



Orofacial evaluation of individuals with temporomandibular disorder after LED therapy associated or not of occlusal splint: a randomized double-blind controlled clinical study

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Abstract

This study compared the effects of LED therapy associated with occlusal splint (OS) on the signs and symptoms of temporomandibular disorder (TMD). In this randomized, double-blind clinical trial, 70 TMD patients were randomly divided into six groups. The volunteers received the following treatments: Group 1 (G1) was the control and received only conventional therapy with OS; Group 2 (G2) was the placebo and received treatment with OS and therapy with LED (device turned off); Group 3 (G3) LED therapy (infrared,) once a week; Group 4 (G4) LED therapy (infrared) twice a week; Group 5 (G5) OS associated with LED (infrared) therapy (once a week); Group 6 (G6) received OS therapy plus infrared LED (two sessions per week). The patients were evaluated before, after, and 30 days after treatment. The pain intensity in masticatory system was recorded at each interval. The evaluation of the electromyographic signals (EMG) of the muscles (masseter and temporal) and blood lactate was performed before and after treatment. The associated groups presented better clinical results in relation to the control. The associated groups showed significant differences ($p < 0.05$) from control in the analysis of pain intensity and in decrease of the RMS value (EMG analysis). In the intragroup analysis, the volunteers in G6 exhibited a significant reduction ($p < 0.05$) in blood lactate. In conclusion, the association of LED therapy and OS presented superior results in relation to the isolated therapies, especially the protocol with two weekly sessions.

Keywords LED therapy · Photobiomodulation · TMD · Temporomandibular disorder · Temporomandibular dysfunction · Temporomandibular joint

Introduction

Temporomandibular disorder (TMD) is defined as a set of disorders involving the temporomandibular joint (TMJ), the masticatory muscles, and associated disorders. TMD characterized by the presence of signs and symptoms located in the orofacial region [1–4]. An estimated 60 to 80% of the general population will have at least one sign or symptom of TMD during their adult lives [5]. It is the most common cause of pain in the orofacial region, followed by tooth pain [6, 7].

The most painful conditions are in the masseter and temporal muscles [1, 2]. The TMJ region and other muscles of the face, neck, and shoulders also exhibit painful symptoms at a high frequency. Symptoms such as headache, ear disorders (tinnitus and pressure), and vertigo may also be present [8]. Among the signs of TMD, the most common are muscle hypertrophy, dental wear, and fracture and facial asymmetry.

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The set of these signs and symptoms can drastically decrease patients' quality of life [9] and impair sleep and the performance of daily activities such as speech, chewing, yawning, and even social interaction [10].

The treatment of TMD often consists of the use of medications, including analgesics, anti-inflammatories, anxiolytics, and muscle relaxants. Even though the use of analgesic and anti-inflammatory drugs is recommended for the treatment of severe acute pain, they may have adverse effects. In chronic pain, more frequent in TMD, noninvasive methods are used, considering the need for long-term treatment [11].

Among the noninvasive therapeutic alternatives for the treatment of TMD is the use of occlusal splint (OS), which is considered the gold standard in TMDs. Its use can promote the relaxation of the masticatory muscles. However, studies suggest that the effect of OS may be limited [4, 12].

Low-level laser therapy (LLLT) has been commonly used for the treatment of TMD [7, 13]. Recent studies about the use of LED in TMD have observed a reduction in painful symptoms and an increase in the amplitude of mandibular movements, pointing to a possible similarity in the effects of LED with those obtained with laser [1, 8, 9, 14].

Among the main effects of phototherapy is a reduction in inflammation and pain processes [8, 9]. However, the effects of LED therapy associated with OS have not yet been studied through a randomized, double-blind clinical trial.

Therefore, TMDs present persistent signs and symptoms in addition to difficult clinical management. The use of different combination resources to treat TMD must be investigated. For this reason, the present study aims to compare the effects of LED therapy associated with OS on the signs and symptoms of TMD.

Materials and methods

The Research and Development Institute (IP&D) of the Universidade do Vale do Paraíba (UNIVAP) carried out this double-blind, randomized controlled trial. All volunteers signed a declaration of free and informed consent, and all their rights according to the ethical premises established by resolution 466/2012 of the National Health Council (CNS) were protected. Of the one hundred and fifty-five (155) screened patients, seventy (70) met the study inclusion criteria. The protocol for this study was approved by the Research Ethics Committee of the UNIVAP under number 1,132,239/2015. The present study was also duly registered with the Brazilian Registry of Clinical Trials (RBR-3bs2g4).

Sample size

The sample calculation considered $\alpha = 0.05$ (type I error of 5% probable), $\beta = 0.95$ (95% of the sample power) [15], and data from a visual analog scale (VAS) listed in Demirkol et al.

[11]. The minimum number for each group was determined to be six volunteers, of which four participants were used to compensate for possible dropouts (totaling ten individuals per group) [7]. This calculation used the principles of Armitage and Berry [16], available on the online platform of the Laboratory of Epidemiology and Statistics of the University of São Paulo (LEE - USP).

Inclusion/exclusion criteria

The inclusion criteria for the volunteers:

- Between 18 and 55 years old of either gender
- Pain in the facial area lasting at least 3 months
- Diagnosed with TMD of myogenic origin according to the Research Diagnostic Criteria for Temporomandibular Disorder (RDC/TMD-axis I, category Ia and Ib). To diagnose the TMD, the revised version of the RDC/TMD (axis I) was applied by a single examiner (with more than seven years of research experience).

The exclusion criteria were volunteers:

- Undergoing any treatment for TMD (occlusal splint, physiotherapy, acupuncture, *medication therapy*, and others)
- Previous diagnosis of joint disorders, fibromyalgia, and other painful musculoskeletal syndromes
- With a history of surgery or head/neck trauma
- Who used prescription drugs, such as anxiolytic, anti-inflammatory, analgesic, muscle relaxants, antidepressants, and anticonvulsants
- Pregnant women

Study design, randomization, and blinding procedures

The sample consisted of 70 volunteers, who were randomly distributed into six treatment groups by means of a drawing.

The randomization procedure consisted of drawing six cards (A = Group I, B = Group VI, C = Group II, D = Group V, E = Group III, F = Group IV) carried out by the volunteers in the presence of a researcher who did not participate in clinical evaluations and data analysis.

The volunteers were not informed about the meanings of the cards. During LED therapy (placebo or not), the volunteers used dark green and sealed goggles (opaque card in front of the eyes). In the associated groups, only the irradiation was hidden. During the evaluations and statistical analyses, the researchers did not have access to the corresponding groups of each volunteer.

After the division of the groups, the volunteers received the following treatments: Group I (G1) was the control and received only conventional therapy with occlusal splint (OS); Group II (G2) was the placebo and received treatment with OS and placebo treatment with LED (device turned off); Group III (G3) LED therapy (infrared) once a week; Group IV (G4) LED therapy (infrared) twice a week; Group V (G5) OS associated with LED (infrared) therapy (once a week); Group VI (G6) received OS therapy plus infrared LED (two sessions per week) (Table 1).

The volunteers underwent 4 weeks of treatment and three evaluations, with the first evaluation before the start of treatment and the second immediately after the end of treatment. The third evaluation took place 30 days after the second evaluation. All assessments analyzed pain intensity. In the first two evaluations (before and after therapy), surface electromyography and the blood lactate test were performed. Figure 1 presents the study flowchart.

Procedures

For the diagnosis of TMD, we used the research diagnostic criteria (RDC) for TMD. First, axis II was applied, which consists of a questionnaire. Axis II classifies individuals based on the degree of chronic pain, depression, and physical symptoms. It employed the questionnaire (RDC axis II) in an air-conditioned, well-lit room and without time restrictions. Then, a clinical examination (axis II) was carried out to analyze and record the signs and symptoms related to TMD. A dental surgeon (Ph.D. in biomedical engineering and with more than 10 years of research experience) performed all clinical evaluations.

Pain assessment during the clinical examination (muscle and joint palpation) was performed based on the RDC for TMD. The visual analog scale (VAS) was used to record pain intensity. The VAS consists of a horizontal numerical scale of 10 cm, in which volunteers record their pain intensity. The rating scale contains values from 0 (no pain) to 10 (worst pain imaginable).

In assessing muscle activity, two-channel surface electromyography (EMG) (EMG 230 DL, EMG System from Brazil,

São Paulo, Brazil) was used, with a sampling frequency of 2000 Hz and a 20 Hz and 500 bandpass filter Hz, with the rejection mode greater than 120 dB. The volunteers used disposable silver chloride (Ag/AgCl) electrodes coated with aluminum foil with high conductivity and low impedance synthetic prehydrogel (Miotec, Porto Alegre, Brazil). The electrodes were placed on the thickest part of the masseters and anterior portion of the temporal muscles. The reference electrode was placed in the styloid process of the right ulna. Before placing the electrodes, the skin was cleaned with gauze (Cremer, Santa Catarina, Brazil) and 70% alcohol (Santa Cruz®, São Paulo, Brazil). First, the activity of the masseter muscle was collected, and after a 5-min interval, the temporal muscle was collected.

The rest protocol (RP) and isometric movement protocol (IMP) were used, both for 10 s, to collect the electromyographic signals. In the RP, the volunteers were instructed to keep the upper and lower lip in contact, with no occlusal contact between the teeth [1, 14]. In IMP, the volunteers were instructed, using a verbal and visual stimulus [1] of the electrical signals, to perform an occlusion of the teeth, performing an isometric contraction. A cotton roll was placed bilaterally in the molar region to avoid direct occlusal contact between the teeth.

Blood lactate was collected before and after the EMG protocols, totaling four collections. These collections were performed in the first (before treatment) and the second (immediately after treatment) evaluations. For the examination, a monitor was used to determine blood lactate (Accutrend®, Roche, Mannheim, Germany) and test strips (BM-lactate, Roche, Mannheim, Germany). Before the collections, the device was calibrated using the “master tape” (BM-lactate, Roche, Mannheim, Germany), and then the reading was performed, in which each disposable test strip had its test area covered by a sample of blood. The blood sample was obtained from the volunteers through a disposable lancet (Softclix, Roche, Mannheim, Germany), adapted to a pen, in the distal region of the middle finger of the phalanx, which was prewashed with neutral soap and rinsed under running water. Then, local pressure was made with sterile gauze (Cremer, Santa Catarina, Brazil).

For volunteers using OS, stabilizing plates were made (DEMIRKOL et al., 2014). Patients were instructed to use the splint during sleep for 4 weeks.

For LED therapy, the Fioleed® equipment (MMOptics, São Carlos, SP, Brazil) was used, with a wavelength of 880 ± 20 nm and continuous emission mode, according to the parameters presented in Table 2. The equipment was previously calibrated by the manufacturer.

The irradiation was performed punctually, in contact and perpendicularly, bilaterally, in four points in the form of a cross in the pre-auricular region (Fig. 2), one external non-acoustic point, six points in the masseter, and two points in the temporal muscle (anterior bundle).

Table 1 Division of experimental groups

Groups	Acronyms	LED therapy	Occlusal plate	Frequency
Group 1	G1		X	1×/week*
Group 2	G2	X (placebo)	X	1×/week
Group 3	G3	X		1×/week
Group 4	G4	X		2×/week
Group 5	G5	X	X	1×/week
Group 6	G6	X	X	2×/week

Legend: *Adjusting the occlusal splint

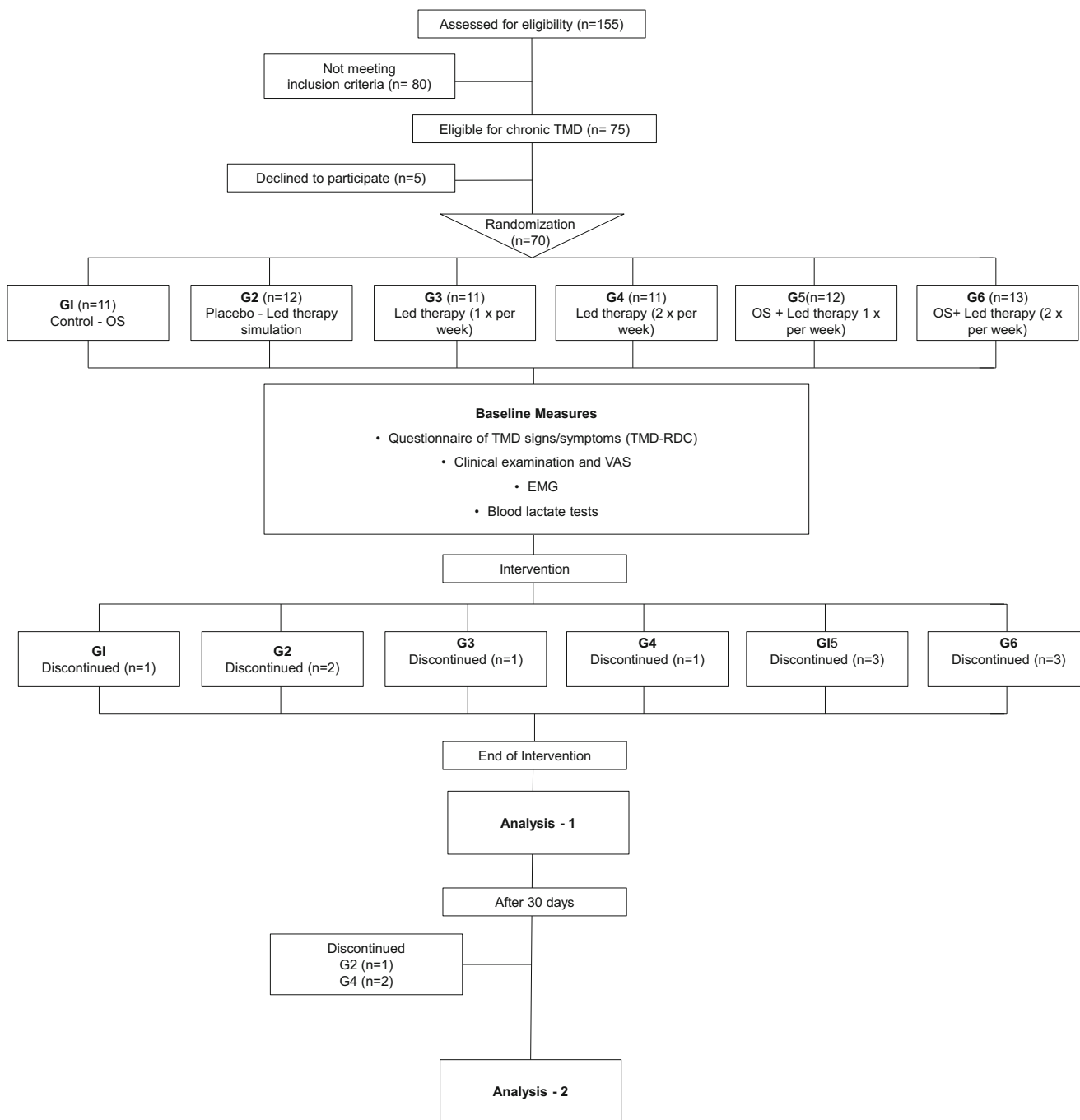


Fig. 1 Study flowchart

The LED therapy points were marked with the aid of a perforated plastic template. Both the parameters and the electromagnetic irradiation points were based on our previous studies and on the specific literature [1–14].

Statistical analysis

The statistical analysis was performed with the aid of software (GraphPad Prism version 5.0, GraphPad

Software, CA, USA) using the Kolmogorov-Smirnov test to analyze the data distribution. After the normality test, the ANOVA test was applied with the Tukey post-test (multiple comparisons) and *T* test (paired test) for the samples that showed the normality distribution. For samples that did not show normality, the Kruskal-Wallis test was used with Dunn's post-test (multiple comparisons) and the Wilcoxon test (paired test). For all tests, a significance level of 5% ($p < 0.05$) was considered.

Table 2 The treatment parameters used in the irradiated groups

Parameters	LED
λ (nm)	880 \pm 20
A (cm ²)	0.7
\emptyset (cm)	1
P (W)	0.03
T (s)	70
E (J)	2.1
ED (J/cm ²)	3
Diodo	InGaAlP
Mode	Continuous

λ , wavelength, A, area, \emptyset , diameter, P, power, T, time, ED, energy density

Results

The study sample consisted of 70 volunteers, 56 (80%) of whom were female and 14 (20%) were male. The average age of the volunteers was 31.4 years (\pm 9.5). Forty-two (60%) of the volunteers were diagnosed with myofascial pain and limited mouth opening (II b) and 28 (40%) with myofascial pain (I b), according to the criteria established by the RDC/TMD.

During the experiment, 11 volunteers were excluded—7 for lack or abandonment of treatment and 3 for using drugs banned

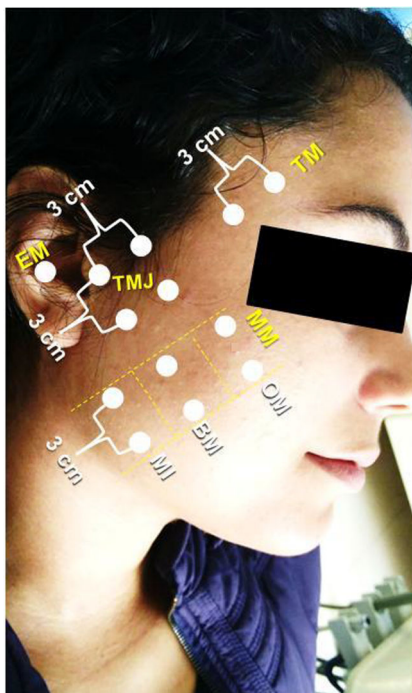


Fig. 2 Points of LED therapy in the orofacial region. (MM) masseter muscle; (OM) origin of the masseter muscle; (BM) masseter muscle body; (MI) insertion of the masseter muscle (ME) external meato acoustic region; (TM) temporal muscle

by the study. Another three volunteers were excluded for not attending the follow-up evaluation (30 days after treatment).

In the intragroup analysis of pain intensity, quantified by VAS scores, all groups presented a significant decrease ($p < 0.05$), both in post-therapy and 30 days post-treatment (Fig. 3).

In the intergroup analysis post-therapy (Fig. 4a), G5 showed extremely significant differences from G1 ($p = 0.0004$) and G2 ($p = 0.0008$). In G6, extremely significant differences were also observed in relation to G1 ($p = 0.0004$), G2 ($p = 0.0003$), and G3 ($p = 0.0007$). In G4, an extremely significant difference was observed from G1 ($p = 0.0004$). Figure 4b presents the last evaluation, performed 30 days after the end of treatment, which verified that G6 continued to exhibit extremely significant differences from G1 ($p = 0.0004$), G2 ($p = 0.0006$), and G3 ($p = 0.0004$). G5 ($p = 0.0004$) and G4 ($p = 0.003$) also showed significant differences from G1.

Analysis of the RMS values (normalized) for the rest and isometric movement protocols was performed. In the intragroup analysis of the resting protocol (Table 3), G1 ($p = 0.02$ and $p = 0.008$) and G2 ($p = 0.04$ and $p = 0.01$) displayed a significant decrease in RMS in the right masseter (RM) and left temporal (LT). In G3, there was a significant reduction in the RMS of the left masseter (LM) ($p = 0.0039$), RM ($p = 0.004$), and LT ($p = 0.004$). G4 had a significant decrease in the LM ($p = 0.01$) and right temporal (RT) ($p = 0.008$). In G5, a significant decrease was observed in all muscles (LM $p = 0.01$, RM $p = 0.008$, RT $p = 0.02$, and LT $p = 0.04$). G6 showed a significant decrease in the RMS value in the LM ($p = 0.019$), RM ($p = 0.0039$), and LT ($p = 0.049$) muscles. In the intergroup analysis, G4 presented a significant difference in RT ($p = 0.001$) compared to G1. In G5, significant differences were observed in the ML from G1 ($p = 0.02$) and G2 ($p = 0.04$), and there was also a significant difference in the TR for G1 ($p = 0.008$).

The intragroup evaluation of the isometric movement protocol (Table 4) revealed that G2 had a significant decrease in RMS in LT compared to G1 ($p = 0.002$). In G3, there was a notable increase in the RMS in the LM compared to G1 ($p = 0.0016$) and G2 ($p = 0.02$). G4 exhibited a significant increase in LT compared to G2 ($p = 0.0002$). In G5, a notable decrease in the RMS compared to G1 ($p = 0.0056$) and G3 ($p < 0.0001$) was found in the LT.

In the evaluation of blood lactate, the intragroup comparison evidenced (Fig. 5) that only G6 achieved a significant decrease ($p = 0.03$). Intergroup analysis showed no differences between groups.

Discussion

The etiology of TMD is complex and multifactorial. Physical and emotional factors can contribute to the appearance, development, and perpetuation of this disorder, which hinders its treatment and clinical management [9, 17]. Currently,

Fig. 3 Intragroup analysis (mean \pm standard error) of pain intensity (VAS score)

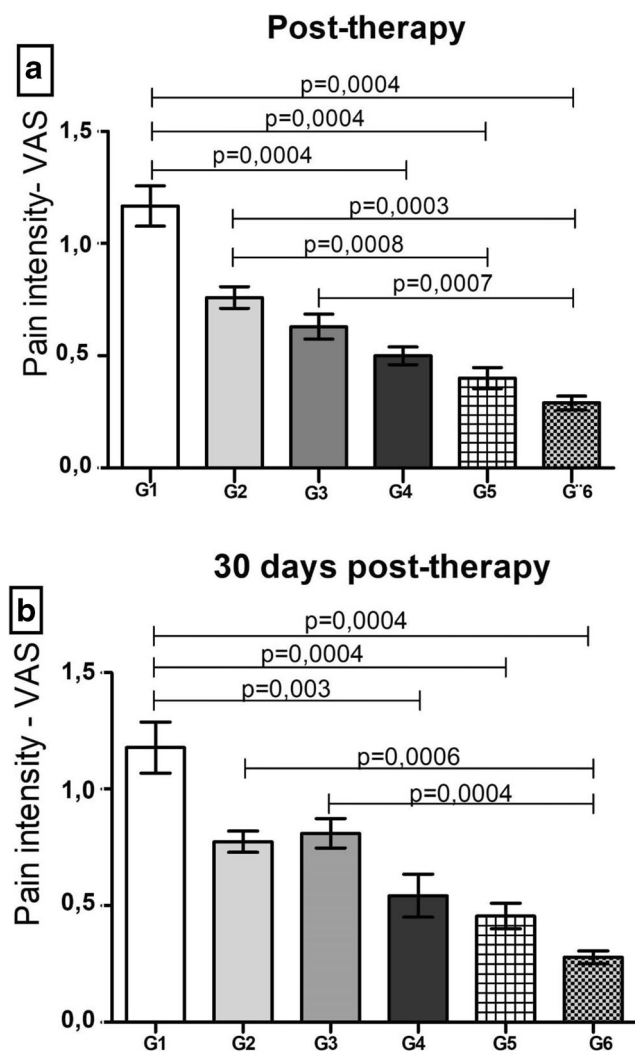
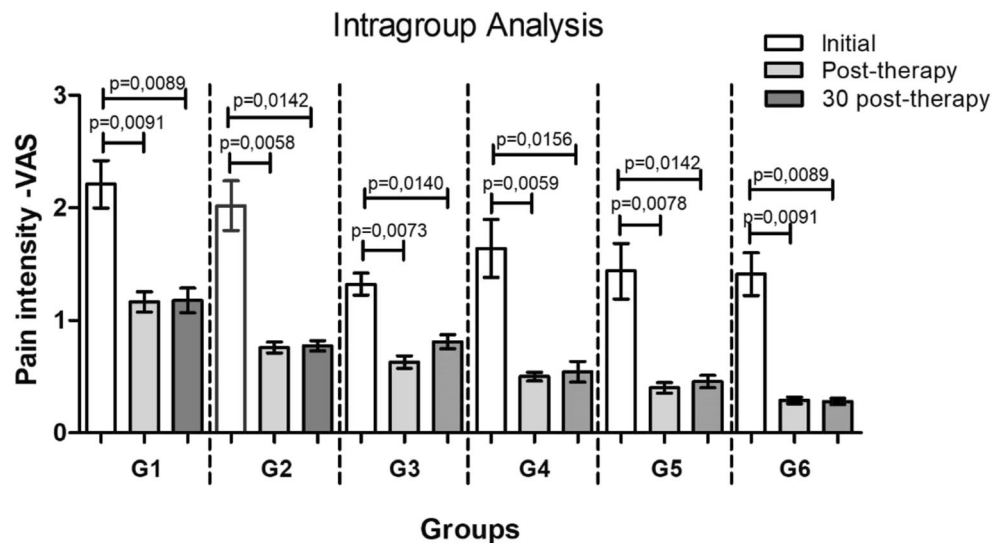


Fig. 4 a, b Intergroup evaluation (mean \pm standard error) of pain intensity (VAS score) after therapy and 30 days after therapy

noninvasive and reversible interventions, such as the use of OS and low-level laser therapy, among other therapeutic and complementary resources are indicated to treatment TMD [12, 18, 19]. The current literature offers few clinical, randomized, controlled, and double-blind studies that compare the effect and effectiveness of different noninvasive interventions alone or in combination to treat TMD [17]. In particular, LED therapy had not been analyzed in association with OS.

The analysis of pain intensity verified that the irradiated groups (G3, G4, G5, and G6) achieved a significant pain reduction compared to the control (G1) and placebo (G2) groups at post-therapy and 30 days post-therapy. The group that received two LED sessions per week associated with OS (G6) presented more efficient results than those obtained in G1, G2, and G3. Studies that used OS [12, 20, 21], or laser therapy [2, 7, 18], or LED therapy [8, 9] in isolation, also found a significant decrease in pain intensity. Conti et al. [20] and Pereira et al. [14] also found that pain reduction occurred more than 30 days after treatment.

On the other hand, Chen et al. [22] and Magri et al. [3] reported controversial effects on pain reduction in patients undergoing phototherapy. The study by Magri et al. [3], in which three treatment modalities were compared, including OS and laser therapy, found that both OS and laser therapy alone showed a significant reduction in pain intensity. However, there was no significant difference between groups. Nevertheless, these studies had some differences from the present study regarding the methodology adopted. In the meta-analysis by Chen et al. [22], there was no distinction between the types of TMD (articular or myogenic) in the analyses. In the study by Magri et al. [3], the proposed treatment time is different between therapies.

RMS analysis was used to assess the behavior of the electrical activity of the masticatory muscles. In the rest protocol, the

Table 3 Analysis of the variation of the RMS (normalized) of the rest protocol

Groups		Rest protocol			
		LM	RM	LT	RT
LB		1.0	1.0	1.0	1.0
G1	<i>v</i>	0.92 ± 0.14	0.65 ± 0.11	0.62 ± 0.10	1.58 ± 0.27
	<i>p1</i>	ns	0.02	0.008	ns
	<i>p2</i>	ns	ns	ns	ns
G2	<i>v</i>	1.03 ± 0.19	0.81 ± 0.08	0.62 ± 0.12	0.84 ± 0.25
	<i>p1</i>	ns	0.04	0.01	ns
	<i>p2</i>	ns	ns	ns	ns
G3 too	<i>v</i>	0.61 ± 0.10	0.82 ± 0.07	0.57 ± 0.10	1.03 ± 0.47
G3	<i>p1</i>	0.0039	0.004	0.004	ns
	<i>p2</i>	ns	ns	ns	ns
G4	<i>v</i>	0.74 ± 0.05	0.67 ± 0.17	0.78 ± 0.12	0.55 ± 0.13
	<i>p1</i>	0.0156	ns	ns	0.008
	<i>p2</i>	ns	ns	ns	0.001A
G5	<i>v</i>	0.48 ± 0.11	0.72 ± 0.02	0.60 ± 0.12	0.45 ± 0.14
	<i>p1</i>	0.016	0.008	0.02	0.049
	<i>p2</i>	0.02A;0.04B	ns	ns	0.008A
G6	<i>v</i>	0.61 ± 0.12	0.62 ± 0.11	0.47 ± 0.10	0.99 ± 0.16
	<i>p1</i>	0.019	0.0039	0.008	ns
	<i>p2</i>	ns	ns	ns	ns

LM, left masseter; *RM*, right masseter; *LT*, left temporal; *RT*, right temporal; *v*, values of normalized means and standard error of RMS, *LB*, baseline, *p1*, *p* value in intragroup analysis, *p2*, *p* value in intergroup analysis; *ns*, not significant ($p > 0.05$)

^A Significant difference from control ($p < 0.05$)

^B Significant difference from placebo

Table 4 Analysis of the variation of the RMS (normalized) of the isometric movement protocol

Groups		Isometric movement protocol			
		LM	RM	LT	RT
LB		1	1	1	1
G1	<i>v</i>	0.62 ± 0.13	1.12 ± 0.06	1.3 ± 0.17	0.98 ± 0.10
	<i>p1</i>	0.04	ns	ns	ns
	<i>p2</i>	ns	ns	ns	ns
G2	<i>v</i>	0.86 ± 0.24	1.04 ± 0.27	0.46 ± 0.10	0.82 ± 0.10
	<i>p1</i>	ns	ns	0.0156	ns
	<i>p2</i>	ns	ns	0.002A	ns
G3	<i>v</i>	0.99 ± 0.23	0.98 ± 0.15	1.10 ± 0.17	1.10 ± 0.21
	<i>p1</i>	ns	ns	ns	ns
	<i>p2</i>	ns	ns	0.009B	ns
G4	<i>v</i>	1.13 ± 0.24	1.28 ± 0.22	1.37 ± 0.10	1.31 ± 0.37
	<i>p1</i>	ns	ns	0.0078	ns
	<i>p2</i>	ns	ns	0.0002B	ns
G5	<i>v</i>	1.57 ± 0.17	0.79 ± 0.14	1.92 ± 0.65	1.17 ± 0.17
	<i>p1</i>	0.02	ns	ns	ns
	<i>p2</i>	0.0016A; 0.02B; 0.04C	ns	0.0093B	ns
G6	<i>v</i>	1.14 ± 0.23	0.69 ± 0.12	0.66 ± 0.16	1.47 ± 0.21
	<i>p1</i>	ns	ns	0.0078	ns
	<i>p2</i>	ns	ns	0.0056 ^A ; <0.0001 ^D	0.02B

LM, left masseter; *RM*, right masseter; *LT*, left temporal; *RT*, right temporal; *v*, values of normalized means and standard error of RMS, *LB*, baseline, *p1*, *p* value in intragroup analysis, *p2*, *p* value in intergroup analysis; *ns*, not significant ($p > 0.05$)

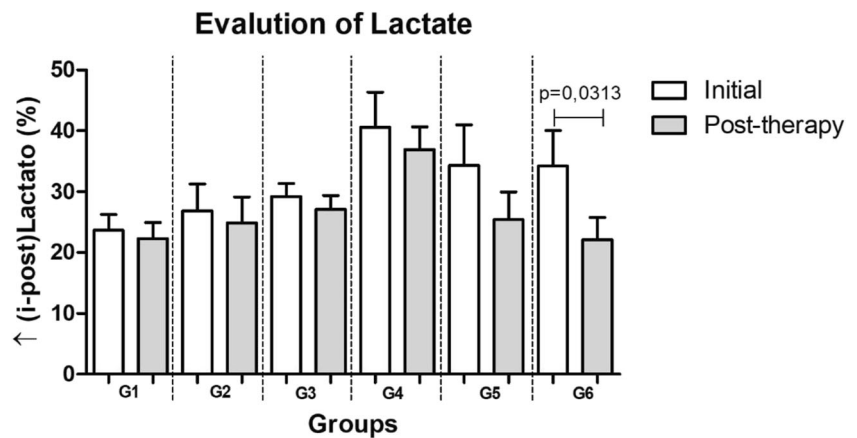
^A Significant difference from control ($p < 0.05$)

^B Significant difference to placebo

^C Significant difference from G3 ($p < 0.05$)

^D Significant difference from G4 ($p < 0.05$)

Fig. 5 Intragroup analysis (mean \pm standard error) of the variation in the increase in blood lactate levels



associated groups (G5 and G6) displayed significantly decreased control in at least one muscle. In the isometric movement, we found a change in the behavior of the volunteers' muscle activity, and with the positive results of the other analyses, we can infer that these changes were beneficial. These findings demonstrate a decrease in muscle activity, probably caused by the improvement of the muscular situation.

Studies comparing healthy individuals with TMD patients found that at rest, the electrical activity of the masticatory muscles was high in TMD patients [23–25]. Criado et al. [25] evaluated the electrical activity of muscles in patients undergoing biofeedback treatment and found a decrease in RMS after treatment. In the isometric movement, neuromuscular rebalancing could be observed, since some groups presented an increase in RMS and others a decrease. In the case of G6, a significant reduction in the right temporal muscle was observed compared with the control, suggesting that the treatment was able to break the cyclical muscle spasm. According to Santos [26], the cyclic cramp is due to the perpetuation of myalgia that generates impulses for the central nervous system so that the reaction returns to the spastic muscle, stimulating it.

The analysis of blood lactate levels verified that only G6 achieved a significant decrease post-therapy. In the comparison between the groups, there was no significant difference in the variation of lactate levels. These findings may indicate less use of the anaerobic route and an increase in blood flow in the affected region, allowing high removal of blood lactate and reduction of muscle fatigue.

The use of OS favors the reduction of muscle hyperactivity. LED therapy stimulates the supply of oxygen and nutrients to muscle tissue due to increased blood flow caused by the action of nitrous oxide [27]. According to Huang et al. [28], the production of nitrous oxide may be due to the action of nitrate reductase, an enzyme in the mitochondrial chain, activated by electromagnetic irradiation.

Conclusion

The results of the present study provide evidence that:

- LED therapy with the tested parameters, when used alone or associated with the occlusal splint, promote the pain reduction in the masseter and temporal muscles, in addition to enabling the modulation of the electrical activity of the muscles in individuals with TMD of myogenic origin (without joint involvement);
- The combination of LED therapy and occlusal splint achieves superior results compared to isolated treatments, and the protocol of two sessions per week proved to be better.

Declarations

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. The authors declare that they have no conflict of interest.

Conflict of interest The authors declare no competing interests.

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