



Immediate Effect of LED Cluster on Masticatory Muscles in Children and Adolescents Diagnosed With Down Syndrome: A Pilot Study

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Abstract

Introduction: Individuals diagnosed with Down syndrome (DS) often exhibit muscle hypotonia, particularly affecting the facial and oropharyngeal muscles involved in the mastication cycle. This condition impairs essential functions such as swallowing, speech, and chewing. Moreover, lingual hypotonia in individuals with DS can lead to problems such as snoring, obstructive sleep apnea, and bruxism. The aim of the present study was to investigate the activity of the masticatory muscles (masseter and temporalis) in individuals with DS and bruxism through electromyographic analyses before and after the application of red light-emitting diode (LED) clusters.

Methods: Ten participants, four to 17 years of age, with diagnoses of DS and bruxism were assessed at the Integrated Health Clinic of Nove de Julho University. The masticatory muscles of the participants were submitted to electromyography (EMG) before and after the application of red LED clusters. Each cluster contained six LEDs with a wavelength of 660 nm, an optical point of 5 ± 2 mm, a dose of 2.675 J/cm^2 , and an optical output of 2-5 mW.

Results: The electromyographic analysis revealed changes in muscle activity after the application of the LED cluster. A reduction in asymmetry was found between the right and left masseter and temporalis muscles, and greater neuromuscular stability was found in the right masseter muscle. Although not statistically significant, these changes suggest immediate neuromodulatory effects.

Conclusion: Although no statistically significant differences were found, the electromyographic patterns suggested improved muscle symmetry and neuromuscular stability following a single application of the LED cluster. These findings indicate the need for further investigation into the short-term effects of photobiomodulation in this population.

Keywords: Down syndrome, Bruxism, LED cluster, Electromyography



Introduction

Trisomy 21, commonly known as Down Syndrome (DS), is one of the leading causes of mental impairment and is associated with various types of social limitations and comorbidities. The prevalence is one out of every 600–800 live births. However, if the different conditions found are well managed, individuals with this diagnosis are fully capable of being included in society and reaching their full potential.¹

DS is characterized by a set of physical and cognitive challenges, including cognitive deficits, immune system impairment, and muscle hypotonia, which can lead to several comorbidities, such as heart problems, thyroid dysfunction, and respiratory problems. Moreover, individuals with DS often have oral and dental problems, such as crossbite and problems with chewing aggravated by muscle hypotonia, which can lead to bruxism and sleep

disorders.² To minimize confounding effects related to neuromuscular abnormalities, we excluded individuals with diagnosed temporomandibular disorder from the present study.

In individuals with DS, muscle hypotonia particularly affects the orofacial and masticatory muscles, leading to poor tongue posture, compromised jaw stability, and inefficient oral motor control. These conditions increase susceptibility to airway obstruction and altered jaw dynamics, which may cause compensatory hyperactivity of the masticatory muscles. As a result, such individuals may develop repetitive involuntary jaw movements, such as clenching or grinding the teeth during waking hours or sleep, characterizing bruxism. This pathophysiological relationship has been described in previous studies addressing neuromuscular and behavioral mechanisms in this population.^{1,2}

Biological tissues are media for interactions with light. Photobiomodulation has emerged as a potential alternative for therapeutic interventions and is the most widely accepted term for describing the different roles of light in regulating biological tissues. The therapeutic effects resulting from the use of light sources include the acceleration of the tissue healing process, cell proliferation, a reduction in inflammation, enhanced neurological regeneration, modulation of the immune system, and a reduction in painful symptoms without transmitting heat to the cellular or biological target, in addition to mechanisms that increase cell metabolism and enhance the action of nerve cells.³

The biological effects of red light-emitting diode (LED) therapy are attributed primarily to the interaction with mitochondrial chromophores, especially cytochrome c oxidase, leading to an enhanced oxidative metabolism. Irradiation at a wavelength of 660 nm stimulates ATP production, modulates reactive oxygen species (ROS), and activates signaling pathways that regulate cell repair and neuromuscular balance. In skeletal muscle tissue, these effects are associated with improved muscle recovery, reduced fatigue, and involuntary contraction modulation. Therefore, LED therapy has been investigated as a non-invasive tool capable of inducing neuromuscular regulation, which is particularly relevant in populations with compromised motor control. Based on these mechanisms, the present study investigated whether a single session of red LED application could produce measurable effects on masticatory muscle activity in children and adolescents with DS and bruxism.^{3,4}

LED devices can treat muscle disorders.⁴ As an affordable alternative due to the lower cost and high durability, LED therapy can be considered a viable option for treating target tissues.⁵ Many studies have used electromyography (EMG) for the objective assessment of muscle activity.⁶ EMG is a noninvasive method for measuring the electrical activity of the neuromuscular junction during the activation of skeletal muscles. This assessment tool can accurately quantify the frequency, duration, and magnitude of muscle activity in microvolts (μV).

Individuals diagnosed with DS and bruxism may employ excessive force during the repetitive movements common to bruxism, leading to significantly accelerated wear. Such wear results in shorter dental crowns, worn occlusal surfaces, and exposed dentin.⁷

The aim of the present study was to analyze the activity of masticatory muscles before and after applying light with a red LED cluster, using photobiomodulation as a therapeutic intervention.

Methods

Ethical Aspects

This study followed the regulatory standards for research involving human beings and received approval from the

Human Research Ethics Committee (CNS No. 466/12 and Res. CNS 510/2016) of Nove de Julho University. Adult volunteers and the parents/guardians of underaged individuals signed a statement of informed consent, and underaged volunteers signed a term of assent authorizing participation in the study. After approval by the ethics committee (opinion number: 3.726.654), interested individuals were invited to participate in the study. The protocol for this study was registered with ClinicalTrials.gov, registration number NCT04211870, on December 26, 2019, and is available online at <https://www.clinicaltrials.gov/study/NCT04211870>.

Participants

Screening was first conducted to determine whether the volunteers were able to take part in the study. Sixty-six assessments were performed, and forty-seven individuals were excluded for not meeting the inclusion criteria. A total of 19 children and adolescents (11 males and eight females six to 12 years of age [13 participants] and 12 to 18 years of age [seven participants]) were selected to participate in the study.

Inclusion and Exclusion Criteria

Inclusion Criteria

- Children and adolescents diagnosed with DS
- Four to 17 years of age
- Parental report of teeth grinding and incisal and/or occlusal wear of teeth according to the criteria of the American Academy of Sleep Medicine (AASM), the questionnaire validated by Serra Negra et al⁸ to assess bruxism, and a systematic review by Manfredini et al.⁹

Exclusion Criteria

- Use of a muscle relaxant
- Presence of temporomandibular disorder
- Undergoing other therapies for bruxism
- Presence of other associated neurological disease
- Cognitive deficit that would prevent understanding the assessment methods
- History of skin irritation or contact allergy
- Skin lesions

Sample Calculation

The sample size was calculated adopting the specification of the reference article, that is, the increase in the standard deviation of the responses by which the hypothesis is rejected was $P = 20\%$. Under these conditions, adopting a maximum significance level of $\alpha = 0.05$ and a minimum test power of 80% , $n = 15.75$.¹⁰

Measurements, Instruments, and Procedures

Electromyographic Assessment of Masticatory Muscles

The right and left masseter and temporalis muscles produce electrical activity when activated. This electrical

activity was recorded by means of an electromyograph (BTS TMJOINT from BTS Engineering), using four channels that have bioelectric signal amplifiers, a wireless system, and disposable bipolar surface electrodes (Ag/AgCl - Medical Trace®) measuring 10 mm in diameter. The EMG signal was amplified with a gain of 2000 times and filtered within a 20–450 Hz frequency. The impedance and common mode rejection of the equipment are $>1015 \Omega/0.2 \text{ pF}$ and 60/10Hz 92 dB, respectively. The data were collected and digitized at 1000 frames/second, exported, and analyzed by the software.

The participants were instructed to sit upright on a chair with the knees flexed at 90° and the feet slightly apart. The skin was cleaned with 70% alcohol to reduce impedance between the skin and electrodes. Disposable self-adhesive Ag/AgCl electrodes (Medical Trace) with a diameter of 10 mm were attached to the skin over the belly of the muscles at the point of greatest tonus. The approximate distance between the electrodes was 20 mm center to center, following the guidelines of the Society of European Recommendations for Surface Electromyography (SENIAM).¹¹

EMG activity was determined under three conditions: I) at rest, II) during maximum voluntary contraction (MVC) of the right and left masseter and right and left anterior bundle of the temporalis muscle with a sheet of Parafilm M[®] between the molars, and III) habitual chewing (isotonic assessment) with more consistent and less consistent foods. The period of each reading under each condition was 10 seconds.^{12–14} The highest value obtained among the three readings under each condition was used to normalize the EMG data. Subsequently, the averages of the values were calculated and used in the analysis. Muscle activity signals were processed using routines designed explicitly in 2016 MATLAB software (The MathWorks Inc., Natick, Massachusetts, USA).

LED Plate Application Protocol

The application of light in this study was performed using LED plates from Odontollux (Cosmedical), which have a total area of $3 \times 6.5 \text{ cm}$, with six LEDs with a wavelength of 660 nm, an optical spot of $5 \pm 2 \text{ mm}$, a dose of $2,675 \text{ J/cm}^2$, and an optical output of $2\sim 5 \text{ mW}$ (Figure 1). The parameters of the LED device used in this study are detailed in Table S1. Four LED plates were fixed – one over each muscle of interest (right and left masseter and anterior bundle of right and left temporalis muscles) – with an application time of seven minutes in each region simultaneously.

Considering the irradiated area and energy density, the total energy delivered to each muscle during the application was approximately 52.16 joules. This value was obtained by multiplying the dose of 2.675 J/cm^2 by the surface area of each LED plate (19.5 cm^2). Such information is offered to ensure clarity, reproducibility,



Figure 1. LED Plate Used for photobiomodulation in the Present Study (Odontollux®, Cosmedical, Brazil)

and alignment with previous clinical protocols using photobiomodulation for neuromuscular effects.^{3,4}

Reports provided by parents or legal guardians regarding changes in bruxism behavior were not obtained through standardized or validated instruments, such as questionnaires or structured interviews. Such accounts were spontaneously shared during clinical interactions following LED application and were recorded descriptively, without being included as primary or secondary outcomes of the study. However, we chose to present these reports in the manuscript due to their potential clinical relevance, as they reflect consistent observational perceptions by caregivers regarding behavioral changes after the intervention. Although not submitted to statistical analysis, such observations offer valuable insights for the generation of future hypotheses and highlight the need for studies that assess this type of perception using validated tools.

Statistical Analysis

As the Shapiro-Wilks test revealed non-normal data distribution, the Mann-Whitney test was used for the statistical analysis. The significance level was set at $\alpha = 0.05$.

Results

This pilot study of a non-randomized clinical trial was developed according to the flowchart shown in Figure 2.

Masticatory Cycle With Foods of Different Consistencies

The electromyographic analysis of masticatory muscle activity during the chewing of both more and less consistent foods revealed no statistically significant differences before and after the application of the LED clusters. The p-values for comparisons between the right temporalis (RT), left temporalis (LT), right masseter (RM), and left masseter (LM) muscles ranged from 0.1380 to 0.5653, according to the Mann-Whitney test. These findings suggest that LED application did not lead to significant measurable

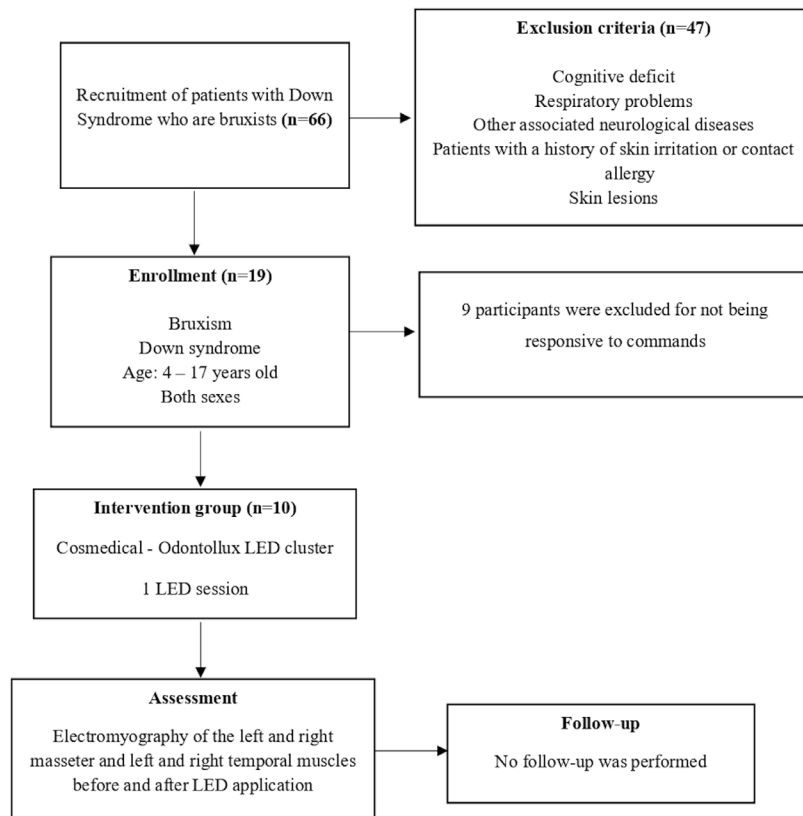


Figure 2. Flowchart of the Study

changes in muscle activation during chewing. However, a visual inspection of the electromyographic data suggested variations in muscle activity, which were further analyzed.

Electromyographic Analysis in Resting Condition

The electromyographic readings at rest revealed irregular, asynchronous muscle activity across all muscles analyzed, especially the right masseter and left temporalis, prior to LED application (Figure 3A). These findings suggest involuntary contractions and poor neuromuscular control commonly observed in individuals with DS and bruxism. After exposure to LED, a reduction in amplitude variability and an improvement in muscle tone were found, particularly in the right masseter (Figure 3B), indicating greater neuromuscular stability. Although these changes were not statistically significant, the trend suggests that the LED may have modulated neuromuscular behavior under the resting condition. As this study was designed as a pilot trial, the aim of using a single session of LED application was to investigate potential immediate effects on muscle activation. This approach followed similar methods adopted in preliminary neuromuscular photobiomodulation research. However, we recognize that a single application may not be sufficient to elicit sustained neuromuscular responses, which constitutes a limitation of the study. Future clinical trials should include repeated applications and longer follow-up periods for the

determination of cumulative or delayed effects.

Maximum Voluntary Contraction

The EMG analysis during MVC revealed pronounced asymmetries in muscle activation between the right and left sides, particularly in the masseter muscles, prior to LED application (Figure 4A). Following the intervention, these discrepancies were visibly reduced, with a more synchronized, symmetrical activation pattern found in both the masseter and temporalis muscles (Figure 4B). Although no statistically significant changes were detected, this trend suggests that a single LED session may contribute to short-term neuromuscular regulation by promoting balanced masticatory muscle activity.

Complete Masticatory Cycle

The EMG analysis of the complete masticatory cycle revealed pronounced asymmetries in muscle activation between the right and left sides involving both the masseter and temporalis muscles prior to LED application. The post-intervention readings demonstrated a reduction in these imbalances, with a more homogeneous distribution of muscle effort across the regions analyzed. Although the changes were not statistically significant, the observed pattern suggests that LED exposure may contribute to the short-term modulation of masticatory dynamics. These findings are illustrated in Figure 5.

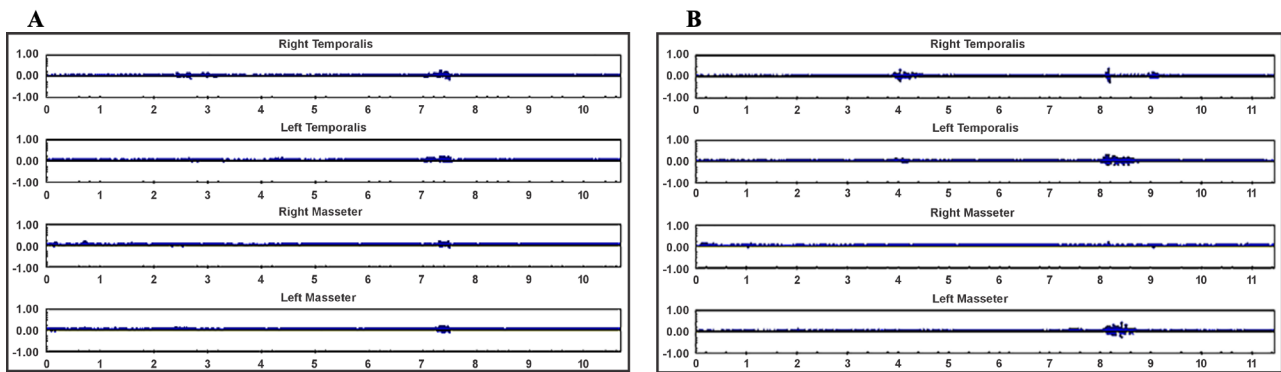


Figure 3. (A) Participant at Rest Before LED Application; (B) Participant at Rest After LED Application

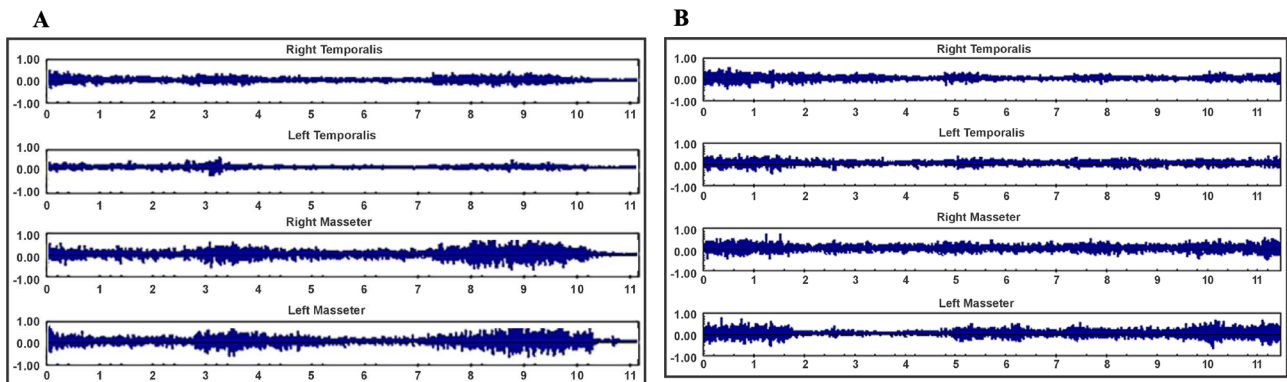


Figure 4. (A) Participant in MVC With ParaFilm M® Before LED Application; (B) Participant in MVC With ParaFilm M® After LED Application

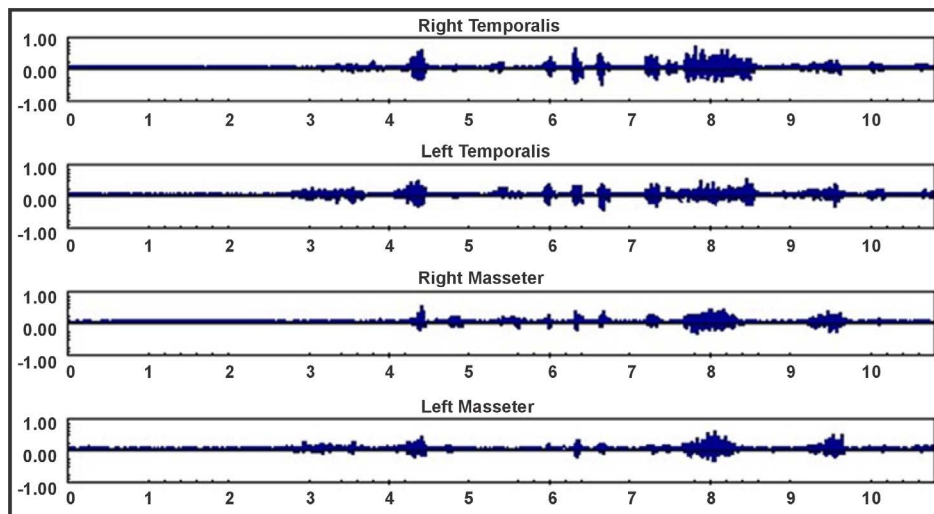


Figure 5. Masticatory Cycle After LED Application

An individual analysis of a representative participant's MVC demonstrated a clear improvement in muscle activation symmetry following LED application. The comparison of electromyographic readings (Figures 6A and 6B) revealed a more balanced distribution of muscle effort across the right and left masticatory muscles after the intervention. Although limited to a single case, this example illustrates the potential immediate regulatory effect of photobiomodulation on

neuromuscular coordination.

Reports from Participants' Parents/Guardians

Although not part of the formal outcome measures, anecdotal observations provided by the parents/guardians of the participants suggested a reduction in the frequency and intensity of bruxism episodes, particularly within the first three days following LED application. These subjective impressions were not collected through standardized

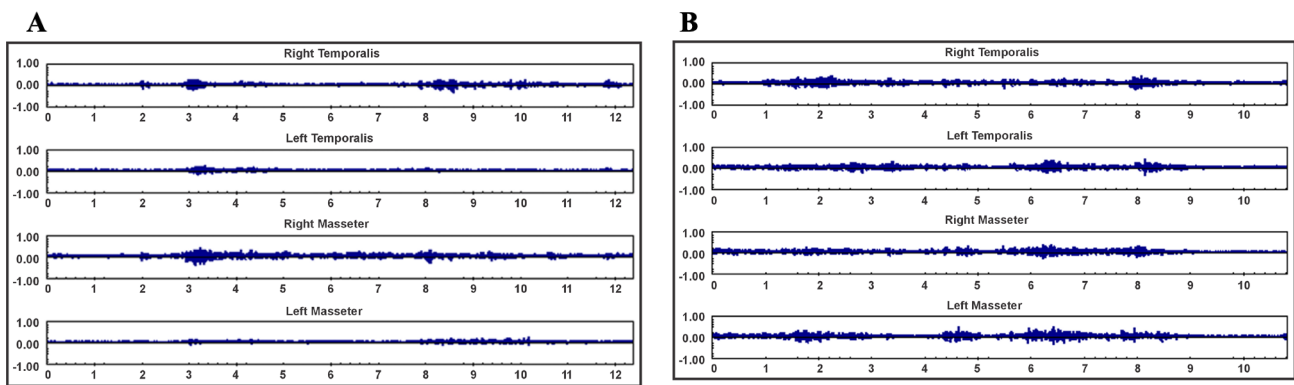


Figure 6. (A-B) Balance of Muscular Activities in all Target Muscles After LED Application in Maximum Voluntary Contraction

instruments and were not quantitatively analyzed, but they indicate a perceived short-term benefit, supporting the rationale for further studies using structured tools to assess behavioral outcomes over time.

Discussion

Trisomy 21, commonly known as DS, is a leading cause of cognitive impairment and is associated with a significant variety of social limitations and comorbidities.¹ Individuals with DS often have an abnormal oral and facial physiology, mainly due to muscle hypotonia, which can result in sleep disorders (e.g., obstructive sleep apnea) and problems such as bruxism, exerting a negative impact on quality of life and general well-being.² Early diagnosis of bruxism in children with DS is crucial not only to prevent tooth wear and alleviate craniofacial pain, but also to mitigate potential craniofacial alterations and enable the recovery of compromised dental and bone structures.¹⁵ Individuals diagnosed with DS and bruxism may exert excessive force during the characteristic repetitive movements of the latter condition.⁷

Furthermore, these individuals have characteristics of muscle spasticity, making the muscles more susceptible to involuntary contractions and requiring additional efforts to achieve relaxation.¹⁴ While hypotonia remains the primary neuromuscular feature in DS, there may be compensatory responses during functional activities, such as chewing and clenching. These responses may include increased muscle tone or spastic behavior, especially in tasks that require control of the jaw. In the present study, this duality was reflected in the electromyographic data, which revealed signs of involuntary contractions and abnormal activation patterns. This suggests that the underlying hypotonia and compensatory hyperactivity may coexist and contribute to bruxism in this population.

The electromyographic analysis confirmed these neuromuscular characteristics by revealing atypical patterns of activation, including irregular recruitment and muscle imbalance, especially during jaw movements, such as chewing and voluntary clenching.¹ While it is not possible to obtain a precise estimate of the prevalence of

bruxism in children with DS, the condition appears to occur more frequently in this group than in the general pediatric population, the prevalence of which ranges from 5.9% to 49.6%.¹⁶ Despite the significant prevalence of bruxism in children with DS, the literature on the management of this condition is limited, with only four studies published between 1988 and 2020 addressing this issue.⁹ Approaches range from stress-reducing medications,¹⁷ differential reinforcement of other behavior (DRO), and functional therapy with mandibular advancement devices.²

This study is among the first to explore the use of red LED clusters as a non-invasive photobiomodulation strategy targeting masticatory muscles in children and adolescents with DS and bruxism.⁵ Although the protocol did not produce statistically significant differences, electromyographic trends suggest a potential for the short-term modulation of muscle symmetry and neuromuscular stability. These findings are promising and warrant further investigation with larger samples and extended intervention periods. As the LED has mechanisms of action similar to those of lasers and has demonstrated the ability to modulate biological tissues, the results indicate the need for further research in this area. Given its low cost and favorable safety profile, LED therapy may constitute a viable adjunct for neuromuscular management in special populations when supported by well-structured clinical evidence.³

Conclusion

While the protocol used for LED application in the masticatory muscles achieved no statistically significant differences, clinical changes were observed in clenching and grinding movements, as reported by parents/guardians. These findings suggest the need for further studies assessing different LED devices and application protocols to explore potential improvements in treatment efficacy.

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Authors' Contribution

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Competing Interests

The authors have no conflicts of interest, financial or otherwise, to declare.

Data Availability Statement

The datasets (Excel spreadsheets) generated from this protocol are available from the corresponding author (Sandra Kalil Bussadori – sandra.skb@gmail.com) upon any reasonable request. However, reuse of these data will not be permitted for anyone who is not an author of this paper.

Ethical Approval

This study received approval from the Human Research Ethics Committee of Universidade Nove de Julho (UNINOVE), São Paulo, Brazil, under approval number 3.726.654. The protocol was registered at ClinicalTrials.gov under registration number NCT04211870, on December 26, 2019. All participants and/or their legal guardians signed the informed consent form and, for participants under 18 years of age, assent forms were also obtained. The research was conducted in accordance with the ethical principles established in the Declaration of Helsinki.

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Supplementary Files

Supplementary file 1 contains Table S1.

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