534. A written informed consent form will be read, understood, and signed by the participants.

Inclusion criteria: Participants will be those receiving treatment in the Department of Periodontics and Implantology at Datta Meghe Institute of Higher Education and Research or referred to the Institute, and who have periodontal pockets of 4 mm or more in atleast 10 permanent teeth. All clinical procedures will be completed within one year of the start of the study.

Exclusion criteria:

1. Patients who have undergone periodontal therapy in the last six months
2. Individuals treated with antibiotics within the last three months
3. Individuals who have allergies to the study drugs
4. Pregnant or lactating women
5. Regular users of mouthwash
6. Chronic alcohol users

Sample size calculation:

The sample size formula used is:

$$n_1 = \frac{(\sigma_1^2 + \sigma_2^2 / \kappa)(z_{1-\alpha/2} + z_{1-\beta})^2}{\Delta^2}$$

The notation for the formula is:

n_1: Sample size for group 1

n_2: Sample size for group 2

Mean PPD in Ozone Therapy = 2.58

Mean PPD in PDT = 2.8

$\sigma 1$= SD of PPD in Ozone Therapy = 0.33

$\sigma 2$= SD of PPD in PDT=0.28 (21)

For detecting mean difference of 0.22 i.e., Δ =2.8-2.58 = 0.28 [18]

K =1

$$N = \frac{(0.33*0.33 + 0.28*0.28)\ (1.96+0.84)^2}{0.22*0.22}$$

=30.33 =15 patients needed in each group

Level of significance: 5% (95% confidence interval)

The present study involves 30 participants, each receiving a designated treatment after completing scaling and root planing. Participants are assigned to either Group 1 (n = 15), where ozonated water will be used for irrigation, or Group 2, where ozonated olive oil will be applied. To minimise bias, a double-blind design is employed, and randomisation is carried out using a coin-flip method. An Independent researcher, not involved in procedural implementation, will generate the allocation sequence.

Study Procedure

Prior to data collection and evaluation, all researchers will undergo training to ensure adherence to standardised procedures and outcome measures. The UNC-15 probe will be utilised for assessing periodontal clinical parameters, including plaque index, bleeding on probing, Probing Depth (PD), pocket depth, and CAL. Patients will receive guidance on proper brushing techniques and demonstrations of effective oral hygiene practices.

During the first appointment, full-mouth supra-gingival scaling will be performed. One week later, at the second appointment, sub-gingival root planing will be conducted under local anaesthesia using 2% lidocaine with epinephrine (1:100,000), utilising Gracey curettes (Hu-Friedy).

Following subgingival SRP, treatment protocols will vary between groups. For Group 1, sites with a probing depth of ≥4 mm will be irrigated for five to 10 minutes using 150 mL of ozonated water, administered via a blunt-tipped sterile plastic syringe. For Group 2, a similar approach will be used, but ozonated olive oil will be applied instead. In both groups, ozone-based treatments will be delivered subgingivally immediately following SRP [20].

Patients will be advised to avoid interdental aids, brushing near the treated area, and consuming hard or sticky foods for one week. Any adverse effects will be documented during recall visits, and any supragingival deposits present will be removed. Recall visits are scheduled for one month and three months, during which plaque

index, bleeding index, pocket probing depth, and clinical attachment loss will be reassessed.

The consent process will be managed by a single researcher—a dentist responsible for treatment administration. Patients will receive comprehensive information about the study's objectives, procedures, potential benefits, and risks. They will be informed that participation is voluntary and that they may withdraw at any time without consequences. If a participant experiences unexpected discomfort during the session, treatment will be discontinued, and they will be withdrawn from the study while continuing to receive monitoring for safety assessments. No concurrent treatments are required or restricted throughout the trial. While no serious side effects are anticipated, possible symptoms such as gum or tooth pain will be tracked, and additional care will be provided as needed.

Outcomes

Primary outcomes: Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL).

Secondary outcomes: Plaque Index and Papillary Bleeding Index.

Measurement methods:

- The PPD: Measured with an UNC-15 probe using an acrylic stent. The probe tip will be placed at the deepest part of the interproximal pocket within the gingival crevice, and the contact point with the stent will be recorded as the reference.
- The CAL: Determined with the UNC-15 probe positioned at the pocket base.
- Plaque Index: Calculated according to the Turesky, Gilmore, and Glickman modification of the Quigley-Hein index (1970) [21].
- Papillary Bleeding Index: Evaluated using Muhlemann's Papillary Bleeding Index (1971) [22].

Assessment schedule: All clinical measurements will be recorded at baseline and at 3-months. Plaque Index (to assess supragingival plaque accumulation) will be assessed at the 1-month follow-up, though probing will not be conducted at that visit.

STATISTICAL ANALYSIS

Statistical software: Statistical Packages of Social Sciences (SPSS) version 15.0 (SPSS, Chicago, USA).Descriptive statistics: Means and Standard Deviations (SD) will be calculated.Inferential statistics: Unpaired and paired t-tests will be used to evaluate data from baseline, 1 month, and 3-months for each group. A p-value <0.05 will be considered statistically significant.

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