*Study Protocol*

Effects of photobiomodulation with LED cluster on mandibular and cervical mobility, pain and quality of life in patients with temporomandibular dysfunction: clinical study protocol, randomized, controlled and blinded

**Abstract**

The term temporomandibular disorders (TMD), according to the American Dental Association (ADA), refers to a group of disorders characterized by pain in the temporomandibular joint (TMD) in the preauricular area or in the muscles of chewing, in addition to the TMD sounds during mandibular function and deviation or restriction of mandibular movements. Its multifactorial etiology is related to a heterogeneous group of functional, structural, and psychological factors, making it difficult to identify the association between a single etiological factor and the signs and symptoms of TMD. It has been demonstrated in various studies that photobiomodulation (PBM) is a preferred treatment option for temporomandibular disorders. However, there is a need to establish protocols that specifically address the multifactorial aspects of this disorder. Additionally, considering the use of clusters may help optimize the treatment. The objective of this study is to evaluate the effects of photobiomodulation using clusters of 850nm LEDs (infrared) and 630nm LEDs (red) on pain, and also in cervical and mandibular mobility in patients with TMD. This randomized, controlled, and blind clinical trial will include 36 patients, randomized into 2 groups: Group 1 Red and infrared LED cluster, Group 2 control (placebo - simulation of irradiation). The irradiated areas (n=12) will include the TMJ, masseter muscles, temporal muscles, ECOM, scalenus and trapezius. Diagnostic Criteria for Temporomandibular Disorders - DC/TMD will be used to include TMD patients in the study. The primary outcome of this study will be pain evaluated using the visual analog scale (1-10cm). The secondary outcomes include cervical spine mobility using a goniometer (fleximeter) that will be used for cervical range of motion and to measure a mandibular range of motion (ADM), a pachymeter will be used. Data analysis: All outcomes will be evaluated at baseline and two weeks after treatment to allow comparisons between the 2 groups Normality will be assessed using the Shapiro-Wilk test. If the data follow a normal distribution, a two-way ANOVA will be used for analysis. Results will be expressed as means with standard deviation (± SD), and statistical significance will be set at $p < 0.05$.

**Keywords**: temporomandibular dysfunction, LED, photobiomodulation, mandibular mobility, cervical mobility, pain.

## Introduction

The American Academy of Orofacial Pain defines Temporomandibular Dysfunction (TMD) as a set of disorders that involve the masticatory muscles, the temporomandibular joints (TMJ), and the structures associated with the stomatognathic system. It is the most common cause of orofacial pain not exclusively of dental origin (Manfredini et al., 2010). TMD occurs in all age groups but is more prevalent in adults, with a higher incidence in women (Okeson, 2013).

Carrara et al. (2010) state that temporomandibular dysfunctions are characterized by three main signs and symptoms: muscle or joint pain, joint sounds, and/or restriction and deviation of the jaw opening pattern. Among the instruments for the evaluation of TMD, there are questionnaires, clinical assessments, and imaging tests (radiography, computed tomography, and magnetic resonance), which are used based on their applicability and the means to which the patient can access (Schiffman et al., 2014).



In this protocol study, the DC/TMD will be used as a validation and diagnostic instrument. It presents a specific double-axis system for the diagnosis of TMD, where information is collected on the physical and psychosocial aspects of those evaluated, serving as an organized structure for TMD research (Schiffman et al., 2014). It provides clear and precise parameters in data collection and diagnosis (Manfredini et al., 2010). It is currently used worldwide in research involving TMD in clinical studies, diagnoses related to psychosocial factors, and epidemiological studies (Langella et al., 2018; Khalighi et al., 2016; Panhoca et al., 2015).

In addition to the signs and symptoms mentioned above (Biasotto-Gonzalez, 2005), it is added that painful manifestations, muscular incoordination, and biomechanical imbalance may occur in the cervical region of those with reported TMJ.

Eriksson et al. (2000) suggest that functional mandibular movements result from the coordinated action of the mandibular and cervical muscles, leading to simultaneous movements in the temporomandibular joint. Considering the occipital junction, the Wiesinger atlas shows a strong relationship between TMD and neck pain.

Due to the complexity of TMD, some authors propose treatment associating different forms of therapy and the involvement of a multidisciplinary team (Freire et al., 2014), including muscle relaxation plates (Bortoletto et al., 2014), physiotherapy treatments with the use of electrotherapy (Gomes et al., 2012), massage therapy, and mobilization resources (Hace et al., 2013; Amaral et al.,

2013), and also photobiomodulation alone(Silva et al., 2017; Seifi et al., 2017; Herpich et al., 2017) or associated with other therapeutics such as occlusal splints (Costa et al., 2022) or myofunctional therapies (Altuhafyet al., 2024).

Photobiomodulation (PBM), also known as low-level laser therapy (LLLT), is the application of red and infrared light to stimulate healing, alleviate pain, and reduce inflammation. PBM has demonstrated its physiological role as a biostimulatory process, promoting vasodilation, analgesia, anti-inflammatory effects, and expediting healing processes. Additionally, LED therapy, particularly in patients with fibromyalgia, complements these benefits (Silva et al., 2017). PBM has shown efficacy in diminishing markers associated with the M1 phenotype in activated macrophages, and various studies indicate reductions in reactive nitrogen species and prostaglandins in diverse animal models. Furthermore, PBM exhibits promise in mitigating inflammation in the brain, abdominal fat, wounds, lungs, and spinal cord (Hamblin MR et al., 2017)

Based on the results of studies found in the literature, related to the reduction of pain in patients with temporomandibular disorders, which used photobiomodulation with LBI as a therapeutic resource (MAGRI et al., 2018), studies were carried out that evaluated, in addition to the pain, also improvement in the range of mandibular movements in these patients using the LBI (SEIFI et al., 2017), or the LED (COSTA et al., 2017; PANHOCA et al. 2015), or in inflammatory processes induced in the ATM and With the use of LED as a resource (CASTRO et al., 2015), or even with the use of different light sources sometimes in the same device (KARU et al., 2003). At the same time, An improvement has also been demonstrated in pain scales and ranges of cervical mobility once low-level laser therapy was applied (CHOW et al., 2009). However, there are many protocols with different results and dosimetric parameters and the need to establish the best way to use the light to obtain the best results, specially using clusters equipaments which allow to treat the affected regions with different lights sources including the red and infrared lights.



Therefore, the objective of this study protocol is to evaluate the effects of PBM using red and infrared LED Cluster on pain and mandibular and cervical mobility in patients with TMD.

**Methods**

This is a randomized, controlled, and blind clinical trial on the effects of LED light in growing patients previously diagnosed with TMD. The study will be realized at the Catholic University of Uruguay, Montevideo. This protocol follows SPIRIT guidelines and was structured in a SPIRIT Flow chart (Figure 1).

The principal investigator will provide 2 informed consents, one consent to carry out the diagnosis and those patients who test positive for Dc/TTM will be given another informed consent to carry out the treatment.

There are no conflicts of interest regarding the products used in the study or the authors involved. The study data will be published without restrictions on data inclusion. All data will be available for consultation and participants will have access to their medical records at any time. All data will be managed by the main researcher on a computer with a password and without the internet.

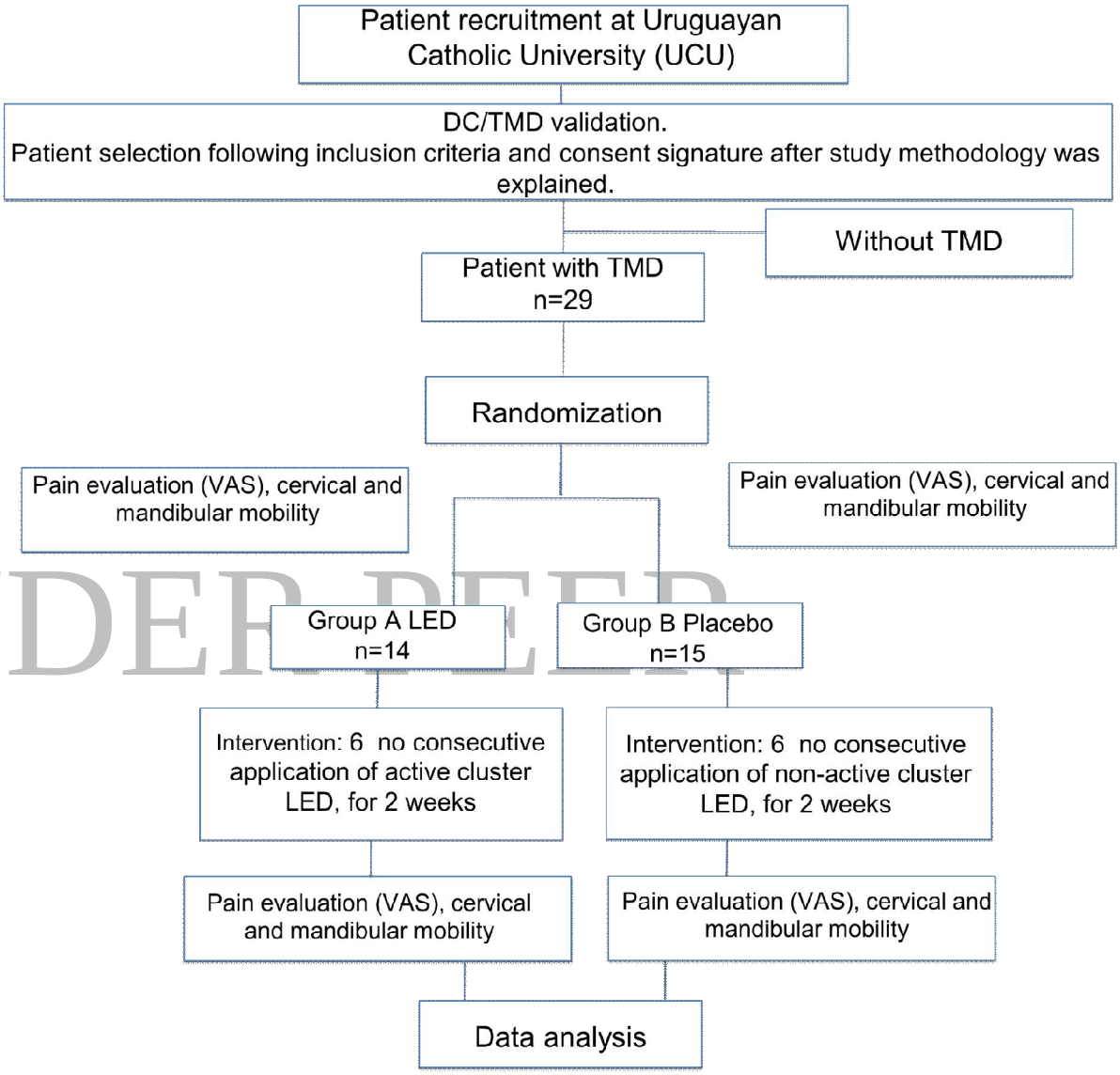




Figure 1: Flowchart of the study designed following the CONSORT checklist.

**Sample Description**

Male and female patients aged between 18 and 45 years from the clinic of the Catholic University of Uruguay participated in this study. Previously selected according to the inclusion criteria based on DC/TMD. All participants will be explained the objectives and procedures to be carried out. They will be asked to complete 2 informed consents, first, they will be given consent to

perform a diagnosis and those who show signs and symptoms of dysfunction are invited to participate in the study, having them sign a second consent.

**Inclusion criteria**

The inclusion criteria for this study will be:

- both genders
- 18-45 years
- present TMD according Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) Axis I
- present all the permanent teeth

**Exclusion criteria**

The inclusion criteria for this study will be:

- undergoing orthodontic treatment
- undergoing other treatment for TMD
- present dental caries or gingival disease
- Initiate or use any type of medication during the phases of the study
- comorbidity



**Sample calculation**

To calculate the sample size, data from the study of Monteiro et al. 2020 (Effectiveness of photobiomodulation in Temporomandibular Disorder- Related Pain Using a 635 nm Diode Laser: A Randomized, Blinded, and Placebo- Controlled Clinical trial) were used. For the sample determination, the program available at https://www.stat.ubc.ca/~rollin/stats/ssize/n2.html was used. Sixteen patients were assigned to each group, considering 10% of possible losses, we have a total of 36 patients. With 10% more there will be 18 in each group. The DP was increased to 3.0 (taking into account the value 2.36). The power of the sample is 80%, with type 1 error of 0.05 (alpha), that is, 5%.

**Randomization**

Randomization will be conducted using the online platform https://www.sealedenvelope.com/ employing a 1:1 block randomization scheme. Opaque envelopes will be identified with ordinal sequential numbers. The information of the corresponding experimental group will be inserted inside, also sealed. The generation of the random sequence and the preparation of the envelopes will be carried out by a person not directly involved in the study. After inclusion of the patient in the study, the investigator that will apply PBM will open 1 envelope and perform the indicated procedure or its simulation. Only this researcher will know the nature of the treatments.

**Clinical sequence of clinical trial**

The study team will include one dentist, one intern, and a dental student. Participants will undergo two phases of data collection. The initial phase, termed the Control Phase, involves a one-week period where participants are validated and will not receive any physiotherapy or dental intervention.

Following the Control Phase, participants will enter the Treatment Phase, which is further divided into two groups. Group 1, receiving red and infrared LED (comprising individuals undergoing photobiomodulation), and Group 2, Placebo (comprising individuals receiving applications from an inactive photobiomodulation device for 6 non-consecutive sessions over 2 weeks).



The following instruments will be used to validate patients:

1) Diagnostic Questionnaire for Temporomandibular Disorders DC/TMD.

2)VAS visual analogue scale to validate pain.

3) Pachymeter to validate mandibular amplitude movement (MMA).

4) Goniometer for cervical ADM.

**Implementation of Blinding:**

The current protocol is characterized as blinded, with blinding occurring only with the patient.

In this way, within the research team, distinct roles are designated for effective blinding:

The principal investigators (Dentist) will perform all procedures, including the assessments outlined in the flowchart, PBM treatment, and data analysis, thus will not be blinded during data collection and analysis, knowing to which groups the patients belong.

Participants: Uninformed about the interventions, participants will experience simulated FBM in the control group, ensuring a rigorous blinding approach throughout the study.

Statistician: Remaining blinded to the treated groups, the statistician will solely receive information on G1 and G2 through sheets.

**Validation instruments**

1. Diagnostic Criteria for Temporomandibular Disorder – DC/TMD. (AXIS I AND AXIS II)

This validation instrument comprises two components: a questionnaire encompassing inquiries related to general health, oral health, facial pain history, limitations in mouth opening, headaches, noises, habits, tinnitus, joint problems, and current behavior (Rongo et al., 2021).

Following questionnaire completion, a clinical examination is conducted, evaluating mouth opening patterns, vertical extension of mandibular movement, TMJ sounds upon palpation, extrusive movements, and TMJ noises in protrusion and lateral extrusion.

The clinical diagnosis is categorized into three groups:

Group 1: Diagnosis of myalgia and arthralgia, including myalgia, local myalgia, myofascial pain, referred myofascial pain, arthralgia, and headache attributed to TMD.

Group 2: Intra-articular disorders, including disc displacement with reduction, disc displacement with reduction with intermittent lock, without reduction with limited opening, and disc displacement without reduction without limited opening.



Group 3: Degenerative joint disease.

Upon completion of both components, a diagnosis is derived, ranging from no diagnosis to a maximum of 5 (a diagnosis from group I + a diagnosis from group II + a diagnosis from group III) for each joint. Additionally, in AXIS II, numerical data and scales will be obtained, capturing various psychosocial aspects (Ohrbach et al., 2016).

**Outcomes**

1) VAS visual analogue scale

The visual analogue scale (VAS) is a validated instrument allowing us to quantify the current intensity of pain. Consisting of a 10 cm straight line, one end features a verbal description of 'no pain,' while the opposite end features another description indicating 'intense pain.' Participants will be guided to draw a perpendicular line between these two extremes to signify the intensity of the pain they are experiencing. The patient made a mark on a line from 0 to 10 cm, which was then measured in millimeters.

2) Mandibular range of motion

A digital caliper will be used to validate the range of motion of mandibular movement in millimeters, including openings, left and right lateral movements, and protrusion. This procedure is part of the DC/TMD validation.

3) Cervical range of motion.

The goniometer will be used for the examination of the cervical range of motion and it will be performed with the patient sitting to stabilize the pelvis and dorsolumbar area. It is crucial that the patient maintains good body alignment, as any compensation can distort the results obtained (Seager et al., 2019).

The cervical spine movements to be measured include, in the frontal plane, right lateral inclination and left lateral inclination, and in the sagittal plane, flexion and extension (Biasotto-Gonzales et al., 2012). These measurements will be taken before commencing the photobiomodulation treatment and once it has concluded.

A demographic pencil will be used to mark the anatomical points. Subsequently, with the assistance of the researcher, the patient will perform the indicated movements in a passive way. To record flexion-extension it will choose the position 0 with the goniometer at 90 degrees. The axis is placed over the external auditory canal, the fixed arm aligned with the vertical midline of the head, and the mobile arm, at the level of the nostrils.

For both left and right lateral inclination, position 0 with the goniometer is set at 0. The axis is placed on the spinous process of C7, the fixed arm aligned with the vertical midline formed by the dorsal spinous processes, and the mobile arm aligned with the midline of the head, referencing the midpoint of the external occipital protuberance.



**Photobiomodulation using a LED cluster**

Photobiomodulation using red and infrared LED will be bilaterally applied to the temporomandibular joint regions and the muscles of the masseter, temporalis, superior trapezius, sternocleidomastoid and scalene. The treatment will consist of 6 non-consecutive sessions spread over 2 weeks, utilizing the Libramed brand model Antares with P2 LED Cluster.

It features 5 LEDs emitting light at 630 nm and 4 LEDs emitting light at 859 nm in an area of 20 $cm^2$. The red light LEDs will be used with a power of 1250 mW, delivering 20 J per point, and a duration of 80 seconds and the infrared light LEDs will be used with a power of 1200 mW, delivering 24,9J per point, and a duration of 83 seconds using for both wavelengths the radiant exposure of 5J/cm2. These parameters were selected based on the study conducted by De Souza et al (2022).

For the placebo group, the same measurements as the LED group will be conducted, with the only difference being that the equipment will be turned off. The interventions will be performed at the University Health Clinic (UCU). Both the professional and the patient will wear protective glasses. The cluster will be covered with disposable transparent PVC plastic for hygiene reasons

and to prevent cross-contamination. Prior to local irradiation, facial cleaning will be performed using 70% alcohol. The patient will be in a supine position during the applications

**List 1 : Dosimetric parameters**

| Parameters | Values/Treatment |
|---|---|
| Wavelength [nm] | 850 infrared<br>660 red |
| Operating mode | Continuous |
| Radiant power [mW] | 300 each infrared LED (total cluster Radiant Power1200)<br>250 each red LED (total cluster Radiant Power 1250) |
| Cluster Irradiance [mW/cm²] | 60 (infrared)<br>62,5 (red) |
| Cluster Area [cm²] | 20 |
| Exposure time [s] | 83 infrared<br>80 red |
| Radiant exposure [J/cm²] | 5 |
| Radiant energy [J] per cluster | 4 infrared LEDs: 99,6J (each LED 24,9J)<br>5 red LEDs: 100J (each LED 20J)<br>Total: 44,9J per cluster application (infrared+LED)<br>Considering the 6 bilateral regions: 538,8J per session |

| | |
|---|---|
| Number of irradiated areas with cluster | Total points in the cluster: 9 points (5 red and 4 infrared).<br>6 bilateral regions irradiated with the cluster.<br>Points irradiated per treatment: 54 points (108 bilateral points).<br>Regions submitted to cluster irradiation:<br>1) Temporal<br>2) Temporomandibular joint<br>3) Masseter insertion<br>4) Sternocleidomastoid<br>5) Scalene<br>6) Superior Trapezius |
| Application technique | In contact, at 90 degrees with the surface |
| Number of sessions and frequency | 6 non-consecutive sessions |



**Statistical analysis**

Initial descriptive analyses will be performed, considering all variables measured in the study. This will include quantitative variables such as mean and standard deviation, as well as qualitative variables represented by frequencies and percentages. Subsequently, normality tests will be executed to determine appropriate statistical tests for each dataset, and specific statistical tests will be applied accordingly. For all tests, a significance level of 5% probability or the corresponding p-value will be adopted.

**Conclusion**

The conclusion will be drawn after carried out the experiment.

- **Ethics approval Statement and Consent -** The project was approved by the Research Ethics Committee of Universidad Católica del Uruguay (UCU), under process number 230821. All participants provided written informed consent before taking part in the study.

- The project has the approval of the Ethics Committee of the Catholic University of Uruguay (230821). The project has been registered on the ClinicalTrials.gov website https://clinicaltrials.gov/ under the number NCT06068959 with a start date of February 29, 2024, and an anticipated completion date of July 10, 2024.

- **Clinical trial registration:** NCT06068959 (initial release: October 16th 2023)

- **Dissemination policy**We will share the trial findings with participants, healthcare professionals, and the general public. Detailed data and results will be made available through complete datasets and results databases, in compliance with publication restrictions. Additionally, we are committed to enabling public access to the full protocol, participant-level data, and statistical code. These efforts reflect our strong commitment to transparency.

- **Disclaimer (Artificial intelligence)** Authors hereby declare that generative AI technologies, specifically OpenAI's ChatGPT (version GPT-4), were used during the editing process to enhance the English language quality of this manuscript

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