TITLE

Comparative analysis of photobiomodulation therapy and transcutaneous electrical nerve stimulation for burning mouth: a randomized clinical trial

NCT number

Trial registration number (TRN) and date of registration: This clinical trial was registered at clinicaltrials.gov (Number: NCT05816200).

DATE: May 13, 2024.

STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

1. Study design

This randomized clinical trial involved patients with burning mouth seen at the Stomatology Service of the Department of Dentistry, Federal University of Rio Grande do Norte (UFRN), Natal-RN, Brazil, from April 2021 to February 2022. The study was approved by the Ethics Committee of UFRN (Approval number 4.066.032) and was conducted in accordance with the Declaration of Helsinki. This clinical trial followed the Consort Statement (http://www.consort-statement.org) and was registered at clinicaltrials.gov (Number: NCT05816200).

2. Participants

Patients older than 18 years who had a burning or dysesthetic sensation in the oral mucosa that recurred daily for more than 2 hours per day for more than 3 months, in the absence of clinically evident causal lesions [4], were included in the study. We also included patients with hyposalivation or any systemic alteration that could be related to a burning sensation in the oral mucosa but who did not have clinical alterations in the oral mucosa such as erosive lichen planus, geographic tongue, infections, allergic reactions, trauma, or injuries caused by physical or chemical agents. Patients who did not comply with the treatment protocol of the study were excluded from the sample.

Before the beginning of treatment, clinical and sociodemographic data of the patients were collected and blood tests were requested (complete blood count, fasting blood glucose, total cholesterol and fractions, triglycerides, ferritin, vitamin B12, folic acid, zinc, thyroxine, triiodothyronine, and antinuclear antibodies).

3. Interventions

In the study group, a portable TENS 7000 device (Roscoe Medical, Middleburg Heights, OH, USA) was used for stimulation. The device was calibrated for a frequency of 50 Hz and its intensity was modulated according to the patient's comfort (pulse amplitude of 1-4). Each session lasted 25 minutes. The self-adhesive electrodes were connected to the channels of the device and attached to the patient's skin of both facial sides next of the major salivary glands (parotid and submandibular glands), passing through the mandibular branch of the trigeminal

nerve as shown in figure 1. The patients were submitted to the respective treatment protocol once a week for eight consecutive weeks, totaling 8 treatment sessions.

In the control group, photobiomodulation was applied using a laser whose parameters are represented in table 1. During laser application, the patients and the clinician wore the protective glasses (figure 2).

4. Outcomes

Pain/burning was the primary outcome and was measured on a visual analogue scale (VAS; 0-10 cm). The patients were asked to choose a number from 0 to 10 on the scale to describe the level of intensity of their symptoms.

Changes in unstimulated salivary flow, xerostomia, and dysgeusia were the secondary outcomes. Saliva was collected as described by Navazesh and Kumar [26] and patients with an unstimulated salivary flow rate < 0.1 ml/min were classified as having hyposalivation.

Information on xerostomia was collected according to the method of Murray Thomson et al. (2006) using a standard question and the Xerostomia Inventory (XI). Patients answered the following question: “How often does your mouth feel dry?” (response options: “never”, “occasionally”, “frequently”, “always”). On each occasion, those who gave either of the latter two responses were categorised as xerostomic. The XI was used to obtain information on the severity of xerostomia symptoms, being an 11-item summated rating scale which combines the responses to 11 individual items into a single continuous scale score which represents the severity of chronic xerostomia, and higher scores represent more severe symptoms. The patients were asked to choose one of five responses (“never”, scoring 1; “hardly ever”, 2; “occasionally”, 3; “fairly often”, 4 and “very often”, 5) to the following statements referring to the previous 4 weeks: “my mouth feels dry”; “my lips feel dry”; “I get up at night to drink”; “my mouth feels dry when eating a meal”; “I sip liquids to aid in swallowing food”; “I suck sweets or cough lollies to relieve dry mouth”; “my throat feels dry”; “the skin of my face feels dry”; “my eyes feel dry”; “my lips feel dry” and “the inside of my nose feels dry”. Each individual's responses were scored and summed to give a single XI score.

Dysgeusia was analyzed using an adaptation of the method proposed by Muller et al. [28], in which the four basic tastes at four different concentrations are tested. Urea was used as bitter taste as proposed by Henn et al. [29] (sucrose solution: 0.4 g/mL, 0.2 g/mL, 0.1 g/mL, 0.005 g/mL; sodium chloride: 0.25 g/mL, 0.1 g/mL, 0.04 g/mL, 0.016 g/mL; citric acid: 0.3 g/mL, 0.165 g/mL, 0.09 g/mL, 0.05 g/mL; urea: 0.005 g/mL, 0.04 g/mL, 0.02 g/mL, 0.01 g/mL). Filter paper strips were impregnated with the test solutions as proposed by Kettenmann et al.

[30]. The strips were placed in the center of the tongue and the patient was asked to identify the taste. The 10th percentile was used to define the presence or absence of dysgeusia.

The primary and secondary outcomes were collected at baseline (T0), after the 4th treatment session (T1), after the 8th treatment session (T2), and 30 days after the end of treatment (T3).

5. Sample size

The sample size was calculated according to Sikora et al. [31] who compared the efficacy of PBM to a placebo group (laser off). In that study, the mean and standard deviation (SD) of the VAS score in the placebo group at the end of treatment was 2.417 ± 2.918. Considering that acceptable improvement in the PBM group would be at least 10% after the intervention, we obtained an effect value (E) = 2.17. The sample size was then calculated using a t-test for comparing the means of continuous variables, whose standardized effect size was 0.70 assuming a one-sided alpha error of 0.05 and a beta error of 0.20, resulting in 28 patients divided into two intervention groups.

6. Randomization

After sample size calculation and selection of the sample according to the inclusion criteria, the patients were randomized to one of the two treatments (TENS or PBM) using an allocation software (https://www.graphpad.com/quickcalcs/randomize1/). This program did not randomly choose a treatment for each patient but randomly shuffled a defined number of patients among a defined number of treatment slots.

7. Blinding

Blinding was not possible since the devices used for the interventions performed in the study and control groups were different.

8. Statistical analysis

All data were analyzed using the Statistical Package for the Social Sciences 25.0 (SPSS). First, the collected data were submitted to descriptive analysis. Fisher's exact test was then used to determine whether statistically significant differences in systemic alterations, psychiatric disorders and medication use exist at T0 between the TENS and PBM groups.

The quantitative data (VAS score and unstimulated salivary flow) were analyzed using the Kolmogorov-Smirnov normality test to assess if the data are normally distributed, which

was confirmed based on the mean error. Two-factor ANOVA was used to analyze intergroup (TENS and PBM) and intragroup (repeated measures at T0, T1, T2 and T3) differences in mean VAS scores and unstimulated salivary flow. For this purpose, the assumptions of sphericity were tested using the Mauchly test. The post-test of intergroup and intragroup interactions was carried out based on Bonferroni corrections, as well as using the trend test of the interaction between time and intervention. A level of statistical significance of 95% was adopted for all tests.