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PHOTOBIOMODULATION EFFICACY OF MAJOR SALIVARY GLANDS IN CHILDREN WITH CARIES ACTIVITY: STUDY PROTOCOL FOR RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL-GROUP CLINICAL TRIAL

EFEITO DA FOTOBIOMODULAÇÃO DAS PRINCIPAIS GLÂNDULAS SALIVARES EM CRIANÇAS COM ACTIVIDADE DE CÁRIE: ENSAIO CLÍNICO ALEATÓRIO DUPLO-CEGO

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Trial registration: clinicaltrials.gov (NCT05546528)

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Abstract
Background: Dental caries is one of the most prevalent diseases among children worldwide. Saliva plays a significant role in the demineralization/remineralization of the dental surface. Several salivary characteristics, such as flow rate, pH, and buffering capacity, provide relevant information regarding the development of carious lesions. Photobiomodulation has shown promising results in improving salivary flow rate and buffer capacity in the adult population.
Purpose: of this trial was to evaluate the efficacy of photobiomodulation of the major salivary glands on salivary parameters in children with caries.
Methods: This protocol details a randomized, double-blind, parallel-group, controlled trial that evaluated salivary parameters through photobiomodulation in children. Fifty 6- to 12-year-old participants will be randomly divided into two groups:1) photobiomodulation experimental group (G1) (n=25) and 2) photobiomodulation placebo group (G2) (n=25). Infrared light will be applied at 16 intra-and extraoral points and placebo, respectively. Unstimulated salivary samples will be collected before and immediately after application once a week for three consecutive weeks. Salivary samples will be analyzed for their flow rate, pH, and buffering capacity. The primary outcomes are the differences in salivary flow rates between G1 and G2. The secondary outcomes are differences in salivary pH and buffering capacity between G1 and G2.
Discussion: The results of this clinical trial will offer evidence for the efficacy of photobiomodulation in salivary parameters and to support decision-making regarding non-invasive treatments to control dental caries.

Keywords: Dental caries. Photobiomodulation. Protocol. Randomized controlled trial. Salivary flow

Resumo
Contexto: A cárie dentária é uma das doenças mais prevalentes entre as crianças em todo o mundo. A saliva desempenha um papel significativo na desmineralização/remineralização da superfície dentária. Várias características salivares, como a taxa de fluxo, o pH e a capacidade de tamponamento, fornecem informações relevantes sobre o desenvolvimento de lesões de cárie. A fotobiomodulação demonstrou resultados promissores na melhoria da taxa de fluxo salivar e da capacidade de tamponamento na população adulta.
Objetivo: deste estudo foi avaliar a eficácia da fotobiomodulação das glândulas salivares principais sobre os parâmetros salivares em crianças com cárie.
Métodos: Este protocolo detalha um estudo randomizado, duplo-cego, de grupos paralelos e controlado que avaliou parâmetros salivares por meio da fotobiomodulação em crianças. Cinquenta participantes de 6 a 12 anos de idade serão divididos aleatoriamente em dois grupos: 1) grupo experimental de fotobiomodulação (G1) (n=25) e 2) grupo placebo de fotobiomodulação (G2) (n=25). A luz infravermelha será aplicada em 16 pontos intra e extraorais e o placebo, respectivamente. Amostras salivares não estimuladas serão coletadas antes e imediatamente após a aplicação, uma vez por semana, durante três semanas consecutivas. As amostras salivares serão analisadas quanto à sua taxa de fluxo, pH e capacidade de tamponamento. Os resultados primários são as diferenças nas taxas de fluxo salivar entre G1 e G2. Os resultados secundários são as diferenças no pH salivar e na capacidade de tamponamento entre G1 e G2.
Discussão: Os resultados desse ensaio clínico oferecerão evidências da eficácia da fotobiomodulação nos parâmetros salivares e apoiarão a tomada de decisões em relação a tratamentos não invasivos para o controle da cárie dentária.

Descritores: Cárie dentária. Fotobiomodulação. Protocolo. Estudo controlado e randomizado. Fluxo salivar

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1 Introduction

Dental caries is a noncommunicable, dental biofilm-mediated, and diet-modulated disease that results in net loss of minerals from dental hard tissues[1,2,3]. Currently, dental caries are one of the most prevalent diseases among children worldwide[4]. Untreated dental carious lesions are the most prevalent health problem in the world, with caries experience rates of 80% to 100% among adolescents in low- and lower-middle-income countries[5].

Saliva is essential for maintaining oral health and plays a leading role in the demineralization-remineralization process of the dental surface, as well as in preserving the integrity of oral tissues[6]. It is a fluid, transparent, relatively alkaline compound of varied viscosity, produced by the salivary glands, and secreted in the oral cavity to fulfill fundamental oral health functions[7,8]. Patient salivary characteristics, such as flow rate, pH, and buffering capacity, can provide information regarding the future risk of developing carious lesions[9,10,11].

Photobiomodulation (PBM) consists of the application of light to promote tissue healing, reduce inflammation, and produce analgesia, usually using a low-intensity light source, such as a laser or LED[12]. Although the mechanism of action of PBM has not yet been fully elucidated, several advances have been made in understanding its molecular mechanisms, including its action on cytochrome c oxidase. Cytochrome c oxidase acts as a photoacceptor and photosignaling transducer in the red and infra-red wavelength spectrum[13]. Absorbed photons convert light into signals that stimulate biological processes[14]. Infrared light acts on membranes, mitochondria, and/or cells to modulate signaling pathways and production of reactive oxygen species (ROS), ATP, calcium, and nitrogen. Secondary effects include stress signaling, metabolic processes, cytoskeletal organization, cell proliferation and/or differentiation, and homeostasis[15,16]. Studies have shown that photobiomodulation improves the functionality of the major salivary glands, as well as salivary flow, and increases the number of glandular ducts, mitosis of epithelial cells, and stimulation of protein synthesis in salivary glands in rats[17,18, 19, 20, 21]. To date, most of the published studies have evaluated the effect of photobiomodulation on salivary glands with parafunction due to hyposalivation, xerostomia, Sjogren's syndrome, or after radiotherapy[18, 22, 23,24].

A systematic review by Golez et al[25] found a beneficial effect of photobiomodulation on salivary gland function, which is fundamentally related to salivary flow rate and buffer capacity. Little is known about the effect of photobiomodulation on the salivary glands and its relationship with dental caries. To the best of our knowledge, no studies have been published that evaluated the effect of photobiomodulation on the salivary glands of the major salivary

glands associated with dental caries. A clinical study by Nemeth et al 2020[19] carried out on an adult population evaluated the effect of photobiomodulation of the major salivary glands on the risk of caries, finding promising results, fundamentally with respect to the microbial count, as well as an improvement in the salivary buffering capacity, which may then help reduce the risk of caries. Although the results of this study are promising, more evidence is needed regarding the use of photobiomodulation and its effects on the treatment of dental caries in children. Likewise, considering the high prevalence of caries in children worldwide and the understanding that photobiomodulation is a non-invasive therapy, it would be appropriate to explore new non-restorative alternatives to complement the traditional preventive therapy already implemented, such as the use of gels, varnishes, fluoride rinses, dietary modifications, and salivary supplements.

The aim of this clinical study is to explore whether stimulation of the major salivary glands through photobiomodulation can help improve salivary parameters in children with caries.

2 Methods

2.1 Study design

This is a randomized, placebo-controlled, double blind clinical protocol consisting of an intervention phase for the application of extra- and intraoral photobiomodulation for three consecutive weeks, once a week. Non-stimulated saliva will be collected and analyzed before and after each intervention. Sixteen extra and intraoral sites will be irradiated weekly for 3 weekly sessions of photobiostimulation of the salivary glands, lasting an average of 26 minutes per session. One week after the last session, a new evaluation of salivary parameters will be performed. This session will only include salivary collection.

This protocol was written in accordance with Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) (https://www.spirit-statement.org) to improve the quality of reporting.

2.2 Trial registration

This trial has been registered at Clinical Trials (https://clinicaltrials.gov), and the registration number is NCT05546528.

2.3 Sample size

The sampling plan was based on a study by Vantipalli et al[37] who evaluated salivary flow in children with and without caries activity. Considering the mean difference between the groups and the standard deviation of the change in salivary flow in the group with caries, and with analysis using the t-test, the sample size was calculated using the statistical program G-Power 3.1.9.4, with α= 0 .05, a minimum of 22 participants per group is sufficient to control statistical variance, ensuring a test power of 0.80. Considering the possible losses, 20% will be added to this number, totaling 50 children (25 per group).

2.4 Participants

This research will be performed with 6- to 12-year-old school children who accept the invitation to participate in the trial in Montevideo, Uruguay.

2.4.1 Inclusion criteria

Patients of both sexes between 6 and 12 years of age with mixed dentition having been diagnosed with at least one active caries lesion will be included. Participants with good general health will be included expect participants with systemic or local diseases affecting salivary secretions.

2.4.2 Exclusion criteria

Participants with severely active cavitated lesions with pulpal symptoms, patients diagnosed with hyposalivation, xerostomia or Sjögren syndrome, diabetics, with fixed appliances or any physical disability that interferes with oral hygiene.

2.4.3 Drop-out criteria

Participants who do not attend more than two consecutive appointments will be discontinued, and their data will be computed for later analysis.

2.4.4 Recruitment, randomization, and concealment

Participants aged 6 who attend Federico Ozanam School in Montevideo, Uruguay, will be invited along with their parents to take part in the study. The 50 participants will be randomly divided into two groups: Group 1 (G1). The 25 participants allocated to G1 will be submitted

to treatment with PBM and 25 participants to Group 2 (G2) will be submitted to simulated treatment with PBM.

Randomization will be performed by an external investigator who is not involved in the treatment of patients, collection, or evaluation data. Simple randomization will be performed with the aim of maintaining a balance between the participants in the laser group and the placebo group. This is a clinical trial in which all participants have the same chance of receiving treatment and will be randomly selected using sealed opaque envelopes. The division will be made with numbered envelopes (each patient will be identified with sequential numbers, 1-50) that will contain pieces of paper with information on whether the treatment will be with laser or placebo. These envelopes will be kept away by the observing researcher in charge of the data collection. Prior to the application of the laser or placebo, the operator responsible for applying the PBM will receive an envelope to determine which treatment should be applied to each patient.

Only the researcher responsible for applying the PBM will know which treatment was assigned to each participant. The researcher responsible for the salivary collection and analysis will be blinded to the type of treatment the patients received. The participants will be blinded in the study.

G2 group will receive the same PBM and salivary collection protocol, except that the operator will not turn on the laser device. For placebo purposes, the music of the laser was recorded to simulate the sounds of the device.

2.5 Study interventions

The PBM therapy consists in the application of low-intensity infrared laser in the 3 major salivary glands (parotid, submaxillary and sublingual) employing Laser Therapy EC device (DMC, São Paulo, Brazil) used in the form of a tip. The parameters considered are: laser emitting at infrared wavelength of 808 nm with a radiant energy of 6 J/site, continuous wave, incidence of the beam perpendicular to the irradiated surface (90º) and in contact with the skin during 60 s/site, in two extraoral points and two intraoral points in the region of the parotid glands bilaterally, as well as one extraoral and intraoral point for the submandibular and sublingual glands (totaling 16 points), as shown in figure 1.

At the time of application, only the participant to be treated will be present, who may be accompanied by his/her caregiver (mother, father/guardian) and the operator responsible for the application for the PBM, all with specific eye protection glasses. During the applications,

the participant must remain seated with the Frankfurt plane parallel to the ground. A total of three laser applications will be performed, one session per week on eight points in the face and cervical region (extraoral), as well as at eight intraoral points. After the PBM sessions (3 sessions, once a week), in the following week, the saliva will be collected again as in the initial collection.

For the salivary sample collection, the participants will seat in a dental chair under conventional light. Resting total saliva will be collected between 9 am and 11 am (to avoid the influence of circadian rhythm) using the drainage method. Children must have no food or drink (except water) 1 hour prior to the procedure and should not perform oral hygiene within 2 hours prior to the procedure. Prior to collection, the children will be asked to swallow the saliva in their mouth and will then be instructed not to swallow, allowing the saliva to drain through the corners of the lips between the lips (which should be separated) into a sterile metal funnel that will be connected to a sterile 15-mL Flacon test tube[28,29.] With the help of a stopwatch, all unstimulated saliva samples were collected for 5 min. Salivary flow (mL/min), pH, and buffering capacity will be analyzed.

Figure 1 - Image of the application of the extra and intra-oral laser points

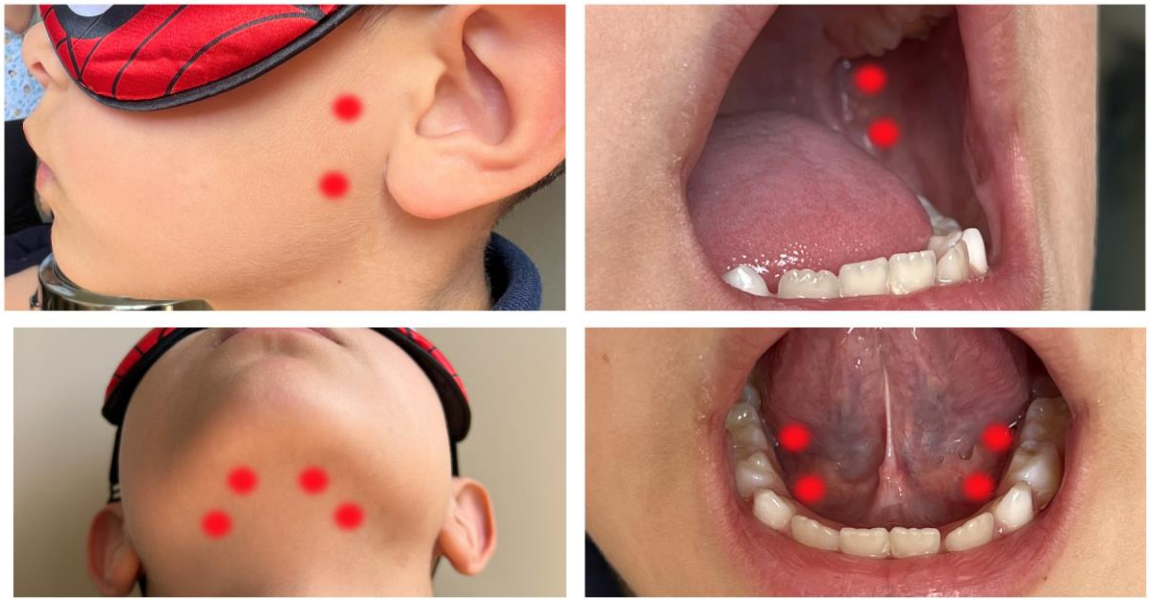

Image property: Magdalena San-Martín.

2.5.1 Adverse effects

No adverse effect has been reported while using PBM with appropriate parameters.

3 Study outcomes

3.1 Primary outcomes

The primary outcomes are the differences in salivary flow rates between base line and final X in G1 and G2.

3.2 Secondary outcomes

The secondary outcomes are the differences in salivary pH and the buffering capacity between base line and final X in G1 and G2.

3.2.1 Salivary flow

The saliva flow rate was estimated by asking the children to salivate into plastic cylinders (previously weighed) for 5 min. Next, these plastic cylinders (containing saliva) were weighed and the flow rate in g/ml was calculated, which was equivalent to ml/min[30].

3.2.2 pH

Saliva pH was measured using a previously calibrated digital pH meter (HI 2211 pH/ORP Meter, Hanna Instruments, Inc. Limena, Italy).

3.2.3 Buffering capacity

Salivary buffering capacity was estimated using the Ericsson method (1959). Buffering capacity is a measure of the efficiency of a buffer to resist changes in pH. Conventionally, buffering capacity (β) is expressed as the amount of strong acid or strong base, in grams equivalents, which must be added to 1 liter of solution to change its pH by one unit. The initial pH of the saliva sample was measured, and the value was noted as explained above. Saliva (0.5 ml of saliva was added to 5 mM HCl (1.5 ml of 5 mmol/l Hcl in a centrifuge tube. The mixture was shaken vigorously, centrifuged for one minute and allowed to stand for 10 min when the final pH was measured using a digital pH meter in a similar manner[31].

The buffering capacity is expressed quantitatively as the ratio of the acid added to the pH change. The buffer capacity was calculated as follows:

Buffer capacity (β) = ΔB / ΔpH

where ΔB = Gram equivalent of strong acid to change the pH of 1 liter of buffer solution, and ΔpH = pH change caused by the addition of strong acid (final pH – initial pH).

3.2.4 Questionnaire

Sociodemographic information (age and sex) was collected. In addition to sociodemographic information, clinical information will be collected, such as the history of systemic diseases, treatments performed previously and at the time of the investigation, medications used, previous experience with caries, oral hygiene, and dietary habits.

3.2.5 Carious lesions diagnosis and activity (ICDAS)

To assess current caries experience, the ICDAS caries diagnostic index was used. After professional brushing, the presence of caries lesions will be assessed, and their severity and activity will be determined by visual examination[32,33]. For this purpose, the following classification will be considered: healthy without evidence of demineralization or injury.

Initial caries: First visible or detectable change in enamel seen as carious opacity or visible discoloration (white and/or café-au-lait lesion), which is not consistent with the clinical appearance of sound enamel (ICDAS 1 and 2).

Moderate caries: a white or café-au-lait spot lesion with localized enamel breakdown, with no visible exposed dentin (ICDAS 3) or an underlying shadow of dentin (ICDAS 4) originating from the surface being evaluated.

Severe caries: detectable cavity in opaque or discolored enamel with visible dentin (ICDAS 5 and 6).

Regarding the assessment of the activity of the lesions (active or inactive), a detailed assessment of the superficial visual appearance, careful tactile sensation, potential accumulation of biofilm on the surface, and stage of gingival health/disease[34,35,36].

3.3 Statistical analysis

A descriptive analysis of the population will be carried out in terms of sociodemographic variables and clinical characteristics of the sample. The data for the clinical variables were analyzed using the statistical program SPSS 23.0. Shapiro wilk´s test will be used to assess data normality. In case of normality, Two-way ANOVA formula will be used to compare the mean values between time and groups. It will be considered as level of statistical significance $\alpha = 0.05$.

3.4 Data collection, management, and monitoring

Data from the participants and measurement instruments will be collected at every visit before and after the photobiomodulation therapy. A standardized computer and paper form were used for the data collection. Upon completion of the trial, the data will be stored for five years at a secure location, being available for access by the investigators, IRB, and the participants' request.

3.5 Ethical consideration

The study underwent external peer review and was approved by the Human Research Ethics Committee of the Catholic University of Uruguay (approval number #220803). Investigators will obtain informed consent and provide each participant with a copy of the consent form. Both the participant and legal representative should return informed consent. The privacy of the participants will be respected and documents from the participants will be managed with enrollment numbers and initials. Participants' personal information will be kept confidential (only accessible to the investigators or related monitoring agents), and all documents will be shredded after five years of storage. The application of laser offers minimal risks. However, if participants wish to withdraw their consent, they will be excluded from the study. The results of this trial will be published in a peer-reviewed journal.

4 Discussion

The results of this project can support clinical choices and decision-making regarding noninvasive and non-pharmacological treatments to control the development of dental caries.

The tested protocol should be evaluated for its effects on salivary parameters. The results will serve to consider this treatment as a low-cost, non-invasive alternative to the traditional treatment for dental caries. Additionally, if positive results are obtained, it is expected that these can be evaluated in other populations and studies for the development of clinical protocols and therapeutic guidelines.

Conflict of interest

The authors have no conflicts of interest to disclose.

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Ethical statement

This work obtained ethics compliance approval from the Human Research Ethics Committee of the Catholic University of Uruguay, under process number 220803. Any changes in the protocol will be reported to this committee. Informed consent will be obtained from all the participants.

Data collections methods

All authors are qualified in photobiomodulation therapy. All data will be entered electronically. The participants file will be stored in numerical order in a safe place and accessible only to the authors of this study.

Data availability

All information collected from the participants will be transcribed into a database replacing the individuals name with the registration number of the evaluation form. The data supporting the findings of this study are *available from the corresponding author upon reasonable request.* After the data analysis, volunteers will be invited to a meeting where the results will be shared and will become public.

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